CORONAVIRUS DISEASE (COVID-19): DIET, INFLAMMATION AND NUTRITIONAL STATUS

EDITED BY: Ioannis Zabetakis, Christophe Matthys and Alexandros Tsoupras

PUBLISHED IN: Frontiers in Nutrition and Frontiers in Immunology

frontiers Research Topics

Frontiers eBook Copyright Statement

The copyright in the text of individual articles in this eBook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this eBook is the property of Frontiers.

Each article within this eBook, and the eBook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this eBook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or eBook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-88971-581-7
DOI 10.3389/978-2-88971-581-7

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

CORONAVIRUS DISEASE (COVID-19): DIET, INFLAMMATION AND NUTRITIONAL STATUS

Topic Editors:
Ioannis Zabetakis, University of Limerick, Ireland
Christophe Matthys, KU Leuven, Belgium
Alexandros Tsoupras, University of Limerick, Ireland

Citation: Zabetakis, I., Matthys, C., Tsoupras, A., eds. (2021). Coronavirus Disease (COVID-19): Diet, Inflammation and Nutritional Status.
Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-581-7

Table of Contents

06 ***Editorial: Coronavirus Disease (COVID-19): Diet, Inflammation and Nutritional Status***
Ioannis Zabetakis, Christophe Matthys and Alexandros Tsoupras

08 ***Mucosal-Associated Invariant T Cells as a Possible Target to Suppress Secondary Infections at COVID-19***
Roman A. Akasov and Evgeny V. Khaydukov

12 ***Understanding Selenium and Glutathione as Antiviral Factors in COVID-19: Does the Viral M^{pro} Protease Target Host Selenoproteins and Glutathione Synthesis?***
Ethan Will Taylor and Wilson Radding

19 ***Perspective: The Potential Effects of Naringenin in COVID-19***
Ricardo Wesley Alberca, Franciane Mouradian Emidio Teixeira, Danielle Rosa Beserra, Emily Araujo de Oliveira, Milena Mary de Souza Andrade, Anna Julia Pietrobon and Maria Notomi Sato

28 ***Thrombosis and COVID-19: The Potential Role of Nutrition***
Alexandros Tsoupras, Ronan Lordan and Ioannis Zabetakis

36 ***Immune-Boosting, Antioxidant and Anti-inflammatory Food Supplements Targeting Pathogenesis of COVID-19***
M. Mrityunjaya, V. Pavithra, R. Neelam, P. Janhavi, P. M. Halami and P. V. Ravindra

48 ***Rethinking Urban and Food Policies to Improve Citizens Safety After COVID-19 Pandemic***
Andrea Galimberti, Hellas Cena, Luca Campone, Emanuele Ferri, Mario Dell'Agli, Enrico Sangiovanni, Michael Belingheri, Michele Augusto Riva, Maurizio Casiraghi and Massimo Labra

57 ***Clinical Impact Potential of Supplemental Nutrients as Adjuncts of Therapy in High-Risk COVID-19 for Obese Patients***
Emre Sahin, Cemal Orhan, Fatih M. Uckun and Kazim Sahin

73 ***Tea Bioactive Modulate Innate Immunity: In Perception to COVID-19 Pandemic***
Pritom Chowdhury and Anoop Kumar Barooah

82 ***The Long History of Vitamin C: From Prevention of the Common Cold to Potential Aid in the Treatment of COVID-19***
Giuseppe Cerullo, Massimo Negro, Mauro Parimbelli, Michela Pecoraro, Simone Perna, Giorgio Liguori, Mariangela Rondanelli, Hellas Cena and Giuseppe D'Antona

98 ***NLRP3 Inflammasome: The Stormy Link Between Obesity and COVID-19***
Alberto López-Reyes, Carlos Martinez-Armenta, Rocio Espinosa-Velázquez, Paola Vázquez-Cárdenas, Marlid Cruz-Ramos, Berenice Palacios-Gonzalez, Luis Enrique Gomez-Quiroz and Gabriela Angélica Martínez-Nava

107 ***The Impact of Obesity and Lifestyle on the Immune System and Susceptibility to Infections Such as COVID-19***
Daan L. de Frel, Douwe E. Atsma, Hanno Pijl, Jacob C. Seidell, Pieter J. M. Leenen, Willem A. Dik and Elisabeth F. C. van Rossum

119 ***Zinc, Vitamin D and Vitamin C: Perspectives for COVID-19 With a Focus on Physical Tissue Barrier Integrity***
José João Name, Ana Carolina Remondi Souza, Andrea Rodrigues Vasconcelos, Pietra Sacramento Prado and Carolina Parga Martins Pereira

133 ***A Network-Based Analysis Reveals the Mechanism Underlying Vitamin D in Suppressing Cytokine Storm and Virus in SARS-CoV-2 Infection***
Firoz Ahmed

150 ***Ramadan Fasting During the COVID-19 Pandemic; Observance of Health, Nutrition and Exercise Criteria for Improving the Immune System***
Majid Taati Moghadam, Behzad Taati, Seyed Mojtaba Paydar Ardakani and Katsuhiko Suzuki

160 ***The Burden of Malnutrition and Fatal COVID-19: A Global Burden of Disease Analysis***
Elly Mertens and José L. Peñalvo

172 ***The Impact of Lockdown During the COVID-19 Outbreak on Dietary Habits in Various Population Groups: A Scoping Review***
Grace Bennett, Elysia Young, Isabel Butler and Shelly Coe

182 ***Negative Association Between Mediterranean Diet Adherence and COVID-19 Cases and Related Deaths in Spain and 23 OECD Countries: An Ecological Study***
Michael W. Greene, Alexis P. Roberts and Andrew D. Frugé

189 ***Vitamin D in Corona Virus Disease 2019 (COVID-19) Related Multisystem Inflammatory Syndrome in Children (MIS-C)***
Gavriela Feketea, Vasiliki Vlacha, Ioana Corina Bocsan, Emilia Vassilopoulou, Luminita Aurelia Stanciu and Mihnea Zdrenghea

196 ***The Looming Effects of Estrogen in Covid-19: A Rocky Rollout***
Hayder M. Al-kuraishy, Ali I. Al-Gareeb, Hani Faidah, Thabat J. Al-Maiahy, Natália Cruz-Martins and Gaber El-Saber Batiha

204 ***Bovine Coronavirus Immune Milk Against COVID-19***
Antonio Arenas, Carmen Borge, Alfonso Carbonero, Ignacio Garcia-Bocanegra, David Cano-Terriza, Javier Caballero and Antonio Arenas-Montes

209 ***Low Serum 25-hydroxyvitamin D (Vitamin D) Level Is Associated With Susceptibility to COVID-19, Severity, and Mortality: A Systematic Review and Meta-Analysis***
Mohammad Rizki Akbar, Arief Wibowo, Raymond Pranata and Budi Setiabudiawan

219 ***Cooking at Home and Adherence to the Mediterranean Diet During the COVID-19 Confinement: The Experience From the Croatian COVIDiet Study***
Danijela Pfeifer, Josip Rešetar, Jasenka Gajdoš Kljusurić, Ines Panjkota Krbavčić, Darija Vranešić Bender, Celia Rodríguez-Pérez, María Dolores Ruíz-López and Zvonimir Šatalić

237 ***Breastfeeding and COVID-19: From Nutrition to Immunity***
Emilia Vassilopoulou, Gavriela Feketea, Lemonica Koumbi, Christina Mesiari, Elena Camelia Berghea and George N. Konstantinou

247 ***Clinical Characteristics and Survival Analysis in Frequent Alcohol Consumers With COVID-19***
Ricardo Wesley Alberca, Paula Ordonhez Rigato, Yasmim Álefe Leuzzi Ramos, Franciane Mouradian Emidio Teixeira, Anna Cláudia Calvielli Branco, Iara Grigoletto Fernandes, Anna Julia Pietrobon, Alberto Jose da Silva Duarte, Valeria Aoki, Raquel Leão Orfali and Maria Notomi Sato

254 ***COVID-19 Severity in Obesity: Leptin and Inflammatory Cytokine Interplay in the Link Between High Morbidity and Mortality***
Radheshyam Maurya, Prince Sebastian, Madhulika Namdeo, Moodu Devender and Arieh Gertler

265 ***Effects of the Mediterranean Lifestyle During the COVID-19 Lockdown in Spain: Preliminary Study***
Ana Zaragoza-Martí, Miriam Sánchez-SanSegundo, Rosario Ferrer-Cascales, Eva Maria Gabaldón-Bravo, Ana Laguna-Pérez and Lorena Rumbo-Rodríguez

272 ***A Computational Approach Identified Andrographolide as a Potential Drug for Suppressing COVID-19-Induced Cytokine Storm***
Mohd Rehan, Firoz Ahmed, Saad M. Howladar, Mohammed Y. Refai, Hanadi M. Baeissa, Torki A. Zughaibi, Khalid Mohammed Kedwa and Mohammad Sarwar Jamal

282 ***Dairy-Derived and Egg White Proteins in Enhancing Immune System Against COVID-19***
Gaber El-Saber Batiha, Mohammed Alqarni, Dina A. B. Awad, Abdelazeem M. Algammal, Richard Nyamota, Mir I. I. Wahed, Muhammad Ajmal Shah, Mohammad N. Amin, Babatunde O. Adetuyi, Helal F. Hetta, Natália Cruz-Martins, Niranjan Koirala, Arabinda Ghosh, Javier Echeverría, Jorge Pamplona Pagnossa and Jean-Marc Sabatier

frontiers in Nutrition

EDITORIAL
published: 14 September 2021
doi: 10.3389/fnut.2021.760720

Editorial: Coronavirus Disease (COVID-19): Diet, Inflammation and Nutritional Status

Ioannis Zabetakis[1,2,3]*, *Christophe Matthys*[4,5] *and Alexandros Tsoupras*[1,2,3]

[1] Department of Biological Sciences, University of Limerick, Limerick, Ireland, [2] Bernal Institute, University of Limerick, Limerick, Ireland, [3] Health Research Institute, University of Limerick, Limerick, Ireland, [4] Clinical and Experimental Endocrinology, Department of Chronic Diseases and Metabolism, KU Leuven, Leuven, Belgium, [5] Department of Endocrinology, University Hospitals Leuven, Leuven, Belgium

Keywords: inflammation, chronic disease, COVID-19, obesity, nutrition, Mediterranean diet (MD)

Editorial on the Research Topic

Coronavirus Disease (COVID-19): Diet, Inflammation and Nutritional Status

Since late 2019, the world has come to terms with the fact that it is facing a major public health crisis against the novel coronavirus disease termed COVID-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of August 12th 2021, there are over 205 million confirmed cases of COVID-19 and 4,300,000 deaths globally while more than 180 million patients have recovered. The first known case of COVID-19 originated from the city of Wuhan in Hubei Province, China. From there, it has spread to every inhabited continent worldwide.

COVID-19 is a highly infectious disease with severe or even lethal complications and a significant impact on the way in which we live our lives. Whilst the vast majority of people infected with COVID-19 experience mild symptoms and can recover without the need for hospitalization, it has become increasingly clear that those with pre-existing non-communicable diseases such as chronic lung diseases, metabolic syndrome, obesity and diabetes mellitus, cardiovascular disease, and renal disorders, as well as people under immunosuppression (i.e., the elderly and people with chronic rheumatoid conditions like rheumatoid arthritis, Lupus, etc.), are at increased risk of severe illness and mortality. The inflammatory and thrombo-inflammatory manifestations and cascades following SARS-CoV-2 infection are directly linked to the increased severity of COVID-19 complications, especially in patients with such underlying conditions and the elderly.

Thus, the potential for specific foods and nutrients that can beneficially affect COVID-19 severity and outcomes is gathering increasing interest from the scientific community, as well as the general population and media. Given that a common complication in patients with severe COVID-19, and individuals with non-communicable diseases, is excessive inflammation, foods with anti-inflammatory properties may possess a protective role. Likewise, the role of nutritional status and other nutrients, such as selenium, vitamin D, and vitamin C has gathered attention, with vitamin D and selenium deficiency recently linked to COVID-19 severity. Nevertheless, the potential for supplementation of specific foods and nutrients to act as a protective measure against COVID-19 remains a topic of debate.

The goal of this Research Topic was to gather Original Research articles and Reviews which have improved our understanding of diet, specific foods and nutritional status in relation to COVID-19 severity and outcomes. Twenty-eight articles have been published under this Research Topic covering a wide variety of Covid-19, the role of nutrition and how specific nutrients may be able to inhibit the spread of the disease and reduce its detrimental health effects. The articles addressed several aspects of Covid-19 from a metabolic, exercise, and nutrition point and

OPEN ACCESS

Edited and reviewed by:
Mauro Serafini,
University of Teramo, Italy

***Correspondence:**
Ioannis Zabetakis
ioannis.zabetakis@ul.ie

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *18 August 2021*
Accepted: *23 August 2021*
Published: *14 September 2021*

Citation:
Zabetakis I, Matthys C and Tsoupras A (2021) Editorial: Coronavirus Disease (COVID-19): Diet, Inflammation and Nutritional Status. Front. Nutr. 8:760720. doi: 10.3389/fnut.2021.760720

they inform the readers on the latest developments of research related to Covid-19. The articles include perspectives, review articles and research articles and they also address the covid-19 related inflammation and cytokine storm.

The role of specific nutrients is widely discussed in this Research Topic from this inflammation point of view for this disease. It was also described that apart from inflammation, thrombosis, and thrombo-inflammatory complications are also linked to COVID-19, and the role of exercise and diet is discussed further. The role of foods, minerals, and vitamins is presented as means to reduce the cytokine storm and covid-19 related inflammation. The impact of lifestyle is also presented. The role of vitamin C as a potential aid in the treatment of Covid-19 is discussed in Cerullo et al. Also, the role of vitamins C and D and zinc are discussed in relation to physical tissue barrier integrity in Name et al.

The role of food supplements in relation to immune-boosting, antioxidant, and anti-inflammatory activities is presented in relation to Covid-19 pathogenesis. This article Mrityunjaya et al., suggests that grouping phytonutrients in the form of a food supplement may help to boost the immune system, prevent virus spread, preclude the disease progression to severe stage, and further suppress the hyper inflammation providing both prophylactic and therapeutic support against COVID-19.

The stormy link between obesity and Covid-19 is presented in López-Reyes et al. The authors have concluded that the SARS-CoV-2 infection could potentiate or accelerate the pre-existing systemic inflammatory state of individuals with obesity, *via* the NLRP3 inflammasome activation and the release of pro-inflammatory cytokines from cells trough Gasdermin-pores commonly found in cell death by pyroptosis.

In Bennett et al., it was found that the effect of COVID-19 lockdown both negatively and positively impacted dietary practices throughout Europe and globally, and negative diet habits were associated with other poor lifestyle outcomes including weight gain, mental health issues, and limited physical activity. Both in the short term and if sustained in the long term, these changes may have significant impacts on the health of the population.

In Vassilopoulou et al., an article linking breastfeeding and covid-19, it was highlighted that since SARS-CoV-2 is highly transmissible, breastfeeding should be encouraged, but observing all appropriate safety measures, for the mother and close contacts is needed. With the vaccination schedule in progress, the pregnant mothers-to-be should probably be prioritized.

In Zaragoza-Martí et al., an article linking Mediterranean lifestyle and lockdown, it was found that adherence to the Mediterranean Diet may play an important role in the establishment of appropriate dietary guidelines in confinement situations such as the COVID-19.

People with underlying conditions are more susceptible to COVID-19; as a matter of fact around 95% of COVID-19 related deaths are people with severe chronic diseases and these data manifest that COVID-19 is a deadly virus in people with chronic inflammation. In other words, a pre-existing chronic inflammatory status detrimentally affects our immune system and homeostatic mechanisms, and thus make us more susceptible to the cytokine storm and its associated inflammatory manifestations that COVID-19 causes in our bodies. The potential role of nutrition in relation to thrombosis and COVID-19 19 is discussed in Tsoupras et al.

In conclusion, this Research Topic, with a variety of articles has provided, a wealth of insights on the link between nutrition, inflammation, and covid-19, with promising outcomes and future perspectives. As Editors, we would like to thank all the contributing authors and the people in Frontiers in Nutrition for their excellent editing support.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Zabetakis, Matthys and Tsoupras. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

OPINION
published: 04 August 2020
doi: 10.3389/fimmu.2020.01896

Mucosal-Associated Invariant T Cells as a Possible Target to Suppress Secondary Infections at COVID-19

Roman A. Akasov[1,2,3*†] *and Evgeny V. Khaydukov*[1,3*†]

[1] *Federal Scientific Research Centre "Crystallography and Photonics" Russian Academy of Sciences, Moscow, Russia,* [2] *Laboratory of Biomedical Nanomaterials, National University of Science and Technology "MISIS", Moscow, Russia,* [3] *Center of Biomedical Engineering, Institute of Molecular Medicine, Sechenov University, Moscow, Russia*

Keywords: COVID-19, SARS-CoV-2, mucosal associated invariant T cells, riboflavin, flavin mononucleotide, FMN riboswitch, immunomodulation, cytokines

INTRODUCTION

Co-infections are of proven importance in the severity of respiratory diseases while their involvement in COVID-19 progression is still little discussed (1). However, one in seven patients with COVID-19 had secondary non-viral infections during hospitalization, and 50% of non-surviving patients had secondary infection in a retrospective cohort study in Wuhan (2). Severe patients with COVID-19 often need invasive mechanical ventilation that takes a long time (on average 9 days), and can lead to infections acquired in the hospital and on the ventilator (1). The role of gut microbiota in the severity of COVID-19 has been also recently discussed, suggesting that a microbial metabolic process in the gut may affect the production of pro-inflammatory cytokines (3). There are some pathways between the microbiota and the host immune system, including TNFα and IFNγ production associated with specific microbial metabolic pathways of palmitoleic acid metabolism and degradation of tryptophan to tryptophol (4), but this area needs further evaluation. Therefore, the study of interactions between the host and microbiota is of great importance for understanding the progression and therapy of COVID-19.

MAIT CELLS ACTIVATION AS A POSSIBLE MECHANISM PARTICIPATING IN COVID-19 PROGRESSION

Mucosal associated invariant T (MAIT) cells are found in the blood, liver, lungs, and mucosa, protecting against microbial activity and infection (5). MAIT cells can be activated via MR1-dependent and MR1-independent pathways. MR1-independent activation requires cytokines (e.g., IL-12 or IL-18) while MR1-dependent activation needs recognition of small molecules of biosynthesis of vitamin B2 (riboflavin) and B9 (folic acid). Activated MAIT cells rapidly produce pro-inflammatory cytokines including IFNγ, TNFα, and IL-17 (6). Typically, MAIT cells are discussed in context of bacterial or fungal infections, as they can induce the immune response when activated with riboflavin precursors in MR1-dependent manner. However, MAIT cells activation was also described for viral infections, including herpes, hepatitis, or lethal influenza (7, 8). In the case of viruses, MAIT activation occurred via MR1-independent pathway as a result of cytokines binding (IL-18 in synergy with IL-12, IL-15, and/or interferon-α/β) (9). No information regarding the increased values of IL-18, IL-12, or IL-15 in patients with COVID-19 was found and, therefore, this possible activation pathway requires further study. On the other hand, the role of MAIT cells resident in lung tissue in children with community-acquired pneumonia was demonstrated (10). Immunity profiling showed that MAIT cells from the bronchoalveolar lavages, but not from the blood, actively produced IL-17. It is important that most patients were diagnosed with adenovirus and *Mycoplasma pneumoniae*

OPEN ACCESS

Edited by:
Ioannis Zabetakis,
University of Limerick, Ireland

Reviewed by:
Rajesh Lamichhane,
University of Otago, New Zealand

****Correspondence:***
Roman A. Akasov
roman.akasov@gmail.com
Evgeny V. Khaydukov
khaydukov@mail.ru

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: *01 June 2020*
Accepted: *14 July 2020*
Published: *04 August 2020*

Citation:
Akasov RA and Khaydukov EV (2020) Mucosal-Associated Invariant T Cells as a Possible Target to Suppress Secondary Infections at COVID-19. Front. Immunol. 11:1896. doi: 10.3389/fimmu.2020.01896

while neither adenovirus nor mycoplasma synthesize riboflavin. The authors suggested that MAIT cells were probably activated through commensal microorganisms or co-infecting bacteria in combination with inflammatory cytokines (10). Since only MAIT cells resident in lung tissue but not derived from the blood produced IL-17, the contribution of co-infecting bacteria appears to be more important. It should be noted that targeting IL-17 was recently proposed as a strategy to combat acute respiratory distress syndrome in COVID-19 (11). Therefore, we hypothesize the importance of the study of MAIT cells in blood and especially in lungs, given the evidence that the status of T cells reflects the severity of infection and predict the clinical outcomes in patients with COVID-19 (12, 13). Thus, we suggest that activation of MAIT cells in secondary non-viral infection via the MR1-dependent pathway may be a factor that enhances the progression of COVID-19, and IL-17 production may be one of the mediators.

INHIBITION OF RIBOFLAVIN BIOSYNTHESIS IN MICROBIOTA AS A POSSIBLE APPROACH TO PREVENT MAIT ACTIVATION

Since MR1-dependent activation occurs as a result of recognizing small molecules of riboflavin biosynthesis, inhibition of this pathway appears to be a promising approach to prevent the immune response of MAIT cells. Indeed, an immunomodulatory strategy of herpesviruses that functionally disrupts the immune response was defined for MR1 targeting (14). It was found that riboflavin biosynthesis can be repressed by inhibiting enzymes involved in riboflavin biosynthesis (15) or at the level of transcription through the flavin mononucleotide (FMN) riboswitch (16). The FMN riboswitch is a metabolite-dependent RNA element that directly binds FMN and controls the expression of genes responsible for riboflavin biosynthesis since FMN is riboflavin-5′-phosphate (17).

There are some synthetic or natural compounds that can inhibit FMN riboswitch. Among them, roseoflavin, a pigment originally isolated from Streptomyces davawensis, was discussed as an antimetabolite analog of riboflavin and FMN with antimicrobial properties (16). The other is 5FDQD, a riboswitch-binding analog of flavin that protects mice against *Clostridium difficile* infection without inhibiting healthy bowel flora (18). Double-targeting of the *Staphylococcus aureus* FMN riboswitch with roseoflavin and ribocil-C demonstrated efficacy in a murine model of MRSA (Methicillin Resistant Staphylococcus Aureus) infection (19).

However, FMN or even riboflavin may be also effective in inhibiting riboflavin biosynthesis. A strategy of the oral supplementation with riboflavin may be proposed, given that some bacteria can switch from biosynthesis to uptake of riboflavin when it is environmentally available. It should be noted that riboflavin supplementation (100 mg daily for 3 weeks) in patients with Crohn's disease, a type of inflammatory bowel disease, led to anti-inflammatory effects (20). Activation of innate MAIT cells in inflammatory bowel diseases resulted in a switch in the pattern of cytokine secretion was previously demonstrated (21). Riboflavin supplementation (10 mg/day, p.o) significantly decreased plasma homocysteine, a marker of inflammation and ischemic injury, in the group of elderly people with low riboflavin status (22).

An alternative approach to inhibit overactivation of MAIT cells is a ligand-dependent downregulation of MR1 cell surface expression via retaining MR1 molecules in the endoplasmic reticulum in an immature form (23).

DISCUSSION

We hypothesize that secondary non-viral infections may enhance the severity of COVID-19 by activating MR1-dependent MAIT cells. MAIT cells promote protection against primary infections through cytokines production (24), as well as mediate protective host responses in sepsis by reducing bacterial burden (25). However, in the case of secondary infections, additive inflammation can exacerbate the situation due to the progression of the cytokine storm. The possible involvement of MAIT cells in the development of undesirable immune response in certain diseases was previously shown (5). Thus, MAIT cells promote inflammation and exacerbate the disease in murine models of arthritis while mice with MR1 deficiency develop a less severe disease compared to control (26). In mice infected with *Helicobacter pylori*, MAIT cells were expanded in the gastric mucosa and adopted the IL-17A- and IFN-γ-producing phenotype, resulting in the gastric progression (27). It is important that MAIT cell activation is detected 2 h after contact with the antigen (28). It should be also noted that MAIT cells are much less frequent in children (<2 y.o.) than in older humans (5). Functional alteration of innate T cells in COVID-19 patients has been described very recently, including a decrease in circulating MAIT cells in blood, which may be a consequence of their recruitment into the airways (29).

If the hypothesis of MR1-dependent MAIT cells activation in secondary infection of COVID-19 is correct, inhibition mechanisms of this activation could be discussed. This can be accomplished by inhibiting the riboflavin biosynthesis in the microbiota (e.g., with roseoflavin or analogs) or by ligand-dependent downregulation of the MR1 cell surface expression in antigen-presenting cells (e.g., with DB28 or analogs (23)). The first option seems easier to implement since the inhibition of riboflavin biosynthesis pathway can lead to toxicity for microbiota, including pathogen bacteria and yeasts, but not to the host.

We must also point out some arguments that contradict our idea. Thus, neutrophils that are recruited early to sites of infections, including COVID-19 infection, can suppress and prevent overactivation of MAIT cells (30). Since repeated MAIT cells stimulation by cytokines (IL-12 and IL-18) was found to enhance IL-17 production by MAIT cells (31), the patients may not benefit from the treatment that suppresses MR1-dependent stimulation. The interactions of MAIT cells with other participants in the immune response are also unclear, as well as the impact of MAIT cells infection at a distant site, i.e., the impact

of gut MAIT cells on pulmonary infection or vice versa (32). Therefore, we appeal to the biomedical community to test the hypothesis of MR1-dependent MAIT cells activation as a possible therapeutic approach.

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

RA acknowledges the Ministry of Education and Science of the Russian Federation in the framework of Increase Competitiveness Program of NUST MISiS (No. K4-2018-052), implemented by a governmental decree dated 16th of March 2013, N 211. EK acknowledges the Ministry of Science and Higher Education within the State assignment FSRC ≪Crystallography and Photonics≫ RAS.

REFERENCES

1. Cox MJ, Loman N, Bogaert D, O'Grady J. Co-infections: potentially lethal and unexplored in COVID-19. *Lancet Microbe.* (2020) 1:e11. doi: 10.1016/S2666-5247(20)30009-4
2. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
3. Kalantar-Zadeh K, Ward SA, Kalantar-Zadeh K, El-Omar EM. Considering the effects of microbiome and diet on SARS-CoV-2 infection: nanotechnology roles. *ACS Nano.* (2020) 14:5179–82. doi: 10.1021/acsnano.0c04407
4. Schirmer M, Smeekens SP, Vlamakis H, Jaeger M, Oosting M, Franzosa EA, et al. Linking the human gut microbiome to inflammatory cytokine production capacity. *Cell.* (2016) 167:1125–36.e8. doi: 10.1016/j.cell.2016.10.020
5. Godfrey DI, Koay HF, McCluskey J, Gherardin NA. The biology and functional importance of MAIT cells. *Nat Immunol.* (2019) 20:1110–28. doi: 10.1038/s41590-019-0444-8
6. Xiao X, Cai J. Mucosal-Associated Invariant T Cells: New Insights into Antigen Recognition and Activation. *Front Immunol.* (2017) 8:1540. doi: 10.3389/fimmu.2017.01540
7. Ussher JE, Willberg CB, Klenerman P. MAIT cells and viruses. *Immunol Cell Biol.* (2018) 96:630–41. doi: 10.1111/imcb.12008
8. van Wilgenburg B, Scherwitzl I, Hutchinson EC, Leng T, Kurioka A, Kulicke C, et al. MAIT cells are activated during human viral infections. *Nat Commun.* (2016) 7:11653. doi: 10.1038/ncomms11653
9. van Wilgenburg B, Loh L, Chen Z, Pediongco TJ, Wang H, Shi M, et al. MAIT cells contribute to protection against lethal influenza infection *in vivo*. *Nat Commun.* (2018) 9:4706. doi: 10.1038/s41467-018-07207-9
10. Lu B, Liu M, Wang J, Fan H, Yang D, Zhang L, et al. IL-17 production by tissue-resident MAIT cells is locally induced in children with pneumonia. *Mucosal Immunol.* (2020). doi: 10.1038/s41385-020-0273-y. [Epub ahead of print].
11. Pacha O, Sallman MA, Evans SE. COVID-19: a case for inhibiting IL-17? *Nat Rev Immunol.* (2020) 20:345–6. doi: 10.1038/s41577-020-0328-z
12. Liu Z, Long W, Tu M, Chen S, Huang Y, Wang S, et al. Lymphocyte subset (CD4+, CD8+) counts reflect the severity of infection and predict the clinical outcomes in patients with COVID-19. *J Infect.* (2020). doi: 10.1016/j.jinf.2020.03.054. [Epub ahead of print].
13. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and functional exhaustion of t cells in patients with coronavirus disease 2019 (COVID-19). *Front Immunol.* (2020) 11:827. doi: 10.3389/fimmu.2020.00827
14. McSharry BP, Samer C, McWilliam HEG, Ashley CL, Yee MB, Steain M, et al. Virus-mediated suppression of the antigen presentation molecule MR1. *Cell Rep.* (2020) 30:2948–62.e4. doi: 10.1016/j.celrep.2020.02.017
15. Kundu B, Sarkar D, Ray N, Talukdar A. Understanding the riboflavin biosynthesis pathway for the development of antimicrobial agents. *Med Res Rev.* (2019) 39:1338–71. doi: 10.1002/med.21576
16. Lee ER, Blount KF, Breaker RR. Roseoflavin is a natural antibacterial compound that binds to FMN riboswitches and regulates gene expression. *RNA Biol.* (2009) 6:187–94. doi: 10.4161/rna.6.2.7727
17. Machtel P, Bakowska-Zywicka K, Zywicki M. Emerging applications of riboswitches – from antibacterial targets to molecular tools. *J Appl Genet.* (2016) 57:531–41. doi: 10.1007/s13353-016-0341-x
18. Blount KF, Megyola C, Plummer M, Osterman D, O'Connell T, Aristoff P, et al. Novel Riboswitch-Binding Flavin Analog That Protects Mice against Clostridium difficile Infection without Inhibiting Cecal Flora. *Antimicrob Agents Chemother.* (2015) 59:5736–46. doi: 10.1128/AAC.01282-15
19. Wang H, Mann PA, Xiao L, Gill C, Galgoci AM, Howe JA, et al. Dual-Targeting small-molecule inhibitors of the Staphylococcus aureus FMN riboswitch disrupt riboflavin homeostasis in an infectious setting. *Cell Chem Biol.* (2017) 24:576–88.e6. doi: 10.1016/j.chembiol.2017.03.014
20. von Martels JZH, Bourgonje AR, Klaassen MAY, Alkhalifah HAA, Sadaghian Sadabad M, Vich Vila A, et al. Riboflavin Supplementation in Patients with Crohn's Disease [the RISE-UP study]. *J Crohn's Colitis.* (2020) 14:595–607. doi: 10.1093/ecco-jcc/jjz208
21. Serriari N-E, Eoche M, Lamotte L, Lion J, Fumery M, Marcelo P, et al. Innate mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel diseases. *Clin Exp Immunol.* (2014) 176:266–74. doi: 10.1111/cei.12277
22. Tavares NR, Moreira PA, Amaral TF. Riboflavin supplementation and biomarkers of cardiovascular disease in the elderly. *J Nutr Heal Aging.* (2009) 13:441–6. doi: 10.1007/s12603-009-0081-2
23. Salio M, Awad W, Veerapen N, Gonzalez-Lopez C, Kulicke C, Waithe D, et al. Ligand-dependent downregulation of MR1 cell surface expression. *Proc Natl Acad Sci USA.* (2020) 117:10465–75. doi: 10.1073/pnas.2003136117
24. Hartmann N, Harriff MJ, McMurtrey CP, Hildebrand WH, Lewinsohn DM, Kronenberg M. Role of MAIT cells in pulmonary bacterial infection. *Mol Immunol.* (2018) 101:155–9. doi: 10.1016/j.molimm.2018.06.270
25. Trivedi S, Labuz D, Anderson CP, Araujo C V., Blair A, Middleton EA, et al. Mucosal-associated invariant T (MAIT) cells mediate protective host responses in sepsis. *bioRxiv.* (2020). doi: 10.1101/2020.02.05.935866. [Epub ahead of print].
26. Chiba A, Tajima R, Tomi C, Miyazaki Y, Yamamura T, Miyake S. Mucosal-associated invariant T cells promote inflammation and exacerbate disease in murine models of arthritis. *Arthritis Rheum.* (2012) 64:153–61. doi: 10.1002/art.33314
27. D'Souza C, Pediongco T, Wang H, Scheerlinck J-PY, Kostenko L, Esterbauer R, et al. Mucosal-Associated Invariant T Cells Augment Immunopathology and Gastritis in Chronic Helicobacter pylori Infection. *J Immunol.* (2018) 200:1901–16. doi: 10.4049/jimmunol.1701512
28. Chen Z, Wang H, D'Souza C, Sun S, Kostenko L, Eckle SBG, et al. Mucosal-associated invariant T-cell activation and accumulation after *in vivo* infection depends on microbial riboflavin synthesis and co-stimulatory signals. *Mucosal Immunol.* (2017) 10:58–68. doi: 10.1038/mi.2016.39
29. Jouan Y, Guillon A, Gonzalez L, Perez Y, Ehrmann S, Ferreira M, et al. Functional alteration of innate T cells in critically ill Covid-19 patients. *medRxiv.* (2020). doi: 10.1101/2020.05.03.20089300. [Epub ahead of print].

30. Schneider M, Hannaway RF, Lamichhane R, Harpe SM, Tyndall JDA, Vernall AJ, et al. Neutrophils suppress mucosal-associated invariant T cells in humans. *Eur J Immunol.* (2020) 50:643–55. doi: 10.1002/eji.201948394
31. Böttcher K, Rombouts K, Saffioti F, Roccarina D, Rosselli M, Hall A, et al. MAIT cells are chronically activated in patients with autoimmune liver disease and promote profibrogenic hepatic stellate cell activation. *Hepatology.* (2018) 68:172–86. doi: 10.1002/hep.29782
32. Wong EB, Ndung'u T, Kasprowicz VO. The role of mucosal-associated invariant T cells in infectious diseases. *Immunology.* (2017) 150:45–54. doi: 10.1111/imm.12673

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Akasov and Khaydukov. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

PERSPECTIVE
published: 02 September 2020
doi: 10.3389/fnut.2020.00143

Understanding Selenium and Glutathione as Antiviral Factors in COVID-19: Does the Viral M^{pro} Protease Target Host Selenoproteins and Glutathione Synthesis?

Ethan Will Taylor and Wilson Radding*

Department of Chemistry and Biochemistry, The University of North Carolina at Greensboro, Greensboro, NC, United States

OPEN ACCESS

Edited by:
Christophe Matthys, KU Leuven, Belgium

Reviewed by:
Alan Diamond, University of Illinois at Chicago, United States
Anna Kipp, Friedrich Schiller University Jena, Germany

****Correspondence:***
Ethan Will Taylor
ewtaylor@uncg.edu

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Nutrition

Received: *26 June 2020*
Accepted: *21 July 2020*
Published: *02 September 2020*

Citation:
Taylor EW and Radding W (2020) Understanding Selenium and Glutathione as Antiviral Factors in COVID-19: Does the Viral M^{pro} Protease Target Host Selenoproteins and Glutathione Synthesis? Front. Nutr. 7:143. doi: 10.3389/fnut.2020.00143

Glutathione peroxidases (GPX), a family of antioxidant selenoenzymes, functionally link selenium and glutathione, which both show correlations with clinical outcomes in COVID-19. Thus, it is highly significant that cytosolic GPX1 has been shown to interact with an inactive C145A mutant of M^{pro}, the main cysteine protease of SARS-CoV-2, but not with catalytically active wild-type M^{pro}. This seemingly anomalous result is what might be expected if GPX1 is a substrate for the active protease, leading to its fragmentation. We show that the GPX1 active site sequence is substantially similar to a known M^{pro} cleavage site, and is identified as a potential cysteine protease site by the Procleave algorithm. Proteolytic knockdown of GPX1 is highly consistent with previously documented effects of recombinant SARS-CoV M^{pro} in transfected cells, including increased reactive oxygen species and NF-κB activation. Because NF-κB in turn activates many pro-inflammatory cytokines, this mechanism could contribute to increased inflammation and cytokine storms observed in COVID-19. Using web-based protease cleavage site prediction tools, we show that M^{pro} may be targeting not only GPX1, but several other selenoproteins including SELENOF and thioredoxin reductase 1, as well as glutamate-cysteine ligase, the rate-limiting enzyme for glutathione synthesis. This hypothesized proteolytic knockdown of components of both the thioredoxin and glutaredoxin systems is consistent with a viral strategy to inhibit DNA synthesis, to increase the pool of ribonucleotides for RNA synthesis, thereby enhancing virion production. The resulting "collateral damage" of increased oxidative stress and inflammation would be exacerbated by dietary deficiencies of selenium and glutathione precursors.

Keywords: coronavirus, COVID-19, selenium, glutathione, glutathione peroxidase 1, protease, selenoprotein, thioredoxin reductase

INTRODUCTION

Several independent studies have now established a significant association between the outcome of COVID-19 and previously documented regional selenium (Se) status in Chinese cities (1), and a similar relationship between serum Se and mortality in a European cohort of COVID-19 patients (2). These observations invite questions about the mechanisms involved, particularly because they fit into a consistent pattern of a role for Se that has been reported over several decades for a variety of RNA viruses (enteroviruses, hantaviruses, and influenza A) and viruses with an RNA stage (HIV-1 and Hepatitis B virus), as reviewed by various authors (3–5).

Most (but not all) of the biological roles of Se, both as selenocysteine in selenoproteins, and as redox-active Se-containing metabolites, involve interactions with cysteine thiols and disulfides in proteins and peptides, and their various oxidized forms. Like Se, the essential antioxidant and free radical scavenger glutathione (GSH), a tripeptide thiol, has also proven to be an important factor in various viral infections, particularly in HIV/AIDS, as reviewed in section 2.1.2. of Taylor (6), and most recently, in COVID-19 (7, 8). Given their intertwined biochemical roles, there are likely to be common factors and mechanisms underlying the therapeutic importance of Se and GSH in viral infections.

A CONFIRMED MOLECULAR INTERACTION BETWEEN SARS-CoV-2 PROTEASE AND A HUMAN SELENOPROTEIN

Correlations between COVID-19 clinical outcomes and *both* host Se and GSH status provide important context to a related observation emerging from a proteomics-based study of possible cellular targets of SARS-CoV-2 (SCoV2) proteins. Using affinity-purification mass spectrometry, Gordon et al. identified high-confidence protein-protein interactions between 26 of the SCoV2 proteins and human proteins (9). As bait for interacting human proteins, one of the proteins they used was the SCoV2 main viral protease M^{pro}, a cysteine protease also known as nonstructural protein 5 (nsp5). The study also included a catalytically inactive C145A M^{pro} mutant (lacking the active site cysteine), which was also used as a bait protein, in order to discriminate false positives that might bind non-specifically to the M^{pro} active site cysteine via disulfide bond formation. One of the interactions they identified involved the cytosolic form of the selenoprotein glutathione peroxidase, GPX1, which bound strongly to the inactive C145A M^{pro} mutant. However, this interaction with GPX1 was *not* observed with the wild type M^{pro}. *This is precisely what one might expect to see if GPX1 is a protease substrate*, because its cleavage would produce two fragments that would necessarily have reduced affinity to the enzyme relative to GPX1.

Significantly, as shown in Figure 6 in their Extended Data (9), Gordon et al. identified one other host protein (TRMT1) that bound *only* to the inactive M^{pro} C145A mutant, and concluded it was a likely M^{pro} substrate, because they were able to identify a putative M^{pro} cleavage site in the TRMT1 protein sequence (PRLQ/ANFT), where the slash (/) represents the cleavage site. Thus, *the only piece of evidence lacking to draw a similar conclusion for GPX1 is a candidate M^{pro} cleavage site*, which most algorithms will fail to find, e.g., if they are highly stringent about requiring a Q in the P1 position (**Figure 1**).

As shown in **Figure 1A** for the 2003 SARS coronavirus (SARS-CoV, SCoV) and in **Figure 1B** for the 2019 SCoV2, the consensus logo patterns of 11 M^{pro} cleavage sites in the viral 1ab polyprotein are essentially identical for the 2 viruses. Specifically, 8 of the 11 sites are fully identical over the 10 residues spanning the P5 to P5′ positions, and only two of the 11 cleavage sites have more than a single amino acid difference within the 10 residue span (**Figure S1**). This means that (as discussed below) *functional studies of the 2003 SCoV protease are highly relevant to that of the 2019 virus*. From **Figures 1A,B**, the most important residue positions for M^{pro} recognition are, in descending order, P1, P2, P1′, P4, and P3, with the first 3 being the major determinants, with the minimal P2-P1′ consensus being LQ/(S,A,N,G). The downstream sequence past P1′ is highly variable.

The GPx1 active site has the sequence LUG, where U is the catalytic selenocysteine; this matches the observed M^{pro} consensus core target sequence combination LQ/G in 2 of 3 positions. Furthermore, as shown in **Figure 1C**, the important upstream side of the known M^{pro} cleavage site at the nsp13/14 junction, NVATLQ/A, is remarkably similar to that of the active site sequence of GPx1, NVASLU/G, where the selenocysteine (U) lines up with a glutamine (Q) in the viral sequence. These two amino acids (U and Q) are not highly similar, but are both midrange in size, and polar in nature, because the selenol residue is predominantly ionized at physiological pH. The other two "mismatches" in the important positions P3 and P1′, i.e., S vs. T and G vs. A, both differ only slightly, by the size of a methyl group, and in any case, having a glycine (G) in the P1′ position is a permitted residue (**Figure 1A**), as observed in the SCoV M^{pro} cleavage site at the nsp5/6 junction (indicated by * in **Figure 2**). Significantly, the Procleave protease cleavage prediction server (procleave.erc.monash.edu.au) (13) identified the GPX1 active site octameric sequence ASLU/GTTV as a highly ranked possible cleavage site for a cathepsin S-like cysteine protease (**Figure 2G**).

For several reaction intermediates in the GPX1 mechanism, Se is attached to either oxygen, or to nitrogen, as a selenenylamide. The latter is quite stable, and may accumulate during GSH depletion, because GSH is needed to regenerate the selenolate (14). Selenenylamide is more similar than selenocysteine to glutamine, so that form might be preferentially targeted by M^{pro}.

Because the mutation rate in cellular genes is thousands of times slower than that for an RNA virus, if there is a physical interaction between these two proteins as Gordon et al. observed using the inactive mutant M^{pro}, it is almost certainly because the virus has evolved to target the host for some reason, rather than the converse. The most parsimonious hypothesis is simply that this is a case of a viral protease targeting a cellular gene for cleavage, for which there are abundant precedents, including the proposed M^{pro} targeting of TRMT1 and HDAC2 (9), and HIV-1 protease, which can be toxic to cells, via its action at various cellular targets (15).

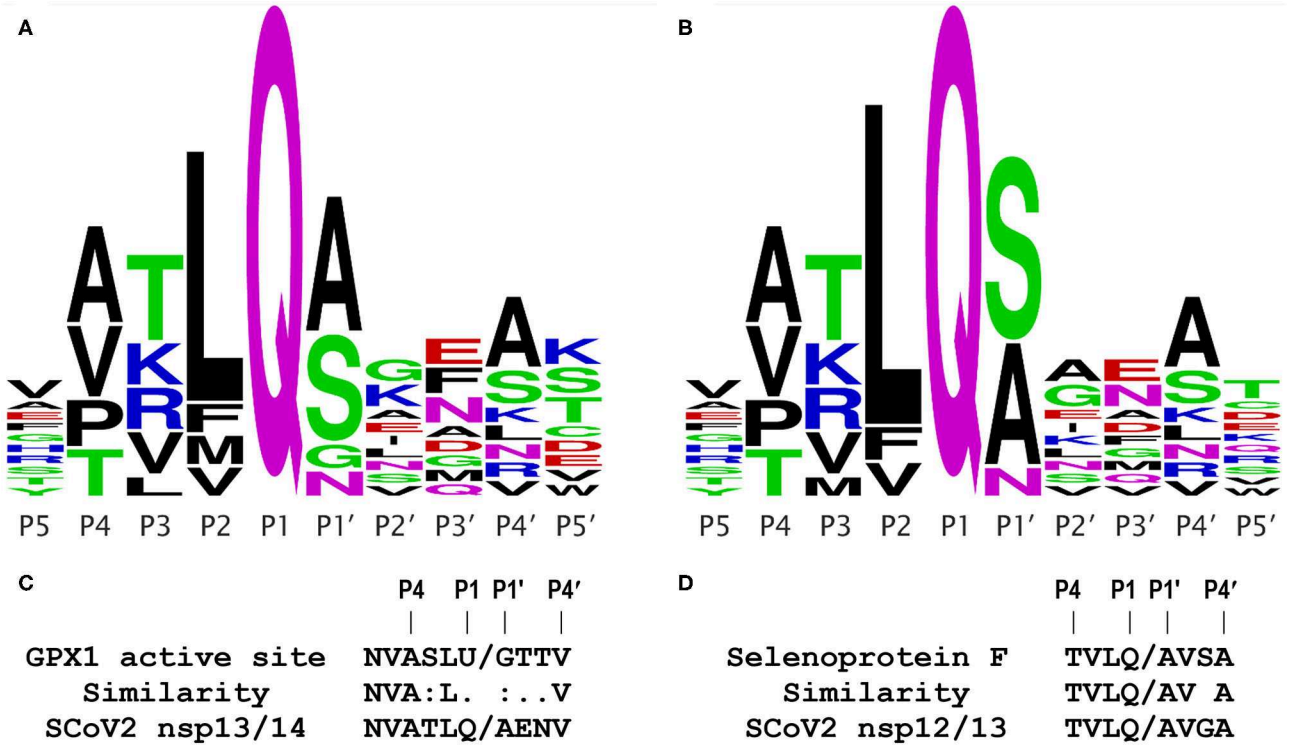


FIGURE 1 | Coronavirus M^{pro} cleavage site consensus sequence logo plots and comparisons. Logo plots from multiple alignments of the known M^{pro} cleavage sites are shown for the 2003 SCoV **(A)** and 2019 SCoV2 **(B)**. The height of a letter at each position reflects its probability in the alignment; each of the logos shown represents the consensus of 11 M^{pro} cleavage sites from a single virus. Plots were generated using WebLogo (weblogo.berkeley.edu/logo.cgi), from the alignments in **Figure S1**. **(C)** Comparison of the GPX1 active site sequence containing selenocysteine (U) to the known SCoV2 M^{pro} cleavage site at the nsp13/14 junction; this site is identical in the 2003 and 2019 coronaviruses. **(D)** Comparison of a predicted M^{pro} cleavage site in human selenoprotein F to a known SCoV2 M^{pro} cleavage site at the nsp12/13 junction.

FUNCTIONAL EFFECTS OF M^{PRO} IN TRANSFECTED CELLS ARE CONSISTENT WITH GPX1 KNOCKDOWN

As detailed above, the original 2003 SCoV M^{pro} has essentially identical substrate sequence specificity as that of SCoV2. More specifically, the 10 residue sequence of the cleavage site at the nsp13/14 junction shown in **Figure 2A**, with the greatest overall similarity to the GPX1 active site sequence, is 100% identical in the 2003 and 2019 SARS coronaviruses (**Figure S1**). Thus, the results of a 2006 study on the effects of transfecting cells with a SCoV M^{pro} expression vector, in order to study the effects of M^{pro} alone in the absence of intact virus (16), are highly relevant for COVID-19. In transfected human cells, the results were *exactly what one would expect from knockdown of GPX1:* increased oxidative stress via production of reactive oxygen species and activation of transcription factor NF-κB (16). Both of these responses can be produced by exposing cells to hydrogen peroxide, and inhibited by increasing GPX1 activity, either by overexpression or via the addition of sodium selenite to cell culture media (17, 18). Cells expressing the SCoV M^{pro} also became apoptotic, which is a known consequence of NF-κB activation, especially in combination with a high burden of ROS (19), which is even more likely to occur if other antioxidant proteins are also degraded, as discussed below.

POTENTIAL M^{PRO} SITES IN OTHER SELENOPROTEINS AND GLUTATHIONE-RELATED PROTEINS

The question then arises, could targeting of host selenoproteins for proteolysis be part of a more extensive viral strategy contributing to the correlations between Se and GSH status and COVID-19 outcome? To investigate this possibility, we undertook a systematic search for potential M^{pro} cleavage sites in the human selenoproteome and in several GSH-related proteins including glutaredoxin, a small thioredoxin-like CXXC protein that is recycled by GSH, rather than directly by a reductase enzyme. For this analysis, we used three online resources: (1) NetCorona (cbs.dtu.dk/services/NetCorona) (12), the only resource specific for predictions of M^{pro} cleavage sites in coronaviruses (although *not* limited to just the SARS type coronaviruses), (2) PROSPER, the Protease Specificity Prediction Server (prosper.erc.monash.edu.au) (11), which was rated in a

	Site	P4-P1'	Relative k_{cat}/K_m	Protease cleavage prediction methods used to identify and/or confirm site
A	GCLC-b	AVLQ/G		**Procleave** #7/592 for cathepsin S
	nsp4/5	AVLQ/S	100%	
	nsp5/6	VTFQ/S	41%	
B	GCLC-a	VTFQ/A		**PROSPER** (1.00); **Procleave** #13 of 592 for cathepsin L
	nsp5/6*	VTFQ/G	41%	
	nsp6/7	ATVQ/S	3%	
C	SelenoP	A*L*LQ/A		**Procleave** #3/374 for cathepsin L
	nsp7/8	ATLQ/A	5%	
D	GLRX-1	VSLQ/*Q*		**PROSPER** (1.30); **Procleave** #1 of 99 for cathepsin S
	nsp8/9	VKLQ/N	2%	
	nsp9/10	VRLQ/A	22%	
	nsp10/11	PMLQ/S	0.2%	
E	TXNRD1	*SI*LQ/A		**PROSPER** (1.14); **NetCorona** (0.640, rank #1)
	nsp12/13	TVLQ/A	8%	
F	SelenoF	TVLQ/A		**PROSPER** (1.02); **NetCorona** (0.846, rank #1)
	nsp13/14	ATLQ/A	9%	
G	GPX1	A*S*LU/G		**Procleave** #2/196 for cathepsin S
	nsp14/15	TRLQ/S	28%	
	nsp15/16	PKLQ/S	27%	

FIGURE 2 | Comparison of known and predicted M^{pro} cleavage sites. The most relevant positions P4-P1′ of the 11 known SARS coronavirus M^{pro} cleavage sites in non-structural proteins are shown (for nsp4 through nsp16), aligned with predicted sites in several human selenoproteins (GPX1, SelenoF, SelenoP, and TXNRD1), as well as glutaredoxin-1 (GLRX-1) and the rate-limiting enzyme for glutathione synthesis, glutamate-cysteine ligase catalytic subunit (GCLC, with 2 predicted cleavage sites **A**, **B**). The known nsp cleavage sites shown are all from SARS-CoV-2, with the addition of the nsp5/6 site from SARS-CoV (marked*). The sites identified in human proteins are displayed next to the known M^{pro} cleavage site to which they are most similar, highlighted in matching color. The experimentally determined catalytic efficiencies of SARS-CoV M^{pro} at each of the known nsp cleavage sites are shown as relative k_{cat}/K_m (10). Three different methods for prediction of cysteine protease cleavage sites were used: PROSPER (11), with numbers in parenthesis showing the score for the predicted site, any score >0.8 being significant; NetCorona (12), where scores >0.5 are considered positive, and Procleave (13), where every possible P4-P4′ octamer in a protein sequence is evaluated, scored and ranked. Some aligned non-identical residues highlighted in italics are nonetheless chemically and structurally similar to residues found in the same position in the known cleavage sites, e.g., serine (*S*) vs. threonine (T), Leucine (*L*) or isoleucine (*I*) vs. valine (V), and glutamine (*Q*) vs. asparagine (N); all of these pairs differ only by a single carbon atom (CH_3 or CH_2 unit). The predicted M^{pro} cleavage sites in human proteins labeled **(A–G)** are discussed in the text. The computational results cited are collated in **Figure S2**.

recent study as one of the most accurate resources for prediction of HIV-1 protease sites (20), and (3) Procleave (cited above), which was only used if either of the first 2 methods failed to identify a site. Both Procleave and PROSPER do not specifically predict coronavirus cysteine protease sites, but make predictions for more generic cysteine proteases (e.g., cathepsins B, K, L, or S). Thus, for both PROSPER and Procleave, the results had to be screened manually for matches to the M^{pro} cleavage site consensus as shown in **Figures 1A,B**. This search is also predicated on the assumption that the approach of Gordon et al. (9) might fail to identify some protease targets, because their high affinity interactions are mostly limited to an 8–10 residue sequence, and in some cases may be unable to withstand the conditions needed to be isolable and observable by mass spectrometry.

Figure 2 presents the most notable and highly ranked potential M^{pro} sites identified computationally (the order A-G is not significant, as placement in the listing was determined by similarity to the known nsp sites). The most remarkable instance is in selenoprotein F (SELENOF), an ER protein involved in glycoprotein folding quality control. Over the 8 residues spanning P4 to P4′, it has 7 of 8 identical to the M^{pro} site at the nsp12/nsp13 junction, with the only mismatch at the highly variable P3′ position (**Figure 1D**), giving a near-perfect match to the M^{pro} consensus. This was the highest scored NetCorona hit, also highly scored by PROSPER, as shown in **Figure 2F**, and is

significant because coronavirus assembly begins in the ER with spike protein accumulation on the ER membrane surface.

Equally important is the hit on the selenoprotein thioredoxin reductase 1 (TXNRD1), which also was highly scored by both NetCorona and PROSPER (**Figure 2E**). Note that in a larger set of 77 human coronaviruses, a serine (S) in P4 is very common (12), and in addition the sequence SI in the TXNRD1 site is isosteric to TV, the aligned residues in the nsp12/13 site, because transfer of a methyl group from threonine to valine would result in serine and isoleucine. Thus, SI should occupy about the same volume as TV in the M^{pro} active site. Functionally, this predicted cleavage site is only 5 residues from the C terminal of TXNRD1, and would result in removal of the C-terminal redox center of TXNRD1 (AGCUG), making the enzyme incapable of regenerating reduced thioredoxin.

A predicted site in selenoprotein P (SELENOP, **Figure 2C**) is at position 56, 3 residues prior to the first selenocysteine, such that cleavage here would likely interfere with the N-terminal redox activity, possibly without affecting the Se-transport function of the rest of the protein.

Two possible sites were identified in the catalytic subunit of glutamate-cysteine ligase (GCLC), the rate limiting enzyme for GSH synthesis. The first of these, GCLC-a (**Figure 2B**), would cleave after position 217, and is an exact match to P4-P1 of nsp5/6, but with an A in P1′, a highly favorable residue for that position, although SCoV has a G there, and SCoV2 has an S. Site GCLC-b (**Figure 2A**), at position 443, is an exact match to P4-P1 of nsp4/5, but with a G in P1′. The two coronavirus sites with exact P4-P1 matches to GCLC are those at either end of nsp5, i.e., M^{pro} itself, which have the highest catalytic efficiency for cleavage of any of the viral M^{pro} targets (100 and 41%). This suggests that M^{pro} might be particularly efficient at disrupting GSH synthesis, as compared to action at some of the other target sites predicted here, thereby contributing to the clinical findings in COVID-19 (7).

Finally (**Figure 2D**), we include a non-canonical hit that would cleave at position 90 of glutaredoxin-1 (GLRX-1). This site was very highly scored by PROSPER, as well as Procleave, despite the glutamine (Q) at P1′, which has an additional carbon atom relative to the asparagine (N) found at P1′ in the nsp8/9 site, to which it is otherwise most similar.

WHY WOULD SARS-CoV-2 TARGET COMPONENTS OF THE THIOREDOXIN AND GLUTAREDOXIN SYSTEMS?

Along with GSH reductase, GSH and glutaredoxin comprise the glutaredoxin system, one of two cellular redox systems involved in maintaining reduced thiol states, protein folding and repair, and providing electrons to ribonucleotide reductase (RNR). The other redox system with similar roles is the thioredoxin system, comprised of thioredoxin and thioredoxin reductase (21).

Because DNA is an "add-on" to RNA biosynthesis, deoxyribonucleotides can *only* be synthesized via the reduction of ribonucleotides by RNR, a process which is unsustainable without the participation of one or both of the thioredoxin and glutaredoxin systems. As a consequence of this basic fact of biochemistry, one should expect that RNA viruses might exploit various mechanisms to interfere with components of the thioredoxin and glutaredoxin systems, in order to minimize the diversion of ribonucleotides into DNA synthesis. This is simply the inverse of a strategy used by some large DNA viruses to maximize DNA production, by encoding their own thioredoxin-like proteins, glutaredoxins and even entire RNR genes (22). Consistent with this hypothesis, the results presented here suggest coronaviral targeting of TXNRD1, glutaredoxin-1, and GCLC, a key enzyme for GSH synthesis, for proteolytic cleavage. This would lead to knockdown of *both* of the essential redox systems required to sustain DNA synthesis, and thereby conserve ribonucleotides to enhance RNA synthesis for virus production.

THE POTENTIAL M^{PRO} TARGETS TXNRD1 AND GCLC ARE STRONGLY UPREGULATED BY VITAMIN D3

In light of accruing evidence that vitamin D3 status is inversely correlated with severity of COVID-19 (23–26), it is significant that, via its hormone-like actions on gene expression, D3 has been shown to be a potent activator of both TXNRD1 and GCLC. In a microarray study, 6 h after exposure to 1,25-dihydroxyvitamin D3, TXNRD1 mRNA was upregulated 7.1X, and GCLC was upregulated 2.5X (27). These effects of vitamin D3, as well as a resulting increase in GSH levels, have been functionally documented in cell culture studies (28, 29) and in human subjects (30). *Vitamin D3-mediated activation of both TXNRD1 and GSH biosynthesis could substantially counteract the proteolytic knockdown of TXNRD1 and GCLC predicted by our hypothesis*, which, if validated, would thus represent an important mechanism contributing to a role for vitamin D3 in moderating the severity of COVID-19. However, the therapeutic potential of D3 in COVID-19 is the subject of ongoing debate (31). Perhaps inconsistencies in the findings of various studies of this question may be explained in part by a 20-year old observation, that *the effective upregulation of TXNRD1 by vitamin D3 requires the presence of an adequate level of Se* (28). So the full potential of vitamin D3 vs. COVID-19 may only be seen in combination with optimal Se intake, and possibly, vice-versa.

DISCUSSION AND CONCLUSIONS

Altogether, given the protein interaction and functional data (9, 16), it is a very strong hypothesis that GPX1 is an M^{pro} substrate. Of the other sites we propose, some are more likely than others, but those in TXNRD1 and SELENOF are particularly convincing, and all of the predicted sites map to surface accessible regions of the targeted proteins (**Figure S3**). If validated, these results offer new insights into COVID-19 pathogenesis. If SCoV2 is targeting GSH biosynthesis as well as TXNRD1 and GPx1 for proteolytic knockdown, in infected cells, the resulting decreases in these critical antioxidant molecules would contribute to increased oxidative stress, NF-κB activation and pro-apoptotic signaling (17, 18). Because NF-κB is an

activator of many pro-inflammatory cytokines, including IL-6 (32), this could contribute to increased inflammation and the cytokine storms observed in COVID-19, and be a significant basis of pathogenic effects associated with SCoV2 infection of various tissues, including the lung, gastrointestinal tract and cardiovascular system. Importantly, *these consequences of virus-mediated proteolysis would be taking place in everyone who is actively infected, regardless of their Se status*, and would thus be consistent with results suggesting that Se intakes up to several times the minimal dietary requirement are associated with an increase in cure rate from COVID-19 (1). But people with suboptimal nutritional status could be particularly at risk, because their GSH and selenoprotein levels might be low to begin with, making them more vulnerable to the detrimental effects of virus-induced proteolysis. Still, an infection resulting from low dose exposure to virus and limited by a strong immune response in a person with excellent dietary status still might have minimal impact on cellular and patient health, because proteolytic knockdown of host proteins is likely to be incomplete, due to low catalytic efficiencies at some target sites (**Figure 2**) and the stochastic nature of such molecular interactions.

These results are not unprecedented—our lab has shown that some RNA viruses target host mRNAs encoding isoforms of thioredoxin reductase via RNA:RNA antisense interactions, which, like proteolysis, would likely also result in host selenoprotein knockdown, but by a different mechanism (22, 33). If our hypothesis is confirmed (i.e., some of these host cleavage sites prove to be functional), that leaves us with an interesting question—what evolutionary advantages are driving some viruses to go so far as to degrade or block the synthesis of GSH and specific host selenoproteins?

Understanding why RNA viruses may have developed such strategies presents an interesting challenge for future research.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

This study was conceived and designed by ET, who wrote the initial draft. ET and WR worked on the data analysis and revisions of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work has been supported by a recurring unrestricted gift from the Dr. Arthur and Bonnie Ennis Foundation, Decatur, IL, USA.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.00143/full#supplementary-material

REFERENCES

1. Zhang J, Taylor EW, Bennett K, Saad R, Rayman MP. Association between regional selenium status and reported outcome of COVID-19 cases in China. *Am J Clin Nutr.* (2020) 111:1297–9. doi: 10.1093/ajcn/nqaa095
2. Moghaddam A, Heller RA, Sun Q, Seelig J, Cherkezov A, Seibert L, et al. Selenium deficiency is associated with mortality risk from COVID-19. *Nutrients.* (2020) 12:2098. doi: 10.3390/nu12072098
3. Steinbrenner H, Al-Quraishy S, Dkhil MA, Wunderlich F, Sies H. Dietary selenium in adjuvant therapy of viral and bacterial infections. *Adv Nutr.* (2015) 6:73–82. doi: 10.3945/an.114.007575
4. Guillin OM, Vindry C, Ohlmann T, Chavatte L. Selenium, selenoproteins and viral infection. *Nutrients.* (2019) 11:2101. doi: 10.3390/nu11092101
5. Hiffler L, Rakotoambinina B. Selenium and RNA viruses interactions: potential implications for SARSCov-2 infection (COVID-19). *Front Nutr.* (2020). doi: 10.3389/fnut.2020.00164
6. Taylor EW. The oxidative stress-induced niacin sink (OSINS) model for HIV pathogenesis. *Toxicology.* (2010) 278:124–30. doi: 10.1016/j.tox.2009.10.018
7. Polonikov A. Endogenous deficiency of glutathione as the most likely cause of serious manifestations and death in COVID-19 patients. *ACS Infect Dis.* (2020) 6:1558–62. doi: 10.1021/acsinfecdis.0c00288
8. Horowitz RI, Freeman PR, Bruzzese J. Efficacy of glutathione therapy in relieving dyspnea associated with COVID-19 pneumonia: a report of 2 cases. *Respir Med Case Rep.* (2020) 30:101063. doi: 10.1016/j.rmcr.2020.101063
9. Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature.* (2020) 583:459–68. doi: 10.1038/s41586-020-2286-9
10. Grum-Tokars V, Ratia K, Begaye A, Baker SC, Mesecar AD. Evaluating the 3C-like protease activity of SARS-Coronavirus: recommendations for standardized assays for drug discovery. *Virus Res.* (2008) 133:63–73. doi: 10.1016/j.virusres.2007.02.015
11. Song J, Tan H, Perry AJ, Akutsu T, Webb GI, Whisstock JC, et al. PROSPER: an integrated feature-based tool for predicting protease substrate cleavage sites. *PLoS ONE.* (2012) 7:e50300. doi: 10.1371/journal.pone.0050300
12. Kiemer L, Lund O, Brunak S, Blom N. Coronavirus 3CLpro proteinase cleavage sites: possible relevance to SARS virus pathology. *BMC Bioinformatics.* (2004) 5:72. doi: 10.1186/1471-2105-5-72
13. Li F, Leier A, Liu Q, Wang Y, Xiang D, Akutsu T, et al. Procleave: predicting protease-specific substrate cleavage sites by combining sequence and structural information. *Genomics Proteomics Bioinformatics.* (2020) 18:52–64. doi: 10.1016/j.gpb.2019.08.002
14. Orian L, Mauri P, Roveri A, Toppo S, Benazzi L, Bosello-Travain V, et al. Selenocysteine oxidation in glutathione peroxidase catalysis: an MS-supported quantum mechanics study. *Free Radic Biol Med.* (2015) 87:1–14. doi: 10.1016/j.freeradbiomed.2015.06.011
15. Blanco R, Carrasco L, Ventoso I. Cell killing by HIV-1 protease. *J Biol Chem.* (2003) 278:1086–93. doi: 10.1074/jbc.M205636200
16. Lin CW, Lin KH, Hsieh TH, Shiu SY, Li JY. Severe acute respiratory syndrome coronavirus 3C-like protease-induced apoptosis. *FEMS Immunol Med Microbiol.* (2006) 46:375–80. doi: 10.1111/j.1574-695X.2006.00045.x
17. Sappey C, Legrand-Poels S, Best-Belpomme M, Favier A, Rentier B, Piette J. Stimulation of glutathione peroxidase activity decreases HIV type 1 activation after oxidative stress. *AIDS Res Hum Retrovir.* (1994) 10:1451–61. doi: 10.1089/aid.1994.10.1451
18. Kretz-Remy C, Arrigo AP. Selenium: a key element that controls NF-kappa B activation and I kappa B alpha half life. *Biofactors.* (2001) 14:117–25. doi: 10.1002/biof.5520140116

19. Kaltschmidt B, Kaltschmidt C, Hofmann TG, Hehner SP, Droge W, Schmitz ML. The pro- or anti-apoptotic function of NF-kappaB is determined by the nature of the apoptotic stimulus. *Eur J Biochem.* (2000) 267:3828–35. doi: 10.1046/j.1432-1327.2000.01421.x
20. Rognvaldsson T, You L, Garwicz D. State of the art prediction of HIV-1 protease cleavage sites. *Bioinformatics.* (2015) 31:1204–10. doi: 10.1093/bioinformatics/btu810
21. Sengupta R, Holmgren A. Thioredoxin and glutaredoxin-mediated redox regulation of ribonucleotide reductase. *World J Biol Chem.* (2014) 5:68–74. doi: 10.4331/wjbc.v5.i1.68
22. Taylor EW. RNA viruses vs. DNA synthesis: a general viral strategy that may contribute to the protective antiviral effects of selenium. *Preprints.* (2020) 2020060069. doi: 10.20944/preprints202006.0069.v1
23. Arboleda JF, Urcuqui-Inchima S. Vitamin D supplementation: a potential approach for coronavirus/COVID-19 therapeutics? *Front Immunol.* (2020) 11:1523. doi: 10.3389/fimmu.2020.01523
24. Lau FH, Majumder R, Torabi R, Saeg F, Hoffman R, Cirillo JD, et al. Vitamin D insufficiency is prevalent in severe COVID-19. *medRxiv.* (2020). doi: 10.1101/2020.04.24.20075838
25. Marik PE, Kory P, Varon J. Does vitamin D status impact mortality from SARS-CoV-2 infection? *Med Drug Discov.* (2020) 6:100041. doi: 10.1016/j.medidd.2020.100041
26. Rhodes JM, Subramanian S, Laird E, Griffin G, Kenny RA. Perspective: vitamin D deficiency and COVID-19 severity – plausibly linked by latitude, ethnicity, impacts on cytokines, ACE2, and thrombosis (R1). *J Intern Med.* (2020). doi: 10.1111/joim.13149. [Epub ahead of print].
27. Kovalenko PL, Zhang Z, Cui M, Clinton SK, Fleet JC. 1,25 dihydroxyvitamin D-mediated orchestration of anticancer, transcript-level effects in the immortalized, non-transformed prostate epithelial cell line, RWPE1. *BMC Genomics.* (2010) 11:26. doi: 10.1186/1471-2164-11-26
28. Schutze N, Fritsche J, Ebert-Dumig R, Schneider D, Kohrle J, Andreesen R, et al. The selenoprotein thioredoxin reductase is expressed in peripheral blood monocytes and THP1 human myeloid leukemia cells–regulation by 1,25-dihydroxyvitamin D3 and selenite. *Biofactors.* (1999) 10:329–38. doi: 10.1002/biof.5520100403
29. Jain SK, Micinski D. Vitamin D upregulates glutamate cysteine ligase and glutathione reductase, and GSH formation, and decreases ROS and MCP-1 and IL-8 secretion in high-glucose exposed U937 monocytes. *Biochem Biophys Res Commun.* (2013) 437:7–11. doi: 10.1016/j.bbrc.2013.06.004
30. Alvarez JA, Chowdhury R, Jones DP, Martin GS, Brigham KL, Binongo JN, et al. Vitamin D status is independently associated with plasma glutathione and cysteine thiol/disulphide redox status in adults. *Clin Endocrinol.* (2014) 81:458–66. doi: 10.1111/cen.12449
31. Brown RA. COVID-19 and vitamin-D – NICE; unthinking discrimination against BAME, Elderly and Obese? Affirmative-action needed to mitigate D deficiency, in high-COVID-risk groups. *BMJ.* (2020) 369:m2475. doi: 10.1136/bmj.m2475
32. Liu T, Zhang L, Joo D, Sun SC. NF-kappaB signaling in inflammation. *Signal Transduct Target Ther.* (2017) 2:17023. doi: 10.1038/sigtrans.2017.23
33. Taylor EW, Ruzicka JA, Premadasa L, Zhao L. Cellular selenoprotein mRNA tethering via antisense interactions with Ebola and HIV-1 mRNAs may impact host selenium biochemistry. *Curr Top Med Chem.* (2016) 16:1530–5. doi: 10.2174/1568026615666150915121633

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Taylor and Radding. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

PERSPECTIVE
published: 25 September 2020
doi: 10.3389/fimmu.2020.570919

Perspective: The Potential Effects of Naringenin in COVID-19

Ricardo Wesley Alberca[1], Franciane Mouradian Emidio Teixeira[2], Danielle Rosa Beserra[1], Emily Araujo de Oliveira[1], Milena Mary de Souza Andrade[1], Anna Julia Pietrobon[2] and Maria Notomi Sato[1]*

[1] Laboratory of Dermatology and Immunodeficiencies, LIM-56, Department of Dermatology, School of Medicine and Institute of Tropical Medicine of São Paulo, University of São Paulo, São Paulo, Brazil, [2] Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Coronavirus disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), was declared a pandemic by the World Health Organization in March 2020. Severe COVID-19 cases develop severe acute respiratory syndrome, which can result in multiple organ failure, sepsis, and death. The higher risk group includes the elderly and subjects with pre-existing chronic illnesses such as obesity, hypertension, and diabetes. To date, no specific treatment or vaccine is available for COVID-19. Among many compounds, naringenin (NAR) a flavonoid present in citrus fruits has been investigated for antiviral and anti-inflammatory properties like reducing viral replication and cytokine production. In this perspective, we summarize NAR potential anti-inflammatory role in COVID-19 associated risk factors and SARS-CoV-2 infection.

Keywords: antiviral, anti-inflammatory, naringenin, SARS-CoV-2, COVID-19, TNF, ACE2

OPEN ACCESS

Edited by:
Christophe Matthys, KU Leuven, Belgium

Reviewed by:
Melissa M. Markofski, University of Houston, United States
Keith R. Martin, University of Memphis, United States

****Correspondence:***
Ricardo Wesley Alberca
ricardowesley@gmail.com

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: *09 June 2020*
Accepted: *07 September 2020*
Published: *25 September 2020*

Citation:
Alberca RW, Teixeira FME, Beserra DR, de Oliveira EA, Andrade MMS, Pietrobon AJ and Sato MN (2020) Perspective: The Potential Effects of Naringenin in COVID-19. Front. Immunol. 11:570919. doi: 10.3389/fimmu.2020.570919

INTRODUCTION

The respiratory diseases named Coronavirus disease 2019 (COVID-19) is generated by a respiratory infection with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) (1, 2). Due to the rapidl viral transmission, the disease was declared a pandemic by the World Health Organization a few months after the first diagnosed case (3, 4).

Besides the similar clinical manifestations to previous Severe Acute Respiratory Syndrome Coronavirus-1 (SARS-CoV-1), SARS-CoV-2 infection presents a much lower death rate (5). Approximately 5% of patients progress to a severe COVID-19, developing mainly severe acute respiratory syndrome, with 3% needing assisted respiratory mechanic ventilation. Coronavirus disease 2019 can progress to septic shock and multiple organ failure (6) and exhibits a death rate of approximately 2% (7).

The SARS-CoV-2 can infect human cells by entry via the angiotensin-converting enzyme 2 (ACE2) receptor and Transmembrane Serine Protease 2 (TMPRSS2) (8). Although this process is wildly accepted, other possible infective routes are being explored such as antibody-dependent enhancement (ADE) (7) and via CD147 (9).

Angiotensin-converting enzyme 2 expression is one of the main explanations for the higher airway infection, as it is highly expressed in the respiratory tract such as epithelial cells of the alveoli, trachea, and bronchi, some bronchial glands and alveolar macrophages (10). However, ACE2 is also expressed in the ileo, kidney, adipose tissue, heart, brain, blood vessels, stomach, liver, and oral and nasal mucosas (11), which could corroborate the systemic inflammatory profile in COVID-19.

Upon viral entry, the virus induces the host to increase the production and release of inflammatory cytokines, which can lead to greater immune activation and tissue damage (12). Hypothetically, the reduction of inflammation could aid COVID-19 patients (13).

Several compounds have been associated with antiviral and anti-inflammatory properties and could impact COVID-19 development such as vitamin D (14), vitamin E (15), vitamin B12 (16), omega-3 (17), and flavonoids (18). Naringenin (NAR) is an important natural flavonoid present in citrus fruits, like grapefruit (43.5 mg/100 mL) and oranges (2.13 mg/100 mL) (19), with a high analgesic, anti-oxidant, anti-inflammatory, anti-tumoral, and anti-viral effect (20–23) (**Figure 1**). The consumption of 8 mL/kg of orange juice increases NAR plasma levels from 0 to 300 μg/L 4 h after ingestion (24).

The antiviral effect of NAR has been studied in several viruses, such as dengue (25, 26), hepatitis C (27), zika (28), chikungunya (29), Semliki Forest (30), herpes simplex 1 and 2 (31), yellow fever (32), and human immunodeficiency virus (33). Several *in vitro* studies have highlighted NAR's antiviral effect in pre-infection and post-infection (28). Similar to other natural compounds, NAR has extensively been investigated in *in vitro*, but has very limited results in *in vivo* models of viral infection (34, 35) (**Figure 1B**). Nevertheless, the *in vitro* and *in vivo* anti-inflammatory potential of NAR has been highlighted in several animal models, including respiratory syndromes (35, 36). In this perspective, we highlight the mechanism in which NAR may present an important anti-inflammatory role in COVID-19.

ANTI-INFLAMMATORY PROPERTIES OF NARINGENIN

Inflammation can be characterized by the regulation of pro- and anti-inflammatory mediators in resident cells and leukocytes recruited from the blood (37). There are strong pieces of evidence of the role of NAR under inflammatory conditions due to a wide range of mechanisms. The immunomodulatory properties of NAR are associated with the regulation of key signaling pathways, like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (38), PI3K/AKT (23), and mitogen-activated protein kinases (MAPK) (39) in different cell types (**Figure 1A**).

Macrophages are an important cell in the COVID-19 pathology, being able to sense and respond to pathogens and produce inflammatory cytokines and chemokines (40). In murine macrophages, NAR can reduce inflammatory mediators production induced by LPS, and in a murine endotoxemia model reduces the mortality rates from 60 to 0% (41). Murine macrophages infected with a gram-negative bacteria (*Chlamydia trachomatis*) NAR reduced the production of IL-1β, IL1α, IL-6, TNF, IL-12p70, and IL-10 in a dose-dependent manner (42). Moreover, NAR's anti-inflammatory effects have been demonstrated *in vivo* (41), in macrophage and *ex vivo* human whole-blood models, reducing IL-1β, IL-6, IL-8, and TNF upon LPS stimulus to close to non-stimulated levels (43) (**Figure 2**).

Barnes et al. described that the cytokine storm developed by severe COVID-19 patients is related to an exacerbation of neutrophil activation (44). It is clear the central role of neutrophils in COVID-19, as neutrophilia and neutrophil-to-lymphocyte ratio in COVID-19 patients is associated with disease severity (45, 46).

Lung biopsies have also identified an infiltration of neutrophils (47) and the formation of neutrophil extracellular traps in COVID-19 patients (48) (**Figure 1A**). Although some animals like cats, ferrets, mice, hamsters, and macaques can be infected by SARS-CoV-2, the usage of animal models in COVID-19 is currently limited (49).

In an animal model of acute respiratory distress syndrome (ARDS), a syndrome with an increase in IL-6, TNF, and neutrophils in the lungs, NAR supplementation can reduce neutrophils infiltration and oxidative stress, greatly reducing airway inflammation and lung injury (50). Naringenin reduction of oxidative stress is partially mediated by a curb in the anion superoxide production (51, 52) (**Figure 2**).

Naringenin can suppress inflammatory molecules production through both transcriptional and post-transcriptional mechanisms (18). In a LPS-induced model of inflammation in a mouse model, NAR suppressed TNF and IL-6 production by macrophages and T lymphocytes without interfering in the toll-like receptor (TLR) cascade but by increasing intracellular cytokine degradation through lysosome-dependent mechanisms (23). These data indicate a potential role in the control of inflammation and oxidative stress-related to airway inflammatory insults (**Figure 2A**). These anti-inflammatory and anti-oxidant effects are also described in chronic comorbidities like in diabetes mellitus (53, 54), dyslipidemia, hyperinsulinemia, and being overweight (55), which are all risk factors associated with severe COVID-19 (4, 56, 57) (**Figure 1C**).

In animal experimental models, NAR was able to modulate different inflammation syndromes and at different sites, such as colitis (58), hepatitis (59), obesity (60), cancer (61), and acute respiratory syndrome (36). This is particularly important in COVID-19, because SARS-CoV-2 infection induces a systemic inflammation and can infect many different organs including lungs, heart, liver, brain, kidneys, and the intestines (62).

In addition, NAR can promote lysosome-dependent cytokine protein degradation, which may be important in COVID-19 (63, 64), considering the systemic and cytokine storm during severe COVID-19 (65). In fact, NAR-induced immunomodulation has been demonstrated in airway inflammatory disorders. In a murine asthma model, treatment with NAR reduced airway hyperactivity and airway inflammation, with a reduction in the levels of IL-4 and IL-13 in bronchoalveolar lavage and serum IgE levels as well improvement in lung function assay (66–68). Overall, the treatment with NAR reduced lung eosinophilia to similar levels to non-asthmatic group (66–68).

In lung fibrosis induced by infection with *Mycoplasma pneumoniae*, NAR reduced autophagy-mediated airway inflammation and lung fibrosis (69, 70), and, in a chronic obstructive pulmonary disease (COPD) model, NAR was able to mitigate lung inflammation, reduce the expression of TGF-β, and increase glucocorticoid receptor expression (GCR)

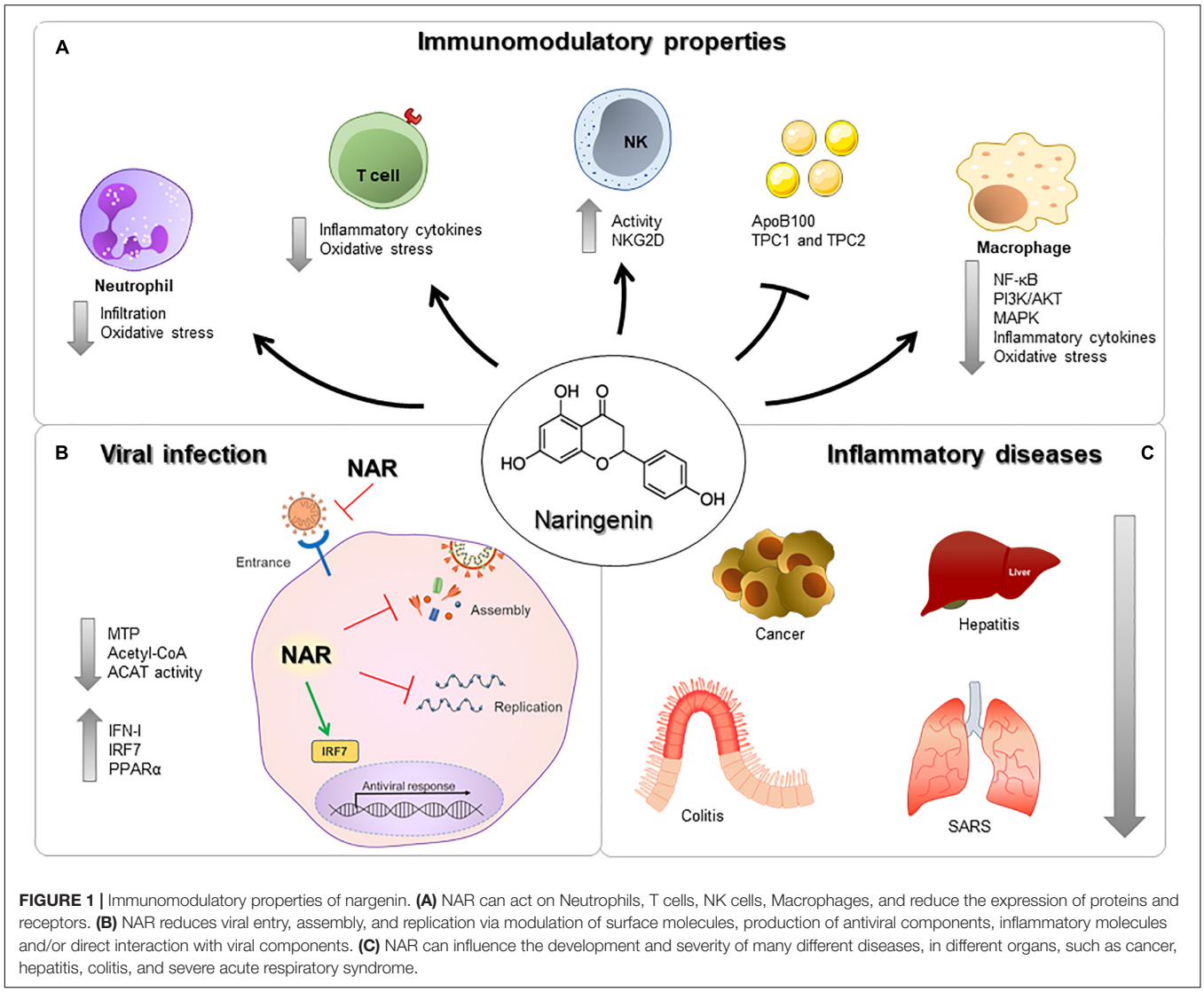


FIGURE 1 | Immunomodulatory properties of nargenin. **(A)** NAR can act on Neutrophils, T cells, NK cells, Macrophages, and reduce the expression of proteins and receptors. **(B)** NAR reduces viral entry, assembly, and replication via modulation of surface molecules, production of antiviral components, inflammatory molecules and/or direct interaction with viral components. **(C)** NAR can influence the development and severity of many different diseases, in different organs, such as cancer, hepatitis, colitis, and severe acute respiratory syndrome.

(71). Naringenin anti-inflammatory effect was also verified in radiation-induced lung injury, reducing lung inflammation and IL-1β levels (72).

The NAR anti-inflammatory effect is thus not directly mediated to a type 2 or type 1/17 immune response but a regulation of the immune response. Studies have highlighted the increase in T regulatory cells and transforming growth factor-β after NAR consumption via aryl hydrocarbon receptor-mediated pathway (73).

Nevertheless, the excessive regulation of the inflammatory response could impair anti-viral immune response, that has not been previously observed with NAR supplementation. Naringenin can also activate the interferon-stimulated response element and enhance IFN-I production via an increase in the expression of IRF7 (74) and increase NK cell activity via enhanced NKG2D ligand expression (75). Considering the crucial role of NK cells and IFN-I in the anti-viral immune response, NAR may also contribute to the viral load control. Overall, these previous studies demonstrated, *in vivo* and *in vitro*, that NAR is a strong candidate as an adjuvant in reducing airway and systemic inflammation.

NARINGENIN AND CORONAVIRUSES

Two coronaviruses have been responsible for recent epidemics. In 2002, the SARS-CoV-1 epidemic caused 8,098 cases, with 774 deaths in 11 countries (76–78). In 2012, in the Middle East, another coronavirus also caused Severe Acute Respiratory Syndrome, being named MERS-CoV (79). Until 2020, MERS-CoV had caused 2,494 cases, with 858 associated deaths (77).

Clinical manifestation of SARS-CoV-1 and MERS-CoV is similar. Patients report clinical symptoms such as fever, cough, body pain, headache, and less commonly, diarrhea and nausea (80). However, the need for intensive care and mechanical ventilation is greater in MERS-CoV than in SARS-CoV-1 (81, 82).

Similarly to MERS-CoV and SARS-CoV-1, SARS-CoV-2 infection is mainly transmitted by respiratory droplets expelled

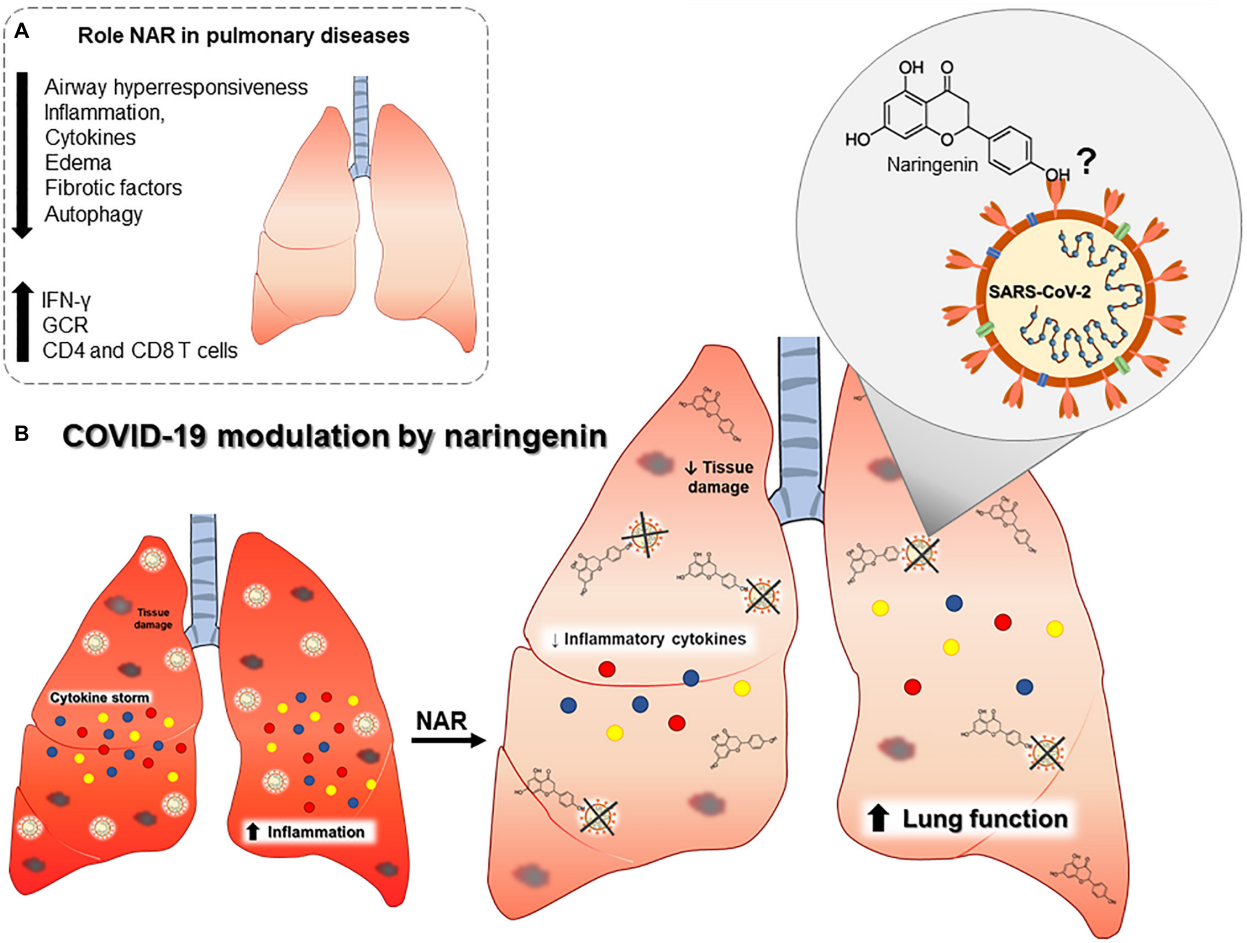


FIGURE 2 | Outline of the putative role of naringenin in COVID-19 pulmonary pathophysiology. **(A)** Established effects of naringenin on different pulmonary diseases. **(B)** Naringenin may reduce inflammatory cytokines and tissue damage, and it may directly bind to SARS-CoV-2. Abbreviations: Interferon gamma (IFN-γ), glucocorticoid receptor expression (GCR), and cluster differentiation (CD).

from an infected person during sneezes or coughs (83, 84). Severe Acute Respiratory Syndrome Coronavirus-2 surface glycoprotein spike (S protein) binds to ACE2 on the surface of the host's cell surface. This invading process is the same used by SARS-CoV-1 (85). In comparison, MERS-CoV uses dipeptidyl peptidase 4 (DPP4), a multifunction surface protein to entry into cells (85). Dipeptidyl peptidase 4 is mainly expressed on the kidney, intestine, liver, prostate, and activated leukocytes. Dipeptidyl peptidase 4 is expressed on the lower respiratory tract, glands located in submucosa of the upper respiratory tract, lung macrophages, and alveolar epithelial cells (86).

After these coronaviruses (MERS-CoV, SARS-CoV-1, and SARS-CoV-2) invade the host's cell, polypeptides are released from the polyproteins by proteolytic processing. The proteolytic process is mediated by papain-like protease (PL^{pro}) and 3-chymotrypsin-like protease ($3CL^{pro}$). The $3CL^{pro}$ cleaves the polyprotein to generate various non-structural proteins, crucial for viral replication (87, 88). Due to the main role of 3CL in coronaviruses viral cycle, inhibitors of 3CL could potentially be used in COVID-19.

Flavonoid inhibition of the 3CL protease has been described in MERS-CoV (89) and SARS (90), but NAR was not among the flavonoids investigated. Nevertheless, in silico analysis demonstrated that NAR has the potential to inhibit SARS-CoV-2 $3CL^{pro}$ (91). A recent study verified that SARS-CoV-1 and SARS-CoV-2 share 99.02% of genetic similarity of 3CL, with only 12 punctual mutations (88), leading to the possible inhibition of 3CL by NAR and other flavonoids.

Another possible mechanism is the inhibition of the two-pore ionic channel (TPC1 and TPC2) (92). Inhibition of TPC1 and TPC2 reduces MERS-CoV infectivity, intracellular traffic (93), and viral replication (93, 94). Due to SARS-CoV-2 viral genome sequencing similarities with MERS-CoV and SARS-CoV-1 (95), it is possible that similar mechanism of inhibition of TPC1 and TPC2 channel be effective in COVID-19, aiding in the reduction of viral replication (96).Interestingly, NAR can inhibit the activity of TPC1 and TPC2 both in humans and plants (97).

NAR is a hydrophilic substance with a higher affinity for the cytoplasmic membrane generating intracellular accumulation of

NAR (98). Therefore, this affinity probably enhances intracellular signaling and the modulation of and TPC1 and TPC2 (27). Therefore, the TPC1 and TPC2's modulation by NAR should be further investigated as a possible anti-coronavirus intervention.

DISCUSSION

Several reports of natural compounds with anti-SARS-CoV-2 potential are currently being investigated. Substances that may compete with the ACE2 receptor or reduces the ACE2 expression may present an alternative or adjuvant therapy in COVID-19 (99). In fact, NAR consumption has been associated with a reduction in ACE2 expression in the kidneys of rats (100) and could bind directly to the ACE2 receptor (101).

However, nutritional interventions aiming to regulate SARS-CoV-2 entry receptor ACE2 need to be carefully evaluated, as downregulating of ACE2 could also lead to greater inflammation and lung damage (102, 103). Previous reports demonstrated that the oral consumption of NAR can reduce acute lung injury in a mouse model (50) and reduce the production of pro-inflammatory cytokines (18). This is extremely relevant, as a part of COVID-19 lung injury can be classified as ARDS (104).

Coronavirus disease 2019 can also lead to cytokine storm, progress to septic shock, and cause death (105, 106). Modulating the cytokine storm is thus a vital process for treating COVID-19. Naringenin has been used in experimental models to regulate the production of IL-6 and TNF (23), cytokines that are increased in COVID-19 and further increased in severe cases (107, 108). Also in an animal model of septic shock, the consumption of NAR has been demonstrated to reduce kidney damage via an increase in antioxidant enzymes (109).

Studies verified a direct role of NAR in abrogating viral replication in human cells, before (21) and after infection (30). In SARS-CoV2, in silico analysis demonstrated that NAR has the potential to inhibit SARS-CoV-2 $3CL^{pro}$ and consequently inhibit viral replication (91), which still needs to be further verified experimentally.

The consumption of NAR via citrus fruits (110) or supplementation (111) can rapidly increase circulating levels of NAR and increase intracellular levels of NAR (98, 111). An increase in the concentration of NAR in plasma samples can be observed 20 min oral consumption and peaking around 4 h post-consumption (112). In addition, in vitro models have also demonstrated a long-term anti-viral benefit, even after discontinuation of supplementation with NAR (21), although there is little evidence of *in vivo* antiviral activity (35).

Previous clinical trials with the consumption of 500 mL/day for 8 weeks of orange juice, rich in NAR, has demonstrated an adjuvant effect in antiviral therapy (34). The consumption of 340 mL of grapefruit juice per day (containing approximately 210 mg of NAR) also improved cardiac-related measurements in post-menopause women (113). Although NAR is one of the most important naturaly occurring flavonoids, there is a lack of clinical trials and data on pharmacokinetic aspects, metabolic fate, and chemical stability that may limit the usage of this bioactive compound in humans (35).

A caveat of NAR is the oral consumption. Although widely accepted by patients, it could be a barrier in severe COVID-19 patients. Therefore, NAR may be better applied as a prophylactic intervention or on the onset of SARS-CoV-2 infection. The possible effect of NAR on the ACE2 receptor also needs to investigated, as ACE2 reduction could lead to greater inflammation (102, 103). Naringenin is mostly absorbed in the small intestine (114), and differences in microbiota may thus also present an important inter-individual variable (24, 112).

Another caveat is the NAR poor aqueous solubility and bioavailability; currently, the usage of liposomes, nanoparticles, and other formulations may present itself as a solution (115–118).

Furthermore, NAR interactions with the cytochrome P450 (CYP) system need to be evaluated, as NAR can affect drug-metabolizing enzymes and pharmacokinetic of important drugs that may be of regular use or specific in COVID-19 patients (119–121).

In conclusion, NAR potential as an anti-inflammatory nutritional intervention has been demonstrated in many different diseases, such as SARS-CoV-1 and MERS-CoV. Further investigations and clinical trials are needed to help understand the role of NAR consumption in humans during a viral infection, especially in SARS-CoV-2 infection and COVID-19.

DATA AVAILABILITY STATEMENT

All datasets generated in this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

RA: conception, writing, and review. FT: writing, drawing, and review. DB, EO, MA, AP, and MS: writing and review. All authors contributed to the article and approved the submitted version.

FUNDING

RA holds a post-doctorate fellowship from FAPESP (19/02679-7). FT holds a doctorate fellowship from FAPESP (18/18230-6). EO holds a scientific initiation fellowship from FAPESP (19/26928-6). MS have a research grant from FAPESP (17/18199-9).

REFERENCES

1. Alberca GGF, Fernandes IG, Sato MN, Alberca RW. What is COVID-19? *Front Young Minds*. (2020) 8:74. doi: 10.3389/frym.2020.00074
2. Cao X. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol*. (2020) 20:269–70. doi: 10.1038/s41577-020-0308-3
3. South AM, Diz DI, Chappell MC. COVID-19, ACE2, and the cardiovascular consequences. *Am J Physiol Heart Circ Physiol*. (2020) 318:H1084–90. doi: 10.1152/ajpheart.00217.2020

4. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet*. (2020) 395:809–15. doi: 10.1016/S0140-6736(20)30360-3
5. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus disease 2019 (COVID-19) outbreak in China. *JAMA*. (2020) 323:1239–42. doi: 10.1001/jama.2020.2648
6. Xie M, Chen Q. Insight into 2019 novel coronavirus – an updated interim review and lessons from SARS-CoV and MERS-CoV. *Int J Infect Dis*. (2020) 94:119–24. doi: 10.1016/j.ijid.2020.03.071
7. Peron JPS, Nakaya H, Peron JPS, Nakaya H. Susceptibility of the elderly to SARS-CoV-2 infection: ACE-2 overexpression, shedding, and antibody-dependent enhancement (ADE). *Clinics*. (2020) 75:e1912. doi: 10.6061/clinics/2020/e1912
8. Palmeira P, Barbuto JAM, Silva CAA, Carneiro-Sampaio M, Palmeira P, Barbuto JAM, et al. Why is SARS-CoV-2 infection milder among children? *Clinics*. (2020) 75:e1947. doi: 10.6061/clinics/2020/e1947
9. Wang K, Chen W, Zhou Y-S, Lian J-Q, Zhang Z, Du P, et al. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. *bioRxiv*[Preprint]. (2020):doi: 10.1101/2020.03.14.988345
10. Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. *Respirology*. (2018) 23:130–7. doi: 10.1111/resp.13196
11. Gembardt F, Sterner-Kock A, Imboden H, Spalteholz M, Reibitz F, Schultheiss HP, et al. Organ-specific distribution of ACE2 mRNA and correlating peptidase activity in rodents. *Peptides*. (2005) 26:1270–7. doi: 10.1016/j.peptides.2005.01.009
12. Conti P, Ronconi G, Caraffa A, Gallenga CE, Ross R, Frydas I, et al. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by COVID-19: anti-inflammatory strategies. *J Biol Regul Homeost Agents*. (2020) 34:327–31. doi: 10.23812/CONTI-E
13. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol*. (2020) 5:562–9. doi: 10.1038/s41564-020-0688-y
14. Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-κB–linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *J Immunol*. (2010) 184:965–74. doi: 10.4049/jimmunol.0902840
15. Andreone P, Fiorino S, Cursaro C, Gramenzi A, Margotti M, Di Giammarino L, et al. Vitamin E as treatment for chronic hepatitis B: results of a randomized controlled pilot trial. *Antiviral Res*. (2001) 49:75–81. doi: 10.1016/S0166-3542(00)00141-8
16. Tamura J, Kubota K, Murakami H, Sawamura M, Matsushima T, Tamura T, et al. Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. *Clin Exp Immunol*. (1999) 116:28–32. doi: 10.1046/j.1365-2249.1999.00870.x
17. Braz-De-Melo HA, Pasquarelli-do-Nascimento G, Corrêa R, das Neves Almeida R, de Oliveira Santos I, Prado PS, et al. Potential neuroprotective and anti-inflammatory effects provided by omega-3 (DHA) against Zika virus infection in human SH-SY5Y cells. *Sci Rep*. (2019) 9:20119. doi: 10.1038/s41598-019-56556-y
18. Gupta SC, Tyagi AK, Deshmukh-Taskar P, Hinojosa M, Prasad S, Aggarwal BB. Downregulation of tumor necrosis factor and other proinflammatory biomarkers by polyphenols. *Arch Biochem Biophys*. (2014) 559:91–9. doi: 10.1016/j.abb.2014.06.006
19. Hartogh DJD, Tsiani E. Antidiabetic properties of naringenin: a citrus fruit Polyphenol. *Biomolecules*. (2019) 9:99. doi: 10.3390/biom9030099
20. Pinho-Ribeiro FA, Zarpelon AC, Fattori V, Manchope MF, Mizokami SS, Casagrande R, et al. Naringenin reduces inflammatory pain in mice. *Neuropharmacology*. (2016) 105:508–19. doi: 10.1016/j.neuropharm.2016.02.019
21. Goldwasser J, Cohen PY, Lin W, Kitsberg D, Balaguer P, Polyak SJ, et al. Naringenin inhibits the assembly and long-term production of infectious hepatitis C virus particles through a PPAR-mediated mechanism. *J Hepatol*. (2011) 55:963–71. doi: 10.1016/j.jhep.2011.02.011
22. Zhang FY, Du GJ, Zhang L, Zhang CL, Lu WL, Liang W. Naringenin enhances the anti-tumor effect of doxorubicin through selectively inhibiting the activity of multidrug resistance-associated proteins but not P-glycoprotein. *Pharm Res*. (2009) 26:914–25. doi: 10.1007/s11095-008-9793-y
23. Jin L, Zeng W, Zhang F, Zhang C, Liang W. Naringenin ameliorates acute inflammation by regulating intracellular cytokine degradation. *J Immunol*. (2017) 199:3466–77. doi: 10.4049/jimmunol.1602016
24. Erlund I, Meririnne E, Alfthan G, Aro A. Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. *J Nutr*. (2001) 131:235–41. doi: 10.1093/jn/131.2.235
25. Zandi K, Teoh BT, Sam SS, Wong PF, Mustafa MR, Abubakar S. In vitro antiviral activity of Fisetin, Rutin and Naringenin against Dengue virus type-2. *J Med Plant Res*. (2011) 5:5534–9.
26. Frabasile S, Koishi AC, Kuczera D, Silveira GF, Verri WA, Dos Santos CND, et al. The citrus flavanone naringenin impairs dengue virus replication in human cells. *Sci Rep*. (2017) 7:41864. doi: 10.1038/srep41864
27. Nahmias Y, Goldwasser J, Casali M, Van Poll D, Wakita T, Chung RT, et al. Apolipoprotein B-dependent hepatitis C virus secretion is inhibited by the grapefruit flavonoid naringenin. *Hepatology*. (2008) 47:1437–45. doi: 10.1002/hep.22197
28. Cataneo AHD, Kuczera D, Koishi AC, Zanluca C, Silveira GF, de Arruda TB, et al. The citrus flavonoid naringenin impairs the in vitro infection of human cells by Zika virus. *Sci Rep*. (2019) 9:16348. doi: 10.1038/s41598-019-52626-3
29. Ahmadi A, Hassandarvish P, Lani R, Yadollahi P, Jokar A, Bakar SA, et al. Inhibition of chikungunya virus replication by hesperetin and naringenin. *RSC Adv*. (2016) 6:69421–30. doi: 10.1039/c6ra16640g
30. Pohjala L, Utt A, Varjak M, Lulla A, Merits A, Ahola T, et al. Inhibitors of alphavirus entry and replication identified with a stable Chikungunya replicon cell line and virus-based assays. *PLoS One*. (2011) 6:e28923. doi: 10.1371/journal.pone.0028923
31. Lyu SY, Rhim JY, Park WB. Antiherpetic activities of flavonoids against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro. *Arch Pharm Res*. (2005) 28:1293–301. doi: 10.1007/BF02978215
32. Castrillo M, Córdova T, Cabrera G, Rodríguez-Ortega M. Effect of naringenin, hesperetin and their glycosides forms on the replication of the 17D strain of yellow fever virus (Efecto de la naringenina, hesperetina y sus formas glicosidadas sobre la replicación de la cepa 17D del virus de la fiebre amarilla). *Av en Biomed*. (2015) 4:69–78.
33. da Silva CCF, Salatino A, Motta LB da, Negri G, Salatino MLF. Chemical characterization, antioxidant and anti-HIV activities of a Brazilian propolis from Ceará state. *Braz J Pharmacogn*. (2019) 29:309–18. doi: 10.1016/j.bjp.2019.04.001
34. Gonçalves D, Lima C, Ferreira P, Costa P, Costa A, Figueiredo W, et al. Orange juice as dietary source of antioxidants for patients with hepatitis C under antiviral therapy. *Food Nutr Res*. (2017) 61:1296675. doi: 10.1080/16546628.2017.1296675
35. Salehi B, Fokou PVT, Sharifi-Rad M, Zucca P, Pezzani R, Martins N, et al. The therapeutic potential of naringenin: a review of clinical trials. *Pharmaceuticals*. (2019) 12:11. doi: 10.3390/ph12010011
36. Fouad AA, Albuali WH, Jresat I. Protective effect of naringenin against lipopolysaccharide-induced acute lung injury in rats. *Pharmacology*. (2016) 97:224–32. doi: 10.1159/000444262
37. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb Perspect Biol*. (2009) 1:a001651. doi: 10.1101/cshperspect.a001651
38. Pinho-Ribeiro FA, Zarpelon AC, Mizokami SS, Borghi SM, Bordignon J, Silva RL, et al. The citrus flavonone naringenin reduces lipopolysaccharide-induced inflammatory pain and leukocyte recruitment by inhibiting NF-κB activation. *J Nutr Biochem*. (2016) 33:8–14. doi: 10.1016/j.jnutbio.2016.03.013
39. Chen S, Ding Y, Tao W, Zhang W, Liang T, Liu C. Naringenin inhibits TNF-α induced VSMC proliferation and migration via induction of HO-1. *Food Chem Toxicol*. (2012) 50:3025–31. doi: 10.1016/j.fct.2012.06.006
40. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol*. (2020) 20:355–62. doi: 10.1038/s41577-020-0331-4

41. Liu X, Wang N, Fan S, Zheng X, Yang Y, Zhu Y, et al. The citrus flavonoid naringenin confers protection in a murine endotoxaemia model through AMPK-ATF3-dependent negative regulation of the TLR4 signalling pathway. *Sci Rep*. (2016) 6:39735. doi: 10.1038/srep39735
42. Yilma AN, Singh SR, Morici L, Dennis VA. Flavonoid naringenin: a potential immunomodulator for Chlamydia trachomatis inflammation. *Mediators Inflamm*. (2013) 2013:102457. doi: 10.1155/2013/102457
43. Bodet C, La VD, Epifano F, Grenier D. Naringenin has anti-inflammatory properties in macrophage and ex vivo human whole-blood models. *J Periodontal Res*. (2008) 43:400–7. doi: 10.1111/j.1600-0765.2007.01055.x
44. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J Exp Med*. (2020) 217:e20200652. doi: 10.1084/jem.20200652
45. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 Novel Coronavirus-infected Pneumonia in Wuhan, China. *JAMA*. (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
46. Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, et al. Neutrophil-to-lymphocyte ratio predicts severe illness patients with 2019 Novel Coronavirus in the early stage. *medRxiv*[Preprint]. (2020):doi: 10.1101/2020.02.10.20021584
47. Yao XH, Li TY, He ZC, Ping YF, Liu HW, Yu SC, et al. [A pathological report of three COVID-19 cases by minimally invasive autopsies]. *Zhonghua bing li xue za zhi*. (2020) 49:411–7. doi: 10.3760/cma.j.cn112151-20200312-00193
48. Fox SE, Akmatbekov A, Harbert JL, Li G, Brown JQ, Heide RS, et al. Pulmonary and cardiac pathology in Covid-19: the first autopsy series from New Orleans. *medRxiv*[Preprint]. (2020) doi: 10.1101/2020.04.06.20050575
49. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science*. (2020) 368:1016–20. doi: 10.1126/science.abb7015
50. Zhao M, Li C, Shen F, Wang M, Jia N, Wang C. Naringenin ameliorates LPS-induced acute lung injury through its anti-oxidative and anti-inflammatory activity and by inhibition of the PI3K/AKT pathway. *Exp Ther Med*. (2017) 14:2228–34. doi: 10.3892/etm.2017.4772
51. Manchope MF, Calixto-Campos C, Coelho-Silva L, Zarpelon AC, Pinho-Ribeiro FA, Georgetti SR, et al. Naringenin inhibits superoxide anion-induced inflammatory pain: role of oxidative stress, cytokines, Nrf-2 and the no-cGMP-PKG-KATP channel signaling pathway. *PLoS One*. (2016) 11:e0153015. doi: 10.1371/journal.pone.0153015
52. Martinez RM, Pinho-Ribeiro FA, Steffen VS, Caviglione CV, Vignoli JA, Barbosa DS, et al. Naringenin inhibits UVB irradiation-induced inflammation and oxidative stress in the skin of hairless mice. *J Nat Prod*. (2015) 78:1647–55. doi: 10.1021/acs.jnatprod.5b00198
53. Nguyen-Ngo C, Willcox JC, Lappas M. Anti-diabetic, anti-inflammatory, and anti-oxidant effects of naringenin in an in vitro human model and an in vivo murine model of gestational diabetes mellitus. *Mol Nutr Food Res*. (2019) 63:e1900224. doi: 10.1002/mnfr.201900224
54. Al-Rejaie SS, Aleisa AM, Abuohashish HM, Parmar MY, Ola MS, Al-Hosaini AA, et al. Naringenin neutralises oxidative stress and nerve growth factor discrepancy in experimental diabetic neuropathy. *Neurol Res*. (2015) 37:924–33. doi: 10.1179/1743132815Y.0000000079
55. Mulvihill EE, Allister EM, Sutherland BG, Telford DE, Sawyez CG, Edwards JY, et al. Naringenin prevents dyslipidemia, apolipoprotein B overproduction, and hyperinsulinemia in LDL receptor-null mice with diet-induced insulin resistance. *Diabetes*. (2009) 58:2198–210. doi: 10.2337/db09-0634
56. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with Coronavirus disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med*. (2020) 180:934–43. doi: 10.1001/jamainternmed.2020.0994
57. Alberca RW, Oliveira LM, Branco ACCC, Pereira NZ, Sato MN. Obesity as a risk factor for COVID-19: an overview. *Crit Rev Food Sci Nutr*. (2020):doi: 10.1080/10408398.2020.1775546 [Epub ahead of print],
58. Dou W, Zhang J, Sun A, Zhang E, Ding L, Mukherjee S, et al. Protective effect of naringenin against experimental colitis via suppression of Toll-like receptor 4/NF-κB signalling. *Br J Nutr*. (2013) 110:599–608. doi: 10.1017/S0007114512005594
59. Jain A, Yadav A, Bozhkov AI, Padalko VI, Flora SJS. Therapeutic efficacy of silymarin and naringenin in reducing arsenic-induced hepatic damage in young rats. *Ecotoxicol Environ Saf*. (2011) 74:607–14. doi: 10.1016/j.ecoenv.2010.08.002
60. Assini JM, Mulvihill EE, Burke AC, Sutherland BG, Telford DE, Chhoker SS, et al. Naringenin prevents obesity, hepatic steatosis, and glucose intolerance in male mice independent of fibroblast growth factor 21. *Endocrinology*. (2015) 156:2087–92. doi: 10.1210/en.2014-2003
61. Qin L, Jin L, Lu L, Lu X, Zhang C, Zhang F, et al. Naringenin reduces lung metastasis in a breast cancer resection model. *Protein Cell*. (2011) 2:507–16. doi: 10.1007/s13238-011-1056-8
62. Zhang Y, Geng X, Tan Y, Li Q, Xu C, Xu J, et al. New understanding of the damage of SARS-CoV-2 infection outside the respiratory system. *Biomed Pharmacother*. (2020) 127:110195. doi: 10.1016/j.biopha.2020.110195
63. Smith AM, Rahman FZ, Hayee B, Graham SJ, Marks DJB, Sewell GW, et al. Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. *J Exp Med*. (2009) 206:1883–97. doi: 10.1084/jem.20091233
64. Zeng W, Jin L, Zhang F, Zhang C, Liang W. Naringenin as a potential immunomodulator in therapeutics. *Pharmacol Res*. (2018) 135:122–6. doi: 10.1016/j.phrs.2018.08.002
65. Jose RJ, Manuel A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. *Lancet Respir Med*. (2020) 8:e46–7. doi: 10.1016/S2213-2600(20)30216-2
66. Iwamura C, Shinoda K, Yoshimura M, Watanabe Y, Obata A, Nakayama T. Naringenin chalcone suppresses allergic asthma by inhibiting the type-2 function of CD4 T cells. *Allergol Int*. (2010) 59:67–73. doi: 10.2332/allergolint.09-OA-0118
67. Guihua X, Shuyin L, Jinliang G, Wang S. Naringin protects ovalbumin-induced airway inflammation in a mouse model of Asthma. *Inflammation*. (2016) 39:891–9. doi: 10.1007/s10753-016-0321-7
68. Shi Y, Dai J, Liu H, Li RR, Sun PL, Du Q, et al. Naringenin inhibits allergen-induced airway inflammation and airway responsiveness and inhibits NF-κB activity in a murine model of asthma. *Can J Physiol Pharmacol*. (2009) 87:729–35. doi: 10.1139/Y09-065
69. Du G, Jin L, Han X, Song Z, Zhang H, Liang W. Naringenin: a potential immunomodulator for inhibiting lung fibrosis and metastasis. *Cancer Res*. (2009) 69:3205–12. doi: 10.1158/0008-5472.CAN-08-3393
70. Lin Y, Tan D, Kan Q, Xiao Z, Jiang Z. The protective effect of naringenin on airway remodeling after mycoplasma pneumoniae infection by inhibiting autophagymediated lung inflammation and fibrosis. *Mediators Inflamm*. (2018) 2018:8753894. doi: 10.1155/2018/8753894
71. Liu J, Yao J, Zhang J. Naringenin attenuates inflammation in chronic obstructive pulmonary disease in cigarette smoke induced mouse model and involves suppression of NF-κB. *J Microbiol Biotechnol*. (2018):doi: 10.4014/jmb.1810.10061 [Epub ahead of print],
72. Zhang C, Zeng W, Yao Y, Xu B, Wei X, Wang L, et al. Naringenin ameliorates radiation-induced lung injury by lowering IL-1b levels. *J Pharmacol Exp Ther*. (2018) 366:341–8. doi: 10.1124/jpet.118.248807
73. Wang HK, Yeh CH, Iwamoto T, Satsu H, Shimizu M, Totsuka M. Dietary flavonoid naringenin induces regulatory T cells via an aryl hydrocarbon receptor mediated pathway. *J Agric Food Chem*. (2012) 60:2171–8. doi: 10.1021/jf204625y
74. Fast DJ, Stern NP, Chuang J, Li Y, Scholten JD, Hu C. Flavanones common to citrus fruits activate the interferon-stimulated response element by stimulating expression of IRF7. *J Food Bioact*. (2019) 8:58–65. doi: 10.31665/jfb.2019.8207
75. Kim JH, Lee JK. Naringenin enhances NK cell lysis activity by increasing the expression of NKG2D ligands on Burkitt's lymphoma cells. *Arch Pharm Res*. (2015) 38:2042–8. doi: 10.1007/s12272-015-0624-5
76. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med*. (2005) 11:875–9. doi: 10.1038/nm1267
77. Mahase E. Coronavirus covid-19 has killed more people than SARS and MERS combined, despite lower case fatality rate. *BMJ*. (2020) 368:m641. doi: 10.1136/bmj.m641

78. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. In: Maramorosch K, Shatkin AJ, Murphy FA editors. *Advances in Virus Research*. (Vol. 81), Cambridge, MA: Academic Press Inc (2011). p. 85–164. doi: 10.1016/B978-0-12-385885-6.00009-2
79. Chan JFW, Lau SKP, To KKW, Cheng VCC, Woo PCY, Yue KY. Middle east respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clin Microbiol Rev*. (2015) 28:465–522. doi: 10.1128/CMR.00102-14
80. De Wit E, Van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol*. (2016) 14:523–34. doi: 10.1038/nrmicro.2016.81
81. Saad M, Omrani AS, Baig K, Bahloul A, Elzein F, Matin MA, et al. Clinical aspects and outcomes of 70 patients with Middle East respiratory syndrome coronavirus infection: a single-center experience in Saudi Arabia. *Int J Infect Dis*. (2014) 29:301–6. doi: 10.1016/j.ijid.2014.09.003
82. Peiris JSM, Chu CM, Cheng VCC, Chan KS, Hung IFN, Poon LLM, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet*. (2003) 361:1767–72. doi: 10.1016/S0140-6736(03)13412-5
83. Rabaan AA, Al-Ahmed SH, Haque S, Sah R, Tiwari R, Malik YS, et al. SARS-CoV-2, SARS-CoV, and MERS-COV: a comparative overview. *Le Infez Med*. (2020) 28:174–84.
84. Rockx B, Kuiken T, Herfst S, Bestebroer T, Lamers MM, Oude Munnink BB, et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science*. (2020) 368:1012–5. doi: 10.1126/science.abb7314
85. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome composition and divergence of the novel Coronavirus (2019-nCoV) originating in China. *Cell Host Microbe*. (2020) 27:325–8. doi: 10.1016/j.chom.2020.02.001
86. Widagdo W, Raj VS, Schipper D, Kolijn K, van Leenders GJLH, Bosch BJ, et al. Differential expression of the middle east respiratory syndrome coronavirus receptor in the upper respiratory tracts of humans and dromedary camels. *J Virol*. (2016) 90:4838–42. doi: 10.1128/jvi.02994-15
87. Anand K, Ziebuhr J, Wadhwani P, Mesters JR, Hilgenfeld R. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. *Science*. (2003) 300:1763–7. doi: 10.1126/science.1085658
88. ul Qamar MT, Alqahtani SM, Alamri MA, Chen L-L. Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. *J Pharm Anal*. (2020) 10:313–9. doi: 10.1016/j.jpha.2020.03.009
89. Jo S, Kim H, Kim S, Shin DH, Kim MS. Characteristics of flavonoids as potent MERS-CoV 3C-like protease inhibitors. *Chem Biol Drug Des*. (2019) 94:2023–30. doi: 10.1111/cbdd.13604
90. Jo S, Kim S, Shin DH, Kim MS. Inhibition of SARS-CoV 3CL protease by flavonoids. *J Enzyme Inhib Med Chem*. (2020) 35:145–51. doi: 10.1080/14756366.2019.1690480
91. Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S. Potential inhibitor of COVID-19 main protease (M pro) from several medicinal plant compounds by molecular docking study. *Preprints*. (2020):doi: 10.20944/preprints202003.0226.v1
92. Shen D, Wang X, Xu H. Pairing phosphoinositides with calcium ions in endolysosomal dynamics: phosphoinositides control the direction and specificity of membrane trafficking by regulating the activity of calcium channels in the endolysosomes. *BioEssays*. (2011) 33:448–57. doi: 10.1002/bies.201000152
93. Gunaratne GS, Yang Y, Li F, Walseth TF, Marchant JS. NAADP-dependent Ca2+ signaling regulates Middle East respiratory syndrome-coronavirus pseudovirus translocation through the endolysosomal system. *Cell Calcium*. (2018) 75:30–41. doi: 10.1016/j.ceca.2018.08.003
94. Burkard C, Verheije MH, Wicht O, van Kasteren SI, van Kuppeveld FJ, Haagmans BL, et al. coronavirus cell entry occurs through the Endo-/Lysosomal pathway in a proteolysis-dependent manner. *PLoS Pathog*. (2014) 10:e1004502. doi: 10.1371/journal.ppat.1004502
95. Grimm C, Tang R. Could an endo-lysosomal ion channel be the Achilles heel of SARS-CoV2? *Cell Calcium*. (2020) 88:102212. doi: 10.1016/j.ceca.2020.102212
96. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol*. (2020) 20:363–74. doi: 10.1038/s41577-020-0311-8
97. Benkerrou D, Minicozzi V, Gradogna A, Milenkovic S, Bodrenko IV, Festa M, et al. A perspective on the modulation of plant and animal two pore channels (TPCs) by the flavonoid naringenin. *Biophys Chem*. (2019) 254:106246. doi: 10.1016/j.bpc.2019.106246
98. Recourt K, van Brussel AA, Driessen AJ, Lugtenberg BJ. Accumulation of a nod gene inducer, the flavonoid naringenin, in the cytoplasmic membrane of Rhizobium leguminosarum biovar viciae is caused by the pH-dependent hydrophobicity of naringenin. *J Bacteriol*. (1989) 171:4370–7. doi: 10.1128/jb.171.8.4370-4377.1989
99. Chen H, Du Q. Potential natural compounds for preventing SARS-CoV-2 (2019-nCoV) infection. *Preprints*. (2020):doi: 10.20944/preprints202001.0358.v3
100. Wang Z, Wang S, Zhao J, Yu C, Hu Y, Tu Y, et al. Naringenin ameliorates renovascular hypertensive renal damage by normalizing the balance of renin-angiotensin system components in rats. *Int J Med Sci*. (2019) 16:644–53. doi: 10.7150/ijms.31075
101. Cheng L, Zheng W, Li M, Huang J, Bao S, Xu Q, et al. Citrus fruits are rich in flavonoids for immunoregulation and potential targeting ACE2. *Preprints*. (2020):doi: 10.20944/preprints202002.0313.v1
102. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. (2005) 436:112–6. doi: 10.1038/nature03712
103. Diaz JH. Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of severe COVID-19. *J Travel Med*. (2020) 27:1–2. doi: 10.1093/jtm/taaa041
104. Gattinoni L, Coppola S, Cressoni M, Busana M, Chiumello D. Covid-19 does not lead to a "Typical" acute respiratory distress syndrome. *Am J Respir Crit Care Med*. (2020) 201:1299–300. doi: 10.1164/rccm.202003-0817LE
105. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Semin Immunopathol*. (2017) 39:517–28. doi: 10.1007/s00281-017-0639-8
106. Ye Q, Wang B, Mao J. Cytokine storm in COVID-19 and treatment. *J Infect*. (2020) 80:607–13. doi: 10.1016/j.jinf.2020.03.037
107. Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy Eur J Allergy Clin Immunol*. (2020) 75:1730–41. doi: 10.1111/all.14238
108. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunologic features in severe and moderate forms of Coronavirus disease 2019. *medRxiv*[Preprint]. (2020). doi: 10.1101/2020.02.16.20023903
109. Mu L, Hu G, Liu J, Chen Y, Cui W, Qiao L. Protective effects of naringenin in a rat model of sepsis-triggered acute kidney injury via activation of antioxidant enzymes and reduction in urinary angiotensinogen. *Med Sci Monit*. (2019) 25:5986–91. doi: 10.12659/MSM.916400
110. Romaszko E, Marzec-Wróblewska U, Badura A, Buciński A. Does consumption of red grapefruit juice alter naringenin concentrations in milk produced by breastfeeding mothers? *PLoS One*. (2017) 12:e0185954. doi: 10.1371/journal.pone.0185954
111. Breinholt VM, Svendsen GW, Dragsted LO, Hossaini A. The citrus-derived flavonoid naringenin exerts uterotrophic effects in female mice at human relevant doses. *Basic Clin Pharmacol Toxicol*. (2008) 94:30–6. doi: 10.1111/j.1742-7843.2004.pto_940106.x
112. Kanaze FI, Bounartzi MI, Georgarakis M, Niopas I. Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. *Eur J Clin Nutr*. (2007) 61:472–7. doi: 10.1038/sj.ejcn.1602543
113. Habauzit V, Verny MA, Milenkovic D, Barber-Chamoux N, Mazur A, Dubray C, et al. Flavanones protect from arterial stiffness in postmenopausal women consuming grapefruit juice for 6 mo: a randomized, controlled, crossover trial. *Am J Clin Nutr*. (2015) 102:66–74. doi: 10.3945/ajcn.114.104646
114. Nielsen ILF, Chee WSS, Poulsen L, Offord-Cavin E, Rasmussen SE, Frederiksen H, et al. Bioavailability is improved by enzymatic modification

of the citrus flavonoid hesperidin in humans: a randomized, double-blind, crossover trial. *J Nutr*. (2006) 136:404–8. doi: 10.1093/jn/136.2.404

115. Wang Y, Wang S, Firempong CK, Zhang H, Wang M, Zhang Y, et al. Enhanced solubility and bioavailability of naringenin via liposomal nanoformulation: preparation and in vitro and in vivo evaluations. *AAPS PharmSciTech*. (2017) 18:586–94. doi: 10.1208/s12249-016-0537-8
116. Kumar RP, Abraham A. PVP- coated naringenin nanoparticles for biomedical applications – in vivo toxicological evaluations. *Chem Biol Interact*. (2016) 257:110–8. doi: 10.1016/j.cbi.2016.07.012
117. Khan AW, Kotta S, Ansari SH, Sharma RK, Ali J. Self-nanoemulsifying drug delivery system (SNEDDS) of the poorly water-soluble grapefruit flavonoid Naringenin: design, characterization, in vitro and in vivo evaluation. *Drug Deliv*. (2015) 22:552–61. doi: 10.3109/10717544.2013.878003
118. Gera S, Talluri S, Rangaraj N, Sampathi S. Formulation and Evaluation of Naringenin Nanosuspensions for Bioavailability Enhancement. *AAPS PharmSciTech*. (2017) 18:3151–62. doi: 10.1208/s12249-017-0790-5
119. Lu WJ, Ferlito V, Xu C, Flockhart DA, Caccamese S. Enantiomers of naringenin as pleiotropic, stereoselective inhibitors of cytochrome P450 isoforms. *Chirality*. (2011) 23:891–6. doi: 10.1002/chir.21005
120. Bourian M, Runkel M, Krisp A, Tegtmeier M, Freudenstein J, Legrum W. Naringenin and interindividual variability in interaction of coumarin with grapefruit juice. *Exp. Toxicol. Pathol*. (1999) 51:289–93. doi: 10.1016/S0940-2993(99)80008-6
121. Fuhr U, Kummert AL. The fate of naringin in humans: a key to grapefruit juice-drug interactions? *Clin Pharmacol Ther*. (1995) 58:365–73. doi: 10.1016/0009-9236(95)90048-9

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Alberca, Teixeira, Beserra, de Oliveira, Andrade, Pietrobon and Sato. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

MINI REVIEW
published: 25 September 2020
doi: 10.3389/fnut.2020.583080

Thrombosis and COVID-19: The Potential Role of Nutrition

Alexandros Tsoupras[1,2,3], *Ronan Lordan*[3,4*] *and Ioannis Zabetakis*[1,3]

[1] Department of Biological Sciences, University of Limerick, Limerick, Ireland, [2] Bernal Institute, University of Limerick, Limerick, Ireland, [3] Health Research Institute, University of Limerick, Limerick, Ireland, [4] Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the coronavirus disease (COVID-19), is a contagion that has rapidly spread around the globe. COVID-19 has caused significant loss of life and disrupted global society at a level never before encountered. While the disease was predominantly characterized by respiratory symptoms initially, it became clear that other systems including the cardiovascular and neurological systems were also involved. Several thrombotic complications were reported including venous thrombosis, vasculitis, cardiomyopathy, and stroke. Thrombosis and inflammation are implicated in various non-communicable diseases (NCDs). This is of significant concern as people with pre-existing conditions such as cardiovascular disorders, renal disorders, obesity, metabolic syndrome, and diabetes are at greater risk of severe COVID-19 infection. Consequently, the research surrounding the use of anticoagulants, antiplatelet, and antithrombotic strategies for prophylaxis and treatment of COVID-19 is of critical importance. The adoption of a healthy diet, physical exercise, and lifestyle choices can reduce the risk factors associated with NCDs and the thrombo-inflammatory complications. In this review, these thrombotic complications and potential foods, nutraceuticals, and the antithrombotic constituents within that may prevent the onset of severe thrombotic complications as a result of infection are discussed. While nutrition is not a panacea to tackle COVID-19, it is apparent that a patient's nutritional status may affect patient outcomes. Further intensive research is warranted to reduce to incidence of thrombotic complications.

Keywords: nutrition, SARS-CoV-2, COVID-19, thrombosis, antithrombotic

OPEN ACCESS

Edited by:
Bisheng Zhou,
University of Illinois at Chicago,
United States

Reviewed by:
Angelica Sofia Martinez Ramirez,
Northwestern University, United States
David Escobar,
University of Toledo, United States
Jing Du,
Northwestern University, United States

***Correspondence:**
Ronan Lordan
ronan.lordan@ul.ie

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Nutrition

Received: *14 July 2020*
Accepted: *21 August 2020*
Published: *25 September 2020*

Citation:
Tsoupras A, Lordan R and Zabetakis I (2020) Thrombosis and COVID-19: The Potential Role of Nutrition. Front. Nutr. 7:583080. doi: 10.3389/fnut.2020.583080

INTRODUCTION

Since December 2019, the coronavirus disease (COVID-19) has rapidly spread worldwide becoming a pandemic as declared by the World Health Organization (WHO). Over 20 million cases of COVID-19 have been reported, which is responsible for 738,000 deaths globally as of August 11th (1). COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is an enveloped positive-sense single-stranded RNA virus infecting cells of the respiratory system *via* the angiotensin-converting enzyme 2 (ACE2) receptor, when the spike protein is primed by transmembrane protease serine 2 protein (TMPRSS2) (2). SARS-CoV-2 entry into host cells is followed by a subsequent response of the immune system, which includes inflammation-related manifestations leading to disproportionate endothelial damage and dysfunction, dysregulation of perfusion, and a loss of hypoxic vasoconstriction (3). The lungs seem to be the initial target organ of SARS-CoV-2, whereby patients develop acute lung injury that can progress to respiratory failure. Symptoms present as upper and lower respiratory tract infections that in severe cases

is accompanied with inflammatory complications that are attributed to a cytokine storm and hyperinflammation (4).

The cytokine storm can result in acute respiratory distress syndrome (ARDS) and potentially multiorgan failure (5, 6). Infection of the respiratory epithelium is the portal of entry, whereby alveolar damage may be mediated by endothelial injury, resulting in the release of cytokines and chemokines, the recruitment of immune cells, and the activation of coagulation and thrombosis (6–11). The intense cytokine storm is also implicated in the virus-triggered pulmonary tissue damage, functional impairment, reduced lung capacity, and respiratory failure in severe COVID-19 cases. SARS-CoV-2 may extend to other organs as documented by reports that patients with severe COVID-19 often develop neurological issues and multiorgan damage including cardiac injury and acute kidney injury (6, 7, 12). Indeed, it is now known that other complications such as hypercoagulability, venous thrombosis, systemic vasculitis, and stroke are associated with increased mortality in COVID-19 patients (12, 13). Therefore, identifying, repurposing or designing therapeutics and preventative strategies to reduce the risk of thrombotic complications in COVID-19 is of significant importance.

Currently, there are no known effective prophylactic treatments for COVID-19 and only limited effectiveness reported for various therapeutics such as Remdesivir, which reduces the time to recovery in hospitalized COVID-19 patients (14). Press releases and preprints occasionally have shed light on effective treatments such as dexamethasone (15), but many other potential therapeutics that have come to light have yet to be peer reviewed and require much further study. Likewise, the effectiveness of antithrombotic and anticoagulant treatments is still under investigation. In this mini review, the evidence surrounding thrombosis and how nutrition may play a role in the primordial prevention of the development of thrombotic complications in SARS-CoV-2 infected patients is discussed. Several nutrients that exhibit antiplatelet activity are summarized and future directions of nutrition research as a strategy to reduce thrombotic complication and severe infection of COVID-19 are suggested.

SARS-CoV-2 AND THROMBOTIC COMPLICATIONS

Non-communicable diseases (NCDs) seem to be associated with higher incidences of severe COVID-19 infections (7, 16, 17). This is due to the presence of comorbidities and risk factors amongst these subpopulations who may be obese, elderly, or immunocompromised (7, 18). Many NCDs are associated with thrombotic complications such as cardiovascular diseases (CVD), acute coronary syndromes, ischemic stroke, deep vein thrombosis, venous thromboembolism, and pulmonary embolism (19, 20). While thrombosis is a complication of COVID-19 (13), the presence of NCDs such as diabetes or obesity may further increase the risk of thrombotic complications in severely ill patients due to their associations with a prothrombotic state (21). Generally, the hemostatic system that is comprised of coagulation, platelet aggregation, and fibrinolysis, is a host defense mechanism that maintains the integrity of the circulatory system following vascular damage (19, 22). The coagulatory and inflammatory processes are highly regulated and are crucial to the host defense systems for limiting tissue injury, removing pathogens, and re-establishing homeostasis (10, 22).

Pulmonary embolism and thrombosis are now known to contribute to adverse events and increased mortality in critically ill COVID-19 patients due hypercoagulability and a prothrombotic state that can lead to coagulopathy and disseminated intravascular coagulation (11). Therefore, widespread pulmonary and endothelial inflammation and enhanced thrombosis are integral features of COVID-19 pathology. This state of hypercoagulation contributes to a fatal deterioration of the ventilation/perfusion ratio (V/Q), which is a measure of the concentration of air reaching the alveoli per minute to the amount of blood reaching the alveoli per minute *via* the capillaries (8). This is of clinical importance as hypoxia and sepsis can independently promote platelet aggregation mediated by the release of von Willebrand factor, which is increased in the whole blood of intensive care unit (ICU) patients with COVID-19 (8, 23). However, further study is required as only a limited number of patients were enrolled in this study. The substantial coagulation activation in severe COVID-19 infection is likely related to the sustained inflammatory response due to the intense cytokine release induced by virus invasion. Increased levels of D-dimers, a prolongation of prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombocytopenia is representative of disease severity and have been correlated with poor prognosis and increased mortality in COVID-19 patients (11, 24). However, whether the hemostatic changes observed are specific to COVID-19 or are a reflection of the cytokine storm and systemic inflammation is still subject to debate. Nevertheless, these changes in hemostatic factors seems to resemble that of inflammatory markers including IL-6, which potentially indicates a severe inflammatory response accompanied by a secondary hypercoagulable state (10, 24). Indeed, the loss of normal antiplatelet and anti-inflammatory functions of endothelial cells also leads to dysregulation of platelet activation, coagulation, and leukocyte recruitment in the microvasculature, with complement activation possibly playing an important role in the context of COVID-19-associated pneumonitis and purpuric skin lesions (6, 24).

Proteomic and metabolomic characterization of COVID-19 patient sera compared to control groups revealed molecular changes in COVID-19 patients demonstrating dysregulation of macrophages, evidence of platelet degranulation, the involvement of complement system pathways, and significant metabolic suppression, which may be useful as potential blood biomarkers for disease severity evaluation (25). Vascular inflammation and viral endotheliitis are frequent in severe COVID-19 patients as a result of endothelial dysfunction. Large increases of von Willebrand factor (VWF) and factor VIII activity has also been observed in COVID-19, which is also attributed to endothelial damage (26, 27). Notably, even in the absence of severe disease, patients with COVID-19 may be at heightened risk of thrombus formation leading to stroke due to

viral interaction with the endothelium (28). It was reported in New York City that some patients experiencing mild symptoms presented with stroke and large vessel thrombosis without occlusion. Whereas, severely infected patients presented with venous thrombosis and microangiopathy (28, 29).

In general, viral infections can activate monocytes, tissue macrophages, and endothelial cells, thus triggering the production of proinflammatory cytokines and activation of the coagulation cascade. Inflammation and coagulation synchronously respond to invading pathogens as part of the host's defense system, which includes complex processes referred to as thrombo-inflammation or immunothrombosis. In this capacity platelets can become activated following antigen recognition. Activated platelets can directly gather and inactivate pathogens and/or facilitate the clearance of pathogenic microorganisms by promoting the formation of neutrophil extracellular traps (NETs), activating neutrophils and macrophages, and facilitating the formation of platelet aggregates and microthrombi (30), all of which contribute to the crosstalk between the inflammation and coagulation pathways (7).

The complement system is part of the innate immune system and represents one of the first responses of the host immune system to SARS-CoV-2 infection *via* activation of multiple pathways (31). As a result of complement activation, there is significant platelet activation, thrombus formation, endothelial dysfunction, and intravascular coagulation, that can culminate in multiorgan failure and death in severe COVID-19 infection (6). It is hypothesized that C5a and C5b-9 that are generated by cells infected with SARS-CoV-2 may be key mediators of COVID-19-associated endothelial dysfunction and platelet activation. Activation of these cells may lead to the exocytosis of P-selectin and von Willebrand factor multimers and the expression of tissue factor and adhesion molecules from endothelial cells, and the release of chemokines and platelet-activating factor (PAF), which together promote inflammation, increase vascular permeability, and trigger the coagulation process (6, 32). C5b−9 can also be a powerful platelet agonist by inducing the secretion of storage granules and the release of procoagulant platelet microparticles leading to vascular injury and dysfunction followed by the formation of significant blood clots (6, 32).

Overall, COVID-19 patients have a higher risk for thromboembolic complications and a higher frequency is observed in severely ill patients, which can lead to significant damage (33). As a result, thrombotic complications are a potential target for the reduction of disease severity.

MITIGATION OF COVID-19 THROMBOTIC COMPLICATIONS

To avoid thrombotic complications, many have recommended prophylactic antithrombotic pharmacological therapies for COVID-19 patients, along with particular dosing instructions (34–37). However, to date, little research has been conducted and the optimal doses of anticoagulants or antiplatelet agents has yet to be determined. Indeed, data for prospective efficacy and safety of existing antiplatelet therapies to treat or prevent severe symptoms of COVID-19 remains elusive. Despite that, some institutes have adopted pharmacological thromboprophylactic strategies (38). These include the use of intermediate or full-dose preemptive anticoagulation therapy rather than prophylactic dosing for routine care of COVID-19 patients (13). Considering some studies have observed high bleeding rates as a result of standard anticoagulation treatment in COVID-19 patients, randomized trials are required to determine any potential benefit of intensified anticoagulant prophylaxis in COVID-19 patients (39).

One potential therapeutic avenue has shown that antiplatelet treatments can improve hypoxemia in severe COVID-19 patients with hypercoagulability by affecting the ventilation/perfusion ratio in patients with severe respiratory failure (8). However, antiplatelet and anticoagulant therapies administered pre-admission to hospital does not seem to protect with ARDS at presentation who are at significant risk of death (35). Pharmacological agents targeting thrombo-inflammation in COVID-19, including antiplatelet compounds, along with proposed best practices and clinical guidance, has been extensively reviewed elsewhere (34, 36, 37). In brief, current guidelines proposed by the International Society of Thrombosis and Hemostasis recommend low-molecular weight heparin for both mild and severely infected patients who required hospitalization (40). However, further guidance for the utilization of antithrombotic and antiplatelet therapies in patients with known or suspected COVID-19 are necessary, particularly for patients with comorbidities (36).

FOODS AND NUTRIENTS WITH ANTITHROMBOTIC PROPERTIES

Diet and lifestyle are modifiable risk factors that can have a significant impact on an individual's likelihood to develop an NCD (41, 42) and their susceptibility to developing infections (7). It has previously been described how inflammation and thrombosis are implicated in the onset and progression of NCDs (19, 41). However, it has become apparent that one's nutritional status is an important factor for priming the immune system to tackle acute infections such as COVID-19 (7, 43–45). Adoption of healthy dietary habits will prevent the onset of NCDs, which is a significant risk factor for the development of COVID-19, and may provide support to the immune system to lessen the severity of an infection (7). A healthy diet in compliance with current nutritional recommendations can lead to less societal and economic burden to health systems and economies (7, 46). Therefore, promotion of a healthy diet and lifestyle among the general population characterized by anti-inflammatory and antithrombotic properties may potentially benefit or prevent the thrombo-inflammatory manifestations of patients with NCDs and comorbidities that can significantly impact the outcomes of COVID-19 patients (7, 36, 47).

Healthy dietary patterns such as the Mediterranean diet or the DASH diet (Dietary approaches to reduce hypertension) are characterized by high intakes of fruit and vegetables, whole grains, fermented foods, moderate intake of fish, dairy, and low

intake of processed foods (48, 49). Indeed, health outcomes in relation to adherence to the Mediterranean diet during the COVID-19 pandemic is currently under investigation (ClinicalTrials.gov: NCT04449731). Notably, most national dietary guidelines support these same nutritional patterns, whereby high fruit, vegetable, nuts, dairy, and fish intake is promoted (48). These foods are rich in bioactive compounds with potential antithrombotic and anti-inflammatory properties as highlighted in **Table 1** (41, 60, 82–85). These include phytochemicals such as phenolic compounds, carotenes, alkaloids, terpenes, peptides, and bioactive lipid molecules. The latter group of molecules includes monounsaturated fatty acids (MUFA; e.g., oleic acid), omega-3 polyunsaturated fatty acids (e.g., n-3 PUFA; alpha linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid), lipid soluble vitamins (vitamin D and vitamin E), and bioactive polar lipids (e.g., phospholipids, sphingolipids, and glycolipids) (48). Many of these compounds exhibit potent inhibition or modulation of several signaling pathways of key pro-inflammatory and prothrombotic mediators such as platelet-activating factor (PAF), thrombin, collagen, ADP, arachidonic acid, and related eicosanoids (41, 50, 60).

Dietary polar lipids found in a variety of foods (**Table 1**) are of particular interest as they exhibit potent *in vitro* and *in vivo* anti-PAF effects, which can reduce PAF-induced activation platelets and other cells including leukocytes and endothelial cells. Indeed, there is also evidence that polar lipids can reduce the activities of PAF anabolic enzymes and in increase the levels of PAF catabolic enzymes, which leads to a reduction in the overall levels of PAF. Along with modulating PAF metabolism, these molecules may also reduce the oxidation of plasma lipoproteins, thus avoiding a PAF-related cascade of synthesis and expression of other chemokines, cytokines, and

TABLE 1 | A selection of some of the main food-derived bioactive constituents that can affect platelet activity that may be of value to prevent thrombotic complications in COVID-19.

Antithrombotic constituents	Main dietary sources	Some of the documented antiplatelet effects	Further reading
Bioactive lipids compounds			
- Polar Lipids	- Fish and seafood - Dairy - Olives and olive oil - Tea - Meat - Wine and fermented beverages	- Reduction of PAF-induced platelet aggregation *in vitro*, *in vivo*, and *ex vivo* - Potential anti-inflammatory effects - Affects PAF metabolism *in vitro*, *in vivo*, and *ex vivo* - Reduction of thrombin, ADP, and collagen-induced platelet aggregation *in vitro*	(41, 50–59)
- n-3 PUFA	- Fish and seafood - Nuts and seeds - Plant oils	- Reduced thrombin formation - Reduced oxidative stress - Reduced Lp-PLA_2 - Anti-inflammatory - Immunomodulatory	(60–65)
- Vitamin E	- Plant-derived oils (e.g., olive oil), nuts, and seeds	- Reduction of PAF, ADP, thrombin, and collagen-induced platelet aggregation in PRP and whole blood *in vitro* and *ex vivo* - Reduction of PAF and prostaglandin synthesis	(66–69)
- Vitamin D	- Dairy - Oily fish - Meat	- Reduction of PAF platelet aggregation and metabolism *in vitro* and *in vivo* - Anti-inflammatory - Low vitamin D associated with higher platelet reactivity in diabetics	(70, 71)
Bioactive Peptides	- Fish and seafood - Dairy - Seaweeds - Cereal grains	- Inhibition of COX-1 - Inhibition of type-III collagen-induced aggregation - Binding to glycoprotein IIb/IIIa - Interactions with fibrinogen and its receptor. - Reduction of thrombin formation - Reduction of ADP-induced platelet aggregation - Anti-inflammatory	(72–75)
Phytochemicals, vitamins, and phenolic compounds			
- Phytosterols - Phytostanols - Flavonoids - Isoflavonoids - Anthocyanins - Phenolipids - Alkaloids	- Fruit and berries - Vegetables - Seeds, nuts, and oils - Plant oils - Wine - Tea - Fermented beverages	- Reduction of platelet aggregation - Anti-inflammatory effects - Affects PAF metabolism *in vitro*, *in vivo*, and *ex vivo* - Reduction of tissue factor synthesis - Regulation of endothelial function - Inhibition of COX and LOX pathways	(41, 54, 76–81)

ADP, adenosine diphosphate, COX-1, cyclooxygenase-1; Lp-PLA_2, lipoprotein-associated phospholipase A_2; PAF, platelet-activating factor; PRP, platelet-rich plasma.

their respective receptors and pathways, which are involved in thrombosis, coagulation, and thrombo-inflammation (41, 51, 86, 87). For instance, nutritional trials involving the Mediterranean diet have been shown to reduce the risk for NCDs by reducing PAF activity in blood (88, 89). This is particularly interesting as early metabolomics data indicates that there is a significant shift in the glycerophospholipid composition of patient sera, which are related to platelet degranulation and may be related to PAF-like lipids (25). Other lipids such as n-3 PUFA are also known to exhibit antithrombotic, anti-inflammatory, and pro-resolving effects that may be beneficial against SARS-CoV-2 (7, 90). However, their bioavailability and efficacy is subject to debate (61). Further clinical trials are required to determine if n-3 PUFA and anti-PAF compounds have a functional consequence against thrombotic complication in acute infections.

Food-derived bioactive peptides are also functionally important as they exhibit antihypertensive, antioxidant, antithrombotic, anti-inflammatory properties, and immunomodulatory properties (72). Bioactive peptides found in protein hydrolysates generally contain 2–20 amino acid residues (91). They have a low molecular weight, high bioavailability, and their molecular structures allows them to readily interact with different proteins and their receptors (72). Bioactive peptides from foods such as dairy products, seafood, seaweeds, nuts, cereals, and other natural sources possess antithrombotic properties that may be useful as antiplatelet agents as they seem to interact with multiple platelet activation pathways. These include inhibition of cyclooxygenase-1 (COX-1), inhibition of type-III collagen-induced aggregation, binding to glycoprotein IIb/IIIa, interacting with fibrinogen and its receptor, and affecting thrombin (73, 74).

Other food constituents such as phenolic compounds found in fruit, vegetables, cereals, and fermented beverages such as wine and beer also beneficially affect platelet function as outlined in **Table 1**. Indeed, resveratrol is thought to be one of the main bioactive components of wine responsible for its antiplatelet effects (92, 93), which is currently under investigation as a prophylactic and therapeutic in COVID-19 patients (ClinicalTrials.gov: NCT04400890). Herbal phenolic compounds in conjunction with colchicine is also being investigated for potential anti-inflammatory effects in COVID-19 patients (ClinicalTrials.gov: NCT04392141). However, while many of the molecules described in this review are under investigation for their prophylactic and therapeutic properties against SARS-CoV-2, none of the outcomes measured relate to thrombosis yet.

Generally, only a limited number of food-derived compounds have been investigated for their antiplatelet effects clinically as of yet, whereby fish oils are synonymous. However, there has been some success in developing antiplatelet nutraceuticals. One such product is a water-soluble tomato-based nutraceutical given the trademarked name Fruitflow®. Fruitflow is now recognized as a functional product by the European Food Safety Authority (EFSA) with a recognized health claim that states that Fruitflow® "helps maintain normal platelet aggregation, which contributes to healthy blood flow" (94). A 3 g dose (containing 65 mg of antiplatelet components) of Fruitflow® recently demonstrated antiplatelet effects that were approximately one-third the effect of 75 mg of aspirin in a double-blinded randomized clinical trial of 47 healthy subjects (95). Nutraceuticals such as these may serve as a safe antiplatelet prophylactic treatment for those at high risk of COVID-19 who may also be at increased risk of thrombotic complications and an alternative to pharmacological compounds that may cause greater risk of bleeding (19).

In **Table 1**, there are several bioactive lipids, peptides, phytochemicals, and phenolic compounds that can affect platelet activity that may have the capacity to prevent the negative thrombotic outcomes of COVID-19. While these constituents are not the panacea to treat COVID-19, the adoption of a healthy diet characterized by many of these molecules along with a healthy lifestyle may help maintain hemostasis and prevent thrombotic complications. While research is on-going to find an effective pharmacological treatment for COVID-19, it is important to consider that the nutritional status of a patient may affect their health outcomes. Indeed, future COVID-19 studies must consider the effect of nutrition on factors relating to thrombosis and inflammation and how might nutrition play a role in the mitigation of potential vascular and thrombotic complications. Nutritional strategies and the use of nutraceuticals must be further investigated for their potential to prevent thrombotic complications in COVID-19 patients. Clinical trialists currently investigating some of the compounds mentioned in **Table 1** in conjunction with various therapies for other COVID-19 targets should consider the inclusion of measurable thrombosis-related parameters within their existing or planned trials. While there is no evidence that consuming nutritional supplements might protect anyone from becoming infected with COVID-19 or preventing its thrombotic complications, it is imperative that national health authorities promote a healthy diet and lifestyle to maintains one's nutritional status and wellbeing as the pandemic evolves.

CONCLUSIONS

It is evident that thrombotic complications are a significant risk factor for COVID-19 patients. Therefore, there is an urgent need to establish further clinical trials to investigate potential pharmacological and nutritional mitigation strategies to prevent thrombotic complications as a result of severe COVID-19 infection. It is also important to discern whether prophylactic antiplatelet therapies are appropriate in the case of initially mild infections or those with comorbidities and NCDs to prevent potential thrombosis leading to stroke or other major complications. Indeed, it is important that clinical trialists currently investigating some of the compounds mentioned in **Table 1** for other COVID-19 targets include measurable thrombosis-related parameters within their existing or planned trials. While, some institutes have endorsed guidelines for antithrombotic and antiplatelet strategies, these have yet to be verified as effective by clinical study. For those non-infected and especially for those with or at increased risk of underlying NCDs, the adoption of a healthy diet and lifestyle may prevent the onset of severe thrombotic complications due to the presence of bioactive compounds with antiplatelet effects. Coupling a healthy diet and lifestyle along with preventing infection by following

social distance guidelines, wearing a mask, and adopting good public hygiene with prevent the onset of severe infection.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

The authors acknowledge the Department of Biological Sciences for their continued support and Frontiers in Nutrition for supporting open Science during the pandemic. This review is intended for the scientific community.

REFERENCES

1. John Hopkins University. *John Hopkins University & Medicine: Coronavirus Resource Center*. (2020). Available online at: https://coronavirus.jhu.edu/map.html (accessed Aug 11, 2020).
2. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and Is blocked by a clinically proven protease inhibitor. *Cell.* (2020) 181:271–80.e8. doi: 10.1016/j.cell.2020.02.052
3. Bansal R, Gubbi S, Muniyappa R. Metabolic syndrome and COVID 19: Endocrine-immune-vascular interactions shapes clinical course. *Endocrinology*. (2020) 161:bqaa112. doi: 10.1210/endocr/bqaa112
4. Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, Xia Z. Review of the clinical characteristics of coronavirus disease 2019. (COVID-19). *J Gen Intern Med.* (2020) 35:1545–9. doi: 10.1007/s11606-020-05762-w
5. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine Growth Factor Rev*. (2020) 53:25–32. doi: 10.1016/j.cytogfr.2020.05.003
6. Noris M, Benigni A, Remuzzi G. The case of complement activation in COVID-19 multiorgan impact. *Kidney Int.* (2020) 98:314–22. doi: 10.1016/j.kint.2020.05.013
7. Zabetakis I, Lordan R, Norton C, Tsoupras A. COVID-19: The inflammation link and the role of nutrition in potential mitigation. *Nutrients*. (2020) 12:1466. doi: 10.3390/nu12051466
8. Viecca M, Radovanovic D, Forleo GB, Santus P. Enhanced platelet inhibition treatment improves hypoxemia in patients with severe Covid-19 and hypercoagulability. A case control, proof of concept study. *Pharmacol. Res.* (2020) 158:104950. doi: 10.1016/j.phrs.2020.104950
9. Al-Ani F, Chehade S, Lazo-Langner A. Thrombosis risk associated with COVID-19 infection. A scoping review. *Thromb. Res.* (2020) 192:152–60. doi: 10.1016/j.thromres.2020.05.039
10. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood.* (2020) 135:2033–40. doi: 10.1182/blood.2020006000
11. Linnemann B, Bauersachs R, Grebe M, Klamroth R, Müller O, Schellong S, et al. Venous thromboembolism in patients with COVID-19 (SARS-CoV-2 infection) - a position paper of the German society of angiology (DGA). *Vasa.* (2020) 49:259–63. doi: 10.1024/0301-1526/a000885
12. Avula A, Nalleballe K, Narula N, Sapozhnikov S, Dandu V, Toom S, et al. COVID-19 presenting as stroke. *Brain Behav Immun.* (2020) 87:115–9. doi: 10.1016/j.bbi.2020.04.077
13. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DA, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* (2020) 191:145–7. doi: 10.1016/j.thromres.2020.04.013
14. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of Covid-19 — preliminary report. *N Engl J Med*. (2020) NEJMoa2007764. doi: 10.1056/NEJMoa2007764
15. Horby, P, Lim, WS, Emberson, J, et al. Effect of dexamethasone in hospitalized patients with COVID-19: preliminary report. *medRxiv*. (2020) 10:22-0137273. doi: 10.1101/2020.06.22.20137273
16. Centers for Disease Control and Prevention. (2020). *Morbidity and Mortality Weekly Report (MMWR): Preliminary Estimates of the Prevalence of Selected Underlying Health Conditions Among Patients With Coronavirus Disease 2019.* - February 12-March 28, (2020). Available online at: https://www.cdc.gov/mmwr/volumes/69/wr/mm6913e2.htm?s_cid=mm6913e2_w (accessed July 3, 2020).
17. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. *Int J Infect Dis.* (2020) 94:91–5. doi: 10.1016/j.ijid.2020.03.017
18. Cena H, Chieppa M. Coronavirus disease (COVID-19–SARS-CoV-2) and nutrition: is infection in Italy suggesting a connection? *Front Immunol.* (2020) 11:944. doi: 10.3389/fimmu.2020.00944
19. Lordan R, Tsoupras A, Zabetakis I. Platelet activation and prothrombotic mediators at the nexus of inflammation and atherosclerosis: potential role of antiplatelet agents. *Blood Rev.* (2020) 2020:100694. doi: 10.1016/j.blre.2020.100694
20. Chen C, Yang FQ, Zhang Q, Wang FQ, Hu YJ, Xia ZN. Natural products for antithrombosis. *Evid Based Complement Alternat Med.* (2015) 2015:876426. doi: 10.1155/2015/876426
21. Apicella, M, Campopiano, MC, Mantuano, M, Mazoni, L, Coppelli, A, and Del Prato, S, COVID-19 in people with diabetes: understanding the reasons for worse outcomes. *The Lancet Diabetes & Endocrinology*. (2020). doi: 10.1016/S2213-8587(20)30238-2
22. Nicolai L, Massberg S. Platelets as key players in inflammation and infection. *Curr Opin Hematol.* (2020) 27:34–40. doi: 10.1097/MOH.0000000000000551
23. Yau JW, Teoh H, Verma S. Endothelial cell control of thrombosis. *BMC Cardiovasc Disord.* (2015) 15:130. doi: 10.1186/s12872-015-0124-z
24. Langer F, Kluge S, Klamroth R, Oldenburg J. Coagulopathy in COVID-19 and its implication for safe and efficacious thromboprophylaxis. *Hamostaseologie.* (2020) 40:264–9. doi: 10.1055/a-1178-3551
25. Shen B, Yi X, Sun Y, Bi X, Du J, Zhang C, et al. Proteomic and metabolomic characterization of COVID-19 patient sera. *Cell*. (2020) 182:59–72. doi: 10.1016/j.cell.2020.05.032
26. Marchandot B, Sattler L, Jesel L, Matsushita K, Schini-Kerth V, Grunebaum L, et al. COVID-19 related coagulopathy: a distinct entity? *J Clin Med.* (2020) 9:1651. doi: 10.3390/jcm9061651
27. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med.* (2020) 46:1089–98. doi: 10.1007/s00134-020-06062-x
28. Fara MG, Stein LK, Skliut M, Morgello S, Fifi JT, Dhamoon MS. Macrothrombosis and stroke in patients with mild Covid-19 infection. *J Thromb Haemost.* (2020) 18:2031–3. doi: 10.1111/jth.14938
29. Yaghi S, Ishida K, Torres J, Mac Grory B, Raz E, Humbert K, et al. SARS-CoV-2 and stroke in a New York healthcare system. *Stroke.* (2020) 51:2002–11. doi: 10.1161/STROKEAHA.120.030335
30. Guo L, Rondina MT. The era of thromboinflammation: platelets are dynamic sensors and effector cells during infectious diseases. *Front Immunol.* (2019) 10:2204. doi: 10.3389/fimmu.2019.02204
31. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. *J Med Virol.* (2020) 92:424–32. doi: 10.1002/jmv.25685
32. Fletcher-Sandersjöö A, Bellander BM. Is COVID-19 associated thrombosis caused by overactivation of the complement cascade? A literature review. *Thromb Res.* (2020) 19:436–41. doi: 10.1016/j.thromres.2020.06.027
33. Dolhnikoff M, Duarte-Neto AN, de Almeida Monteiro RA, da Silva LFF, de Oliveira EP, Saldiva PHN, et al. Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19. *J Thromb Haemost.* (2020) 18:1517–9. doi: 10.1111/jth.14844
34. Bikdeli B, Madhavan MV, Gupta A, Jimenez D, Burton JR, Der Nigoghossian C, et al. Pharmacological agents targeting thromboinflammation in COVID-19: review and implications for future research. *Thromb Haemost.* (2020) 120:1004–24. doi: 10.1055/s-0040-1713152

35. Russo V, Di Maio M, Attena E, Silverio A, Scudiero F, Celentani D, et al. Clinical impact of pre-admission antithrombotic therapy in hospitalized patients with COVID-19: a multicenter observational study. *Pharmacol Res.* (2020) 159:104965. doi: 10.1016/j.phrs.2020.104965
36. Watson RA, Johnson DM, Dharia RN, Merli GJ, Doherty JU. Anti-coagulant and anti-platelet therapy in the COVID-19 patient: a best practices quality initiative across a large health system. *Hosp Pract.* (2020) 1–11. doi: 10.1080/21548331.2020.1772639
37. Spyropoulos AC, Levy JH, Ageno W, Connors JM, Hunt BJ, Iba T, et al. Scientific and standardization committee communication: clinical guidance on the diagnosis, prevention and treatment of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost.* (2020) 18:14929. doi: 10.1111/jth.14929
38. Fraissé M, Logre E, Pajot O, Mentec H, Plantefève G, Contou D. Thrombotic and hemorrhagic events in critically ill COVID-19 patients: a French monocenter retrospective study. *Crit Care.* (2020) 24:275. doi: 10.1186/s13054-020-03025-y
39. Al-Samkari H, Karp Leaf RS, Dzik WH, Carlson JCT, Fogerty AE, Waheed A, et al. COVID and coagulation: bleeding and thrombotic manifestations of SARS-CoV2 infection. *Blood.* (2020) 136:489–500. doi: 10.1182/blood.2020006520
40. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost.* (2020) 18:1023–6. doi: 10.1111/jth.14810
41. Tsoupras A, Lordan R, Zabetakis, I. Inflammation, not cholesterol, is a cause of chronic disease. *Nutrients.* (2018) 10:604. doi: 10.3390/nu10050604
42. Yu E, Malik VS, Hu FB. Cardiovascular disease prevention by diet modification: JACC health promotion series. *J Am Coll Cardiol.* (2018) 72:914–26. doi: 10.1016/j.jacc.2018.02.085
43. Calder PC, Carr AC, Gombart AF, Eggersdorfer M.Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients.* (2020) 12:1181. doi: 10.3390/nu12041181
44. Briguglio M, Pregliasco FE, Lombardi G, Perazzo P, Banfi G. The malnutritional status of the host as a virulence factor for new coronavirus SARS-CoV-2. *Front Med.* (2020) 7:146. doi: 10.3389/fmed.2020.00146
45. Stachowska E, Folwarski M, Jamioł-Milc D, Maciejewska D, Skonieczna-Zydecka K. Nutritional support in coronavirus 2019 disease. *Medicina.* (2020) 56:289. doi: 10.3390/medicina56060289
46. Ebadi M, Montano-Loza AJ. Perspective: improving vitamin D status in the management of COVID-19. *Eur J Clin Nutr.* (2020) 74:856–9. doi: 10.1038/s41430-020-0661-0
47. Butler MJ, Barrientos RM. The impact of nutrition on COVID-19 susceptibility and long-term consequences. *Brain Behav Immun.* (2020) 87:53–4. doi: 10.1016/j.bbi.2020.04.040
48. Tierney A, Lordan R, Tsoupras A, Zabetakis I. Chapter 8 - Diet and cardiovascular disease: the Mediterranean diet. In Zabetakis I, Lordan R, and Tsoupras A, editors. *The Impact of Nutrition and Statins on Cardiovascular Diseases.* Cambridge, MA: Academic Press. (2019). p. 267–88. doi: 10.1016/B978-0-12-813792-5.00008-2
49. Sacks FM, Moore TJ, Appel LJ, Obarzanek E, Cutler JA, Vollmer WM, et al. A dietary approach to prevent hypertension: a review of the dietary approaches to stop hypertension (DASH) study. *Clin Cardiol.* (1999) 22:6–10. doi: 10.1002/clc.4960221503
50. Lordan R, Nasopoulou C, Tsoupras A, Zabetakis I. The anti-inflammatory properties of food polar lipids. In Mérillon JM, Ramawat KG, editors. *Bioactive Molecules in Food. Springer International Publishing.* Cham. (2018) p. 1–34. doi: 10.1007/978-3-319-54528-8_95-1
51. Lordan R, Tsoupras A, Zabetakis I. Phospholipids of animal and marine origin: structure, function, and anti-inflammatory properties. *Molecules.* (2017) 22:1964. doi: 10.3390/molecules22111964
52. Lordan R, O'Keeffe E, Dowling D, Mullally M, Heffernan H, Tsoupras A, et al. The *in vitro* antithrombotic properties of ale, lager, and stout beers. *Food Biosci.* (2019) 28:83–8. doi: 10.1016/j.fbio.2019.01.012
53. Tsoupras A, Lordan R, Harrington J, Pienaar R, Devaney K, Heaney S, et al. The effects of oxidation on the antithrombotic properties of tea lipids against PAF, thrombin, collagen, and ADP. *Foods.* (2020) 9:385. doi: 10.3390/foods9040385
54. Karantonis, HC. Antiatherogenic properties of olive oil glycolipids. In Kiritsakis A, and Shahidi F, editors. *Olives and Olive Oil as Functional Foods.* Oxford: John Wiley and Sons, Inc. (2017). doi: 10.1002/9781119135340.ch25
55. Xanthopoulou MN, Kalathara K, Melachroinou S, Arampatzi-Menenakou K, Antonopoulou S, Yannakoulia M, et al. Wine consumption reduced postprandial platelet sensitivity against platelet activating factor in healthy men. *Eur J Nutr.* (2017) 56:1485–92. doi: 10.1007/s00394-016-1194-0
56. Argyrou C, Vlachogianni I, Stamatakis G, Demopoulos CA, Antonopoulou S, Fragopoulou E. Postprandial effects of wine consumption on platelet-activating factor metabolic enzymes. *Prostaglandins Other Lipid Mediat.* (2017) 130:23–9. doi: 10.1016/j.prostaglandins.2017.03.002
57. Lordan R, Vidal NP, Huong Pham T, Tsoupras A, Thomas RH, Zabetakis I. Yoghurt fermentation alters the composition and antiplatelet properties of milk polar lipids. *Food Chem.* (2020) 332:127–384. doi: 10.1016/j.foodchem.2020.127384
58. Poutzalis S, Lordan R, Nasopoulou C, Zabetakis I. Phospholipids of goat and sheep origin: structural and functional studies. *Small Rumin Res.* (2018) 167:39–47. doi: 10.1016/j.smallrumres.2018.07.015
59. Lordan R, Vidal NP, Huong Pham T, Tsoupras A, Thomas RH, Zabetakis I. The effect of ovine milk fermentation on the antithrombotic properties of polar lipids. *J Funct Foods.* (2019) 54:289–300. doi: 10.1016/j.jff.2019.01.029
60. Olas B. Chapter Six - Biochemistry of blood platelet activation and the beneficial role of plant oils in cardiovascular diseases. In Makowski GS, editor. *Advances in Clinical Chemsitry.* Cambridge, MA: Elsevier (2020). p. 219–43. doi: 10.1016/bs.acc.2019.08.006
61. Lordan R, Redfern S, Tsoupras A, Zabetakis I. Inflammation and cardiovascular disease: are marine phospholipids the answer? *Food Funct.* (2020) 11:2861–85. doi: 10.1039/C9FO01742A
62. Gajos G, Zalewski J, Mostowik M, Konduracka E, Nessler J, Undas A. Polyunsaturated omega-3 fatty acids reduce lipoprotein-associated phospholipase A2 in patients with stable angina. *Nutr Metab Cardiovasc Dis.* (2014) 24:434–9. doi: 10.1016/j.numecd.2013.09.011
63. Gajos G, Zalewski J, Rostoff P, Nessler J, Piwowarska W, Undas A. Reduced thrombin formation and altered fibrin clot properties induced by polyunsaturated omega-3 fatty acids on top of dual antiplatelet therapy in patients undergoing percutaneous coronary intervention (OMEGA-PCI Clot). *Arterioscler Thromb Vasc Biol.* (2011) 31:1696–702. doi: 10.1161/ATVBAHA.111.228593
64. Gajos G, Rostoff P, Undas A, Piwowarska W. Effects of polyunsaturated omega-3 fatty acids on responsiveness to dual antiplatelet therapy in patients undergoing percutaneous coronary intervention: the OMEGA-PCI (OMEGA-3 fatty acids after PCI to modify responsiveness to dual antiplatelet therapy) study. *J Am Coll Cardiol.* (2010) 55:1671–8. doi: 10.1016/j.jacc.2009.11.080
65. Gao LG, Cao J, Mao QX, Lu XC, Zhou XL, Fan L. Influence of omega-3 polyunsaturated fatty acid-supplementation on platelet aggregation in humans: a meta-analysis of randomized controlled trials. *Atherosclerosis.* (2013) 226:328–34. doi: 10.1016/j.atherosclerosis.2012.10.056
66. Qureshi AA, Karpen CW, Qureshi N, Papasian CJ, Morrison DC, Folts JD. Tocotrienols-induced inhibition of platelet thrombus formation and platelet aggregation in stenosed canine coronary arteries. *Lipids Health Dis.* (2011) 10:58. doi: 10.1186/1476-511X-10-58
67. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The role of vitamin E in human health and some diseases. *Sultan Qaboos Univ Med J.* (2014) 14:e157–65.
68. Violi F, Pratico D, Ghiselli A, Alessandri C, Iuliano L, Cordova C, et al. Inhibition of cyclooxygenase-independent platelet aggregation by low vitamin E concentration. *Atherosclerosis.* (1990) 82:247–52. doi: 10.1016/0021-9150(90)90046-L
69. Kakishita E, Suehiro A, Oura Y, Nagai K. Inhibitory effect of vitamin E (alpha-tocopherol) on spontaneous platelet aggregation in whole blood. *Thromb Res.* (1990) 60:489–99. doi: 10.1016/0049-3848(90)90233-3
70. Verdoia M, Pergolini P, Nardin M, Rolla R, Negro F, Kedhi E, et al. Vitamin D levels and platelet reactivity in diabetic patients receiving dual antiplatelet therapy. *Vasc Pharmacol.* (2019) 120:106564. doi: 10.1016/j.vph.2019.106564

71. Verouti SN, Tsoupras AB, Alevizopoulou F, Demopoulos CA, Iatrou C. Paricalcitol effects on activities and metabolism of platelet activating factor and on inflammatory cytokines in hemodialysis patients. *Int J Artif Organs.* (2013) 36:87–96. doi: 10.5301/ijao.5000187
72. Martínez-Sánchez SM, Gabaldón-Hernández JA, Montoro-García S. Unravelling the molecular mechanisms associated with the role of food-derived bioactive peptides in promoting cardiovascular health. *J Funct Foods.* (2020) 64:103645. doi: 10.1016/j.jff.2019.103645
73. Rengasamy KR, Khan H, Ahmad I, Lobine D, Mahomoodally F, Suroowan S, et al. Bioactive peptides and proteins as alternative antiplatelet drugs. *Med Res Rev.* (2019) 39:2153–71. doi: 10.1002/med.21579
74. Cheng S, Tu M, Liu H, Zhao G, Du M. Food-derived antithrombotic peptides: preparation, identification, and interactions with thrombin. *Crit Rev Food Sci Nutr.* (2019) 59:S81–95. doi: 10.1080/10408398.2018.1524363
75. Erdmann K, Cheung BW, Schröder H. The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. *J Nutr Biochem.* (2008) 19:643–54. doi: 10.1016/j.jnutbio.2007.11.010
76. Violi F, Pignatelli P, Basili S. Nutrition, supplements, and vitamins in platelet function and bleeding. *Circulation.* (2010) 121:1033–44. doi: 10.1161/CIRCULATIONAHA.109.880211
77. Olas B. Dietary supplements with antiplatelet activity: a solution for everyone? *Adv Nutr.* (2018) 9:51–7. doi: 10.1093/advances/nmx014
78. Nile SH, Park SW. Edible berries: bioactive components and their effect on human health. *Nutrition.* (2014) 30:134–44. doi: 10.1016/j.nut.2013.04.007
79. Carrieri C, Milella RA, Incampo F, Crupi P, Antonacci D, Semeraro N, et al. Antithrombotic activity of 12 table grape varieties. Relationship with polyphenolic profile. *Food Chem.* (2013) 140:647–53. doi: 10.1016/j.foodchem.2012.10.132
80. Agrawal, A. Pharmacological activities of flavonoids: a review. *Int J Pharm Sci Nanotechnol.* (2011) 4:1394–8. doi: 10.37285/ijpsn.2011.4.2.3
81. de Almeida Chaves, DS, de Melo, GO, Corrêa, MFP. Chapter 1 - a review of recent patents regarding antithrombotic drugs derived from natural products. In Atta ur R, editor. *Studies in Natural Products Chemistry.* Cambridge, MA: Elsevier (2019). p. 1–47. doi: 10.1016/B978-0-444-64183-0.00001-4
82. Most MM. Estimated phytochemical content of the dietary approaches to stop hypertension (DASH) diet is higher than in the control study diet. *J Am Diet Assoc.* (2004) 104:1725–7. doi: 10.1016/j.jada.2004.08.001
83. Saura-Calixto F, Goñi I. Definition of the Mediterranean diet based on bioactive compounds. *Crit Rev Food Sci Nutr.* (2009) 49:145–52. doi: 10.1080/10408390701764732
84. Maruca A, Catalano R, Bagetta D, Mesiti F, Ambrosio FA, Romeo I, et al. The Mediterranean diet as source of bioactive compounds with multi-targeting anti-cancer profile. *Eur J Med Chem.* (2019) 18:1111579. doi: 10.1016/j.ejmech.2019.111579
85. Lordan R, Zabetakis I. Invited review: the anti-inflammatory properties of dairy lipids. *J Dairy Sci.* (2017) 100:4197–212. doi: 10.3168/jds.2016-12224
86. Lordan R, Tsoupras A, Zabetakis I, Demopoulos CA. Forty years since the structural elucidation of platelet-activating factor (PAF): historical, current, and future research perspectives. *Molecules.* (2019) 24:4414. doi: 10.3390/molecules24234414
87. Tsoupras AB, Iatrou C, Frangia C, Demopoulos CA. The implication of platelet-activating factor in cancer growth and metastasis: potent beneficial role of PAF-inhibitors and antioxidants. *Infect Dis Drug Targets.* (2009) 9:390–9. doi: 10.2174/187152609788922555
88. Antonopoulou S, Fragopoulou E, Karantonis HC, Mitsou E, Sitara M, Rementzis J, et al. Effect of traditional Greek Mediterranean meals on platelet aggregation in normal subjects and in patients with type 2 diabetes mellitus. *J Med Food.* (2006) 9:356–62. doi: 10.1089/jmf.2006.9.356
89. Karantonis HC, Fragopoulou E, Antonopoulou S, Rementzis J, Phenekos C, Demopoulos CA. Effect of fast-food Mediterranean-type diet on type 2 diabetics and healthy human subjects' platelet aggregation. *Diabetes Res Clin Pract.* (2006) 72:33–41. doi: 10.1016/j.diabres.2005.09.003
90. Torrinhas RS, Calder PC, Lemos GO, Waitzberg DL. Parenteral fish oil, an adjuvant pharmacotherapy for COVID-19? *Nutrition.* (2020) 81:110900. doi: 10.1016/j.nut.2020.110900
91. Toldrá F, Reig M, Aristoy MC, Mora L. Generation of bioactive peptides during food processing. *Food Chem.* (2018) 267:395–404. doi: 10.1016/j.foodchem.2017.06.119
92. Shen MY, Hsiao G, Liu CL, Fong TH, Lin KH, Chou DS, et al. Inhibitory mechanisms of resveratrol in platelet activation: pivotal roles of p38 MAPK and NO/cyclic GMP. *Br J Haematol.* (2007) 139:475–85. doi: 10.1111/j.1365-2141.2007.06788.x
93. Olas B, Wachowicz B. Resveratrol, a phenolic antioxidant with effects on blood platelet functions. *Platelets.* (2005) 16:251–60. doi: 10.1080/09537100400020591
94. O'Kennedy N, Raederstorff D, Duttaroy AK. Fruitflow®: the first european food safety authority-approved natural cardio-protective functional ingredient. *Eur J Nutr.* (2017) 56:461–82. doi: 10.1007/s00394-016-1265-2
95. O' Kennedy N, Crosbie L, Song HJ, Zhang X, Horgan G, et al. A randomised controlled trial comparing a dietary antiplatelet, the water-soluble tomato extract Fruitflow, with 75 mg aspirin in healthy subjects. *Eur J Clin Nutr.* (2017) 71:723–30. doi: 10.1038/ejcn.2016.222

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Tsoupras, Lordan and Zabetakis. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

REVIEW
published: 07 October 2020
doi: 10.3389/fimmu.2020.570122

Immune-Boosting, Antioxidant and Anti-inflammatory Food Supplements Targeting Pathogenesis of COVID-19

M. Mrityunjaya[1,2], *V. Pavithra*[1,2], *R. Neelam*[1], *P. Janhavi*[1,2], *P. M. Halami*[3] and *P. V. Ravindra*[1]*

[1] Department of Biochemistry, Council of Scientific and Industrial Research-Central Food Technological Research Institute, Mysuru, India, [2] Academy of Scientific and Innovative Research, Ghaziabad, India, [3] Department of Microbiology and Fermentation Technology, Council of Scientific and Industrial Research-Central Food Technological Research Institute, Mysuru, India

The COVID-19 is an acute and contagious disease characterized by pneumonia and ARDS. The disease is caused by SARS-CoV-2, which belongs to the family of *Coronaviridae* along with MERS-CoV and SARS-CoV-1. The virus has the positive-sense RNA as its genome encoding for ∼26 proteins that work together for the virus survival, replication, and spread in the host. The virus gets transmitted through the contact of aerosol droplets from infected persons. The pathogenesis of COVID-19 is highly complex and involves suppression of host antiviral and innate immune response, induction of oxidative stress followed by hyper inflammation described as the "cytokine storm," causing the acute lung injury, tissue fibrosis, and pneumonia. Currently, several vaccines and drugs are being evaluated for their efficacy, safety, and for determination of doses for COVID-19 and this requires considerable time for their validation. Therefore, exploring the repurposing of natural compounds may provide alternatives against COVID-19. Several nutraceuticals have a proven ability of immune-boosting, antiviral, antioxidant, anti-inflammatory effects. These include Zn, vitamin D, vitamin C, curcumin, cinnamaldehyde, probiotics, selenium, lactoferrin, quercetin, etc. Grouping some of these phytonutrients in the right combination in the form of a food supplement may help to boost the immune system, prevent virus spread, preclude the disease progression to severe stage, and further suppress the hyper inflammation providing both prophylactic and therapeutic support against COVID-19.

Keywords: SARS-CoV-2, COVID-19, pathogenesis, food supplements, immune-boosting, antioxidant, anti-inflammation

OPEN ACCESS

Edited by:
Alexandros Tsoupras, University of Limerick, Ireland

Reviewed by:
Laura Baldomà, University of Barcelona, Spain
José Parente, Federal University of Rio de Janeiro, Brazil

***Correspondence:**
P. V. Ravindra
raviravindra1@gmail.com

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: *06 June 2020*
Accepted: *25 August 2020*
Published: *07 October 2020*

Citation:
Mrityunjaya M, Pavithra V, Neelam R, Janhavi P, Halami PM and Ravindra PV (2020) Immune-Boosting, Antioxidant and Anti-inflammatory Food Supplements Targeting Pathogenesis of COVID-19. Front. Immunol. 11:570122. doi: 10.3389/fimmu.2020.570122

SEVERE ACUTE RESPIRATORY SYNDROME-CORONAVIRUS (SARS-COV)-2 INFECTION

The coronavirus disease (COVID-19) was first reported in late 2019 from Wuhan's city in China. Thus far, the infection has spread to almost all countries globally and was declared a pandemic by the WHO. While writing this review, there were more than 23M confirmed cases and more than 800K deaths. In India, there were more than 3M positive cases, and more than 57K reported deaths. The mortality rate of 2–16%, the rapid spread of the disease and high mortality in the susceptible population (mainly aged over 60 years and also in patients with underlying medical conditions including diabetes, cardiovascular diseases, etc.,) has resulted in a global lockdown and life has come to a standstill causing yet another world economic recession after 2008. The incubation period

is presumed to vary between 2 and 14 days. The transmission mode includes surface contact of aerosol droplets from infected persons, followed by touching nose, eyes, and mouth. Evidence also points toward vertical transmission to new-borns, also by fecal transmission (1–3). Coronaviruses are enveloped and possess positive-sense single-stranded RNA (+ssRNA) as their genome. These viruses belong to the large family of *Coronaviridae* and subfamily *Coronavirinae*, which infect birds and mammals. The genome size of these viruses ranges from 26 to 32 kb (4). The virus binds to angiotensin-converting enzyme 2 (ACE2) receptors on cells through its spike (S) glycoprotein. The S protein has two domains S1 and S2. S1 binds to the peptidase domain of ACE2, which is called the receptor-binding domain (RBD), while S2 catalyzes the membrane fusion, thereby releasing the genetic material into cells (5). Inside the cell, the RNA provides the template for structural proteins such as replicase (R1a/ab), envelope (E), Spike (S), membrane (M), nucleoprotein (N), and several non-structural proteins (NSPs 1–16), uncharacterized protein 14, protein 9b (6). Of them, non-structural proteins are predicted to participate in the host-protein interactions and modulate host cell signaling pathways. The onset of clinical disease and its progression to the severe stage may vary between individuals and that depend upon their immune status, and the presence of underlying medical conditions. In general, the typical clinical symptoms include, dry cough (67%), fever (88%), fatigue (38%), myalgias (14.9%), Dyspnoea (18.7%), other symptoms include, headache, sore throat, rhinorrhoea, and gastrointestinal symptoms. Pneumonia is severe manifestation of the infection (2).

PATHOGENESIS OF COVID-19

The details of the pathogenesis of SARS-CoV-2 infection is not clearly understood. The available evidence suggests that the pathogenesis of infection can be classified into two phases. Phase 1: An asymptomatic phase with or without detectable virus. Phase 2: Symptomatic phase with high viral load (4). The virus enters the airway epithelium after binding its S protein to the ACE2 receptors and subsequent priming by the cellular transmembrane protease, serine 2 (TMPRSS2). Following its entry, the virus inhibits or delays the host innate interferon (IFN) immune response. The mechanisms of how it modulates the host IFN response is not completely understood. Available evidence from other members of the same family suggests that, the virus inhibits the production of type 1 IFN as well as the signaling downstream of the interferon-α/β receptor (IFNAR) (7). The virus interferes with downstream signaling by ubiquitination and degradation of RNA sensor adaptor molecules such as mitochondrial antiviral-signaling (MAVS) protein and tumor necrosis factor receptor-associated factors (TRAF) 3/6 and inhibiting interferon regulatory factor (IRF) 3 nuclear translocation (8). Once type 1 IFN is secreted, the virus interferes IFN signaling by inhibiting signal transducer and activator of transcription (STAT) 1 phosphorylation (9). The viral proteins that modulate host type 1 IFN responses include structural (such as M, N) and NSPs. Following the impairment in the IFN system, virus replication ensues in cells. The viral replication, in turn, triggers the activation of monocytes, macrophages, granulocytes resulting in the hyper inflammatory condition described as "cytokine storm" with the massive secretion of pro-inflammatory cytokines including interleukin (IL)-1, IL-6, IL-8, IL-12, tumor necrosis factor (TNF)-α, etc. This results in hyper inflammation of tissues and subsequent tissue fibrosis and pneumonia (4, 7, 10). Studies also indicate the involvement of oxidative stress in the pathogenesis of COVID-19. Available evidence suggests that, SARS-CoV-2 infection causes oxidative stress directly by enhancing the production of reactive oxygen species (ROS) (11) and indirectly by suppressing the host antioxidant defense mediated by the nuclear factor (erythroid-derived 2)-like 2 (NRF-2) (10). Further, granulocytosis in response to SARS-CoV-2 infection also contributes to the production of super oxide ions, a type of ROS and for the additional production of pro-inflammatory cytokines (12). In a study by Lin et al. (13) showed that a viral protease 3CLpro causes a significant increase in the ROS production in HL-CZ cells. Further, study also found that elevated oxidative stress results in activation apoptosis and inflammation. In another study done on human HCoV-229E infection shows that deficiency in the expression of NRF-2 target, glucose-6-phosphate dehydrogenase (G6PDH) results in enhanced ROS as well as virus production (14). Incidentally, the NRF-2 levels were found to be suppressed in lung biopsies from COVID-19 subjects, on the other hand NRF-2 activators found to inhibit replication of SARS-CoV-2 and the inflammatory response (10). However, it is not known how SARS-CoV-2 infection causes suppression of NRF-2 signaling. Additionally, studies also suggest that SARS-CoV-2 infection triggers the activation of NF-κB-toll-like receptor (TLR) signaling pathways to induce the oxidative stress and hyper inflammatory response, ultimately leading to acute lung injury (11).

The elevated cytokines also trigger induction of endothelium HA-synthase-2 (HAS2) in alveolar epithelial cells (type 2), and fibroblasts (15). Most importantly, key molecule hyaluronan (HA) has high water binding capacity up to 1,000 times its molecular weight. Perhaps the accumulation of fluid in the lungs could be the reason that computer tomography (CT) images of the lung in acute respiratory distress syndrome (ARDS) patients show the presence of distinguishing white patches called ground glass (16). The majority of autopsies have shown that infected lungs are filled with clear liquid jelly, which resembles the lungs of wet drowning (17). Even though the nature of clear liquid jelly is not yet been determined, HA is associated with ARDS (18). The lungs of COVID-19 patients show elevated levels of inflammatory cytokines (IL-1, TNF-α). This correlates with increased activity of HAS2 and the subsequent lung pathology induced by the SARS-CoV-2 infection. Therefore, the above clinical and research findings suggest that COVID-19 pathogenesis involves two phases: Phase 1, suppression of innate immune response, increases in oxidative stress and phase 2 acute inflammation-driven damaging phase (**Figure 1**).

STRATEGIES TO COUNTERACT THE SARS-COV-2 INFECTION USING FOOD SUPPLEMENTS

From the point of prevention, phase 1 is crucial as individuals in this stage are carriers, they can spread the infection

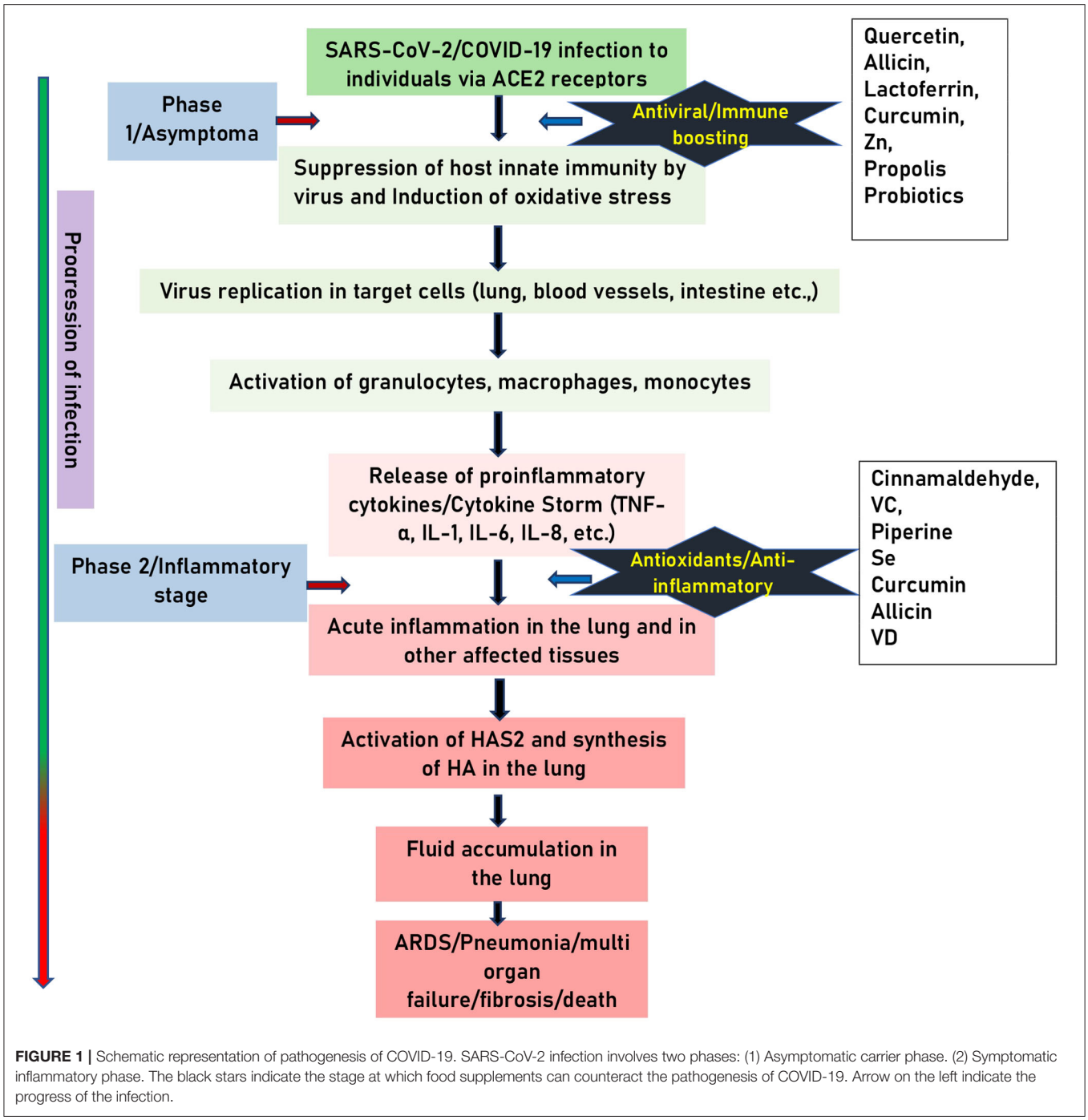


FIGURE 1 | Schematic representation of pathogenesis of COVID-19. SARS-CoV-2 infection involves two phases: (1) Asymptomatic carrier phase. (2) Symptomatic inflammatory phase. The black stars indicate the stage at which food supplements can counteract the pathogenesis of COVID-19. Arrow on the left indicate the progress of the infection.

unknowingly. Management of individuals in phase 1, along with mounting specific adaptive immune response, and use of antivirals is critical to prevent the virus entry, replication as well as the disease progression to phase 2. Therefore, global strategies may include administration of external antiviral, and or immune-boosting food supplements. During the phase 2 of the infection, in addition to maintaining the general health condition of affected patients, the line of treatment may be focused on adapting the strategies including the use of nutritional supplements that can suppress the ongoing oxidative stress, acute-inflammation and cytokine storm so that destruction and damage caused to affected tissues is prevented. In summary, in addition to symptomatic treatment, strategies to counteract the SARS-CoV-2 infection is to boost the immune response in phase 1 while suppressing it in the second phase could be effective.

IMMUNE-BOOSTING, ANTIOXIDANT AND ANTI-INFLAMMATORY FOOD SUPPLEMENTS AGAINST COVID-19

Currently, there is one vaccine; Sputnik V, approved by the Ministry of Health, Russian Federation. It was fast-tracked for use as a corona vaccine, but experts have expressed concern about the vaccine's efficacy and safety since it has not yet been evaluated in phase 3 clinical trials. Currently, most countries around the world are into developing corona vaccines, a few of them have entered into human trials while most of them are in various stages of research and development. Further, there no specific drug for use against COVID-19 as well as substantial data both at the national or international level on the effects of nutritional supplements on risk or severity of COVID-19. The development of new antivirals for COVID-19 is a great challenge and needs a considerable length of time and effort for designing and validation. Several shreds of evidence indicate that many nutritional supplements from various spices, herbs, fruits, roots, and vegetables can reduce the risk or severity of a wide range of viral infections by boosting the immune response, particularly among people with inadequate dietary sources and also by their anti-inflammatory, free radical scavenging, and viricidal functions. These nutrients can be repurposed in mitigating the pathological effects induced by the SARS-CoV-2 infection. Therefore, the use of natural compounds may provide alternative prophylactic and therapeutic support along with the therapy for COVID-19. In the following section, the beneficial effects of some of the nutrients are described.

ZINC (ZN)

Zinc is an essential metal involved in a variety of biological processes due to its function as a cofactor, signaling molecule, and a structural element. It regulates inflammatory activity and has antiviral and antioxidant functions (19). Studies in the rat model show that deficiency of Zn increases oxidative stress, pro-inflammatory TNF-α and vascular cell adhesion molecule (VCAM)-1 expression and causes lung tissue remodeling which was partially reversed by the Zn supplementation (20). Zn deficiency shows up-regulation of TNF-α, IFN-γ, and FasR signaling and induction of apoptosis in lung epithelial cells (21) and also up-regulates the Janus kinase (JAK)-STAT signaling in lungs under septic conditions (22). Zinc can also modulate the viral entry, fusion, replication, viral protein translation and virus budding of respiratory viruses (19, 23). Speth et al. (24) demonstrated that Zn exposure (100 μM) was shown to reduce recombinant human ACE-2 activity in rat lungs. Zn^{2+} cations especially in combination with Zn ionophore pyrithione were shown to inhibit SARS-coronavirus RNA polymerase (RNA dependent RNA polymerase, RdRp) activity by suppressing its replication (25). Studies have shown that oral supplementation of Zn reduces the occurrence of acute respiratory infections by 35%. Zn also shortens the duration of flu-like symptoms by 2 days as well as improves the rate of recovery (26). The recommended dose from various studies ranges from 20 to 92 mg/week (27). Zinc is considered as the potential supportive treatment against COVID-19 infection due to its anti-inflammatory, antioxidant as well as direct antiviral effects (28).

VITAMIN D (VD)

VD a fat-soluble vitamin, plays a vital role in both in immunomodulatory, antioxidant and antiviral responses (29, 30). The human airway epithelium constitutively expresses the vitamin D receptor thereby enabling the protective effects of VD against respiratory infections. VD blocks NF-κB p65 activation *via* up-regulation of NF-κB inhibitory protein I-kappa-B-alpha (IκB-α) (31). VD also decreases the expression levels of pro-inflammatory type 1 cytokines such as IL-12, IL-16, IL-8, TNF-α, IFN-γ while increasing type 2 cytokines such as IL-4, IL-5, IL-10, and regulatory T cells (32, 33). VD increases the levels of antioxidant NRF-2 and facilitates balanced mitochondrial functions, prevents oxidative stress-related protein oxidation, lipid peroxidation and DNA damage (30).

Epidemiological data relates VD deficiency to increases in the susceptibility to acute viral respiratory infections (34) while its supplementation potentiates the innate immune responses to respiratory viral infections including those caused by Influenza A and B, parainfluenza 1 and 2, respiratory syncytial virus (RSV), and chronic hepatitis C (35, 36). Though there are no reports that VD directly affects the virus replication or viral load, studies reveal that VD could contribute to antiviral activity through suppression of virus-induced inflammation. Perhaps this function of VD could help in suppression of the cytokine storm in SARS-CoV-2 infection. In a randomized controlled trial (RCT), VD supplementation of monthly high-dose (100,000 IU/month) in comparison to a standard dose (12,000 IU/month) helps in reducing the incidence of acute respiratory infections especially in older long-term care residents (37). Furthermore, evidence also suggests that VD can supplement the effectiveness of drug treatment as observed in the case of ribavirin therapy for treatment-naïve patients with chronic Hepatitis C virus (HCV) genotype 1 and HCV genotype 2e3 infections (33, 34, 38, 39). The beneficial effect of supplementation was seen in patients across all ages groups and in individuals with pre-existing chronic illness (40). Older people are most often deficient in these important micronutrients. Thus they can derive the most significant benefit from the VD supplementation (41).

VITAMIN C (VC)

Vitamin C can potentially protect against infection due to its essential role on immune health (42). This vitamin supports the function of various immune cells and enhances their ability to protect against infection. Supplementing with VC has been shown to reduce the duration and severity of upper respiratory infections (most of which are assumed to be due to viral infections), including the common cold (43). The recommended dose of VC varied from 1 to 3 g/day. The total recommended daily allowance (RDA) for VC is 60 mg. Various spices, herbs, fruits, and vegetables have found to be excellent sources of VC

(44). For example, thyme fresh (267%RDA), turmeric (43%RDA), cardamom (35%RDA), coriander (35%RDA), beetroot juice are good sources of VC (45). VC is also a potent antioxidant. As an antioxidant, it scavenges ROS, prevents lipid peroxidation, and protein alkylation and thus protects cells from oxidative stress induced cellular damage (46). Studies also have revealed that administration of VC in combination with quercetin provides synergistic antiviral, antioxidant and immunomodulatory effects (47). Recently, based on the clinical trial it is proposed that the oral administration of 250–500 mg quercetin, 500 mg VC for high risk and mild symptomatic subjects twice a day for 7 days and up to 3 g VC and 500 mg quercetin twice a day for 7 days in ARDS patients (assisted ventilation/intubation) improves the overall recovery in SARS-CoV-2 subjects (47). Therefore, having the food supplement incorporated with sources of VC can help in alleviating and providing immune boosting as well as an anti-inflammatory, antioxidant effect against SARS-CoV-2 infection (48).

CURCUMIN

Curcumin has a broad spectrum of biological actions, including antibacterial, antiviral, antifungal, antioxidant and anti-inflammatory activities (49). It inhibits the production of pro-inflammatory cytokines (IL-6 and TNF- α) in lipopolysaccharide (LPS)-stimulated BV2 microglial cells (50) and IL- 1β and IL-6 in TNF-α treated HaCaT cells *via* inhibiting the NF-κB and MAPK signaling pathways (51). The curcumin also inhibits cyclooxygenase-2 (COX-2), as well as STAT signaling pathways (52). Curcumin exerts antiviral effect on a broad range of viruses including influenza virus, adenovirus, hepatitis, human papilloma virus (HPV), human immunodeficiency virus (HIV), herpes simplex virus−2 (HSV-2) and Zika viruses (53). It exerts antiviral effect by various mechanisms ranging from inhibiting the virus entry into cells, inhibiting encapsulation of the virus and viral protease, inhibiting the virus replication, as well as modulating several signaling pathways (54). Recent study has shown that curcumin potentially inhibits ACE2, modulates characteristics of lipid bilayer, as well as viral S protein inhibiting entry of virus into cells (54, 55), inhibits the viral protease (56), stimulates host interferon production to activate the host innate immunity (55), etc. Furthermore, curcumin is a potent antioxidant. It exerts its antioxidant effects both by neutralizing free radicals and enhancing the production of antioxidant enzymes (57–60). These studies reveal potential immune-boosting, antioxidant and anti-SARS-CoV-2 effects of curcumin. Therefore, curcumin could be a potential supplement in combating the COVID-19 pathogenesis.

CINNAMALDEHYDE

Cinnamaldehyde is a naturally present organic compound abundantly found in essential oils in cinnamon. It predominantly exists in the trans-isomer form, which gives cinnamon its flavor and odor (61). Cinnamaldehyde is a well-known dietary phytonutrient, known to possess anti-inflammatory properties. In a study by Liao et al. (62), it was found that cinnamaldehyde inhibits the TNF-α-induced inflammation through suppression of NF-κB activation. Studies have also found that it can suppress endotoxin-mediated hyperexpression of TLR4 and NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome signaling pathways (63). Cinnamaldehyde is also known to downregulate the production of prostaglandins (PGEs) by downregulating IL-1β-induced COX-2 activity thus lowering the chances of hyper inflammation in a dose-dependent manner (64). All the above evidences show cases that cinnamaldehyde is a potential anti-inflammatory bioactive compound and could be useful in mitigation of SARS-CoV-2 induced hyper inflammation in the lung.

ALLICIN

Garlic is a well-known plant/ herb classified under Allium (onion) family and has been used from ages for its several nutraceutical properties. The predominant thiosulfinate in fresh garlic extract identified as allicin, has shown a number of health benefits due to its anti-inflammatory, antioxidant and antiviral properties. Allicin suppresses the inflammation *via* inhibiting the TNF-α induced expression levels of IL-1β, IL-8, IP-10, and IFN-γ and also through suppression of degradation of NF-κB inhibitory protein IκB in intestinal epithelial cells (65). It inhibits inducible NO nitric oxide synthase expression in activated macrophages (66, 67). Several garlic associated compounds have found to possess a strong viricidal activity against a wide range of viruses including parainfluenza virus type 3, human rhinovirus, herpes simplex virus (HSV)-1, HSV-2, and vesicular stomatitis virus (VSV). Some of the garlic compounds that show viricidal activity are ajoene, allicin, allyl, methyl thiosulfinate and methyl allyl thiosulfinate (68, 69). Most of the above-mentioned functional effects were observed at 200 ng/ml concentrations. Studies also have found that only fresh samples with no processing such as heat induction or drying were successful to induce most of the biological activities of garlic (70). Therefore, fresh garlic extract may be useful as a prophylactic against COVID-19.

PIPERINE

Black pepper has long been used in many cuisines and it holds a very valuable space among medicinal plants. Piperine that is obtained from ethanolic extract of black pepper and is a major alkaloid in the group of cinnamamides (71). Piperine possesses a strong anti-inflammatory function and therefore can be repurposed for suppression of hyper inflammation induced during COVID-19. It downregulates PGEs by inhibiting the expression levels of IL-6 and matrix metalloproteinases (MMP-13) (71). Piperine promotes innate immunity by promoting the phagocytic activity of phagocytes and is known to inhibit LPS-induced expression of IRF-1 and IRF-7 mRNA, phosphorylation of IRF-3, type 1IFN mRNA, and down-regulation of STAT-1 activity (72). Few studies conducted on microglial cells have shown that piperine inhibits LPS-Induced TNF-α, IL-6, IL-1β, and PGE2 production in BV2 cells (73). Also, it found to

inhibit the production of IL-2, and IFN-γ in human peripheral blood mononuclear cells (PBMCs) (74). Furthermore, piperine treatment found to reduce the production of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, COX-2, nitric oxide synthase-2, and NF-κB in the cerebral ischemia-reperfusion-induced inflammation rat model (75). These findings indicate the strong anti-inflammatory activity of the piperine. Further, piperine is a potent antioxidant and protects against oxidative damage by neutralizing free radicals, ROS, and hydroxyl radicals. It scavenges superoxide radicals with IC_{50} of 1.82 mM and inhibits lipid peroxidation with IC_{50} of 1.23 mM. These results indicate that piperine possesses a direct antioxidant effect against various free radicals (76). Because of these properties, piperine can be tried as a prophylactic or therapeutic compound to protect from the oxidative stress and hyper inflammation induced during the COVID-19.

SELENIUM (SE)

Selenium is abundantly found in common foods such as corn, garlic, onion, cabbage, broccoli. It's an essential micronutrient that plays a vital role in various physiological processes and on the immune system. Selenium exerts its biological effect through incorporation into selenoproteins in the body. Optimum selenium status (100 μg per day) promotes enhanced T cell proliferation, NK cell activity and innate cell functions. Further supports stronger vaccine response and robust immunity to pathogens. Also, suppresses severe inflammation in tissues such as lungs and intestine (77). Studies have shown that selenium supplementation modulates the inflammatory response in respiratory distress syndrome patients by restoring the antioxidant status of the lungs and suppressing the IL-1β and IL-6 levels (78). Selenium supplementation suppresses pathogen induced activation of NF-κB and its downstream pro-inflammatory cytokine release (79). The antiviral properties of selenium have found to be mediated through its antioxidant effects. Selenium-deficient HIV+ patients tend to present with diminished antioxidant glutathione peroxidase activity (77). On the other hand, selenium supplementation demonstrates the improved CD+ T cell counts (80) and improves glutathione peroxidase and other antioxidant selenoenzymes along with catalase activities (81). Overall, selenium improves the immunity through its non-enzymatic role acting as cofactor for enzymes involved in critical post-translational modifications of proteins. Because of its substantial role in suppressing the inflammation and augmentation of antioxidant status and innate immunity, selenium supplementation may be useful in fight against COVID-19.

PROPOLIS

Propolis produced by honeybees and known to have a broad spectrum of biological properties, including anti-microbial, anti-inflammatory, dermatoprotective, laxative, anti-diabetic, anti-tumor, and immunomodulatory activity (82). The immunomodulatory activity is attributed to flavonoids and some phenolic acids mainly caffeic acid phenethyl esters and artepillin C (3,5-diprenyl-4-hydroxycinnamic acid). Propolis exhibits immunomodulatory effects on a broad spectrum of immune cells mediated by the modulation of extracellular signal-regulated kinase 2 and MAPK signaling pathways. Further, it also modulates nuclear factor of activated T cells (NFAT) and NF κB signaling pathways (82, 83). Propolis also stimulates greater antibody production, suggesting that it could be used as an adjuvant in vaccines. Propolis at higher concentration inhibits lymphoproliferation while at low concentrations the effect is reversed, causing lymphoproliferation (84). Further, compounds in honey propolis inhibits various viruses such as dengue virus type 2, herpes simplex virus, human cytomegalovirus, influenza virus A1 (85). Together, with immunomodulatory and antiviral effects, propolis can be tried as a prophylactic support against COVID-19.

PROBIOTICS

The commonly used probiotics are *Bifidobacterium* and *Lactobacillus* species, followed by the *Streptococcus, Enterococcus, Bacillus, and Escherichia coli*. Probiotics not only support the health of the gut but also improves system functioning and regulation (86). Though it is not clear how gut microbiome provides benefit over respiratory tract infections *via* gut-lung axis. In general, it is observed that the gut microbiome impacts systemic immune responses as well as local immune responses at distal mucosal sites, including lungs (87). Consumption of *Bifidobacterium* and *Lactobacillus* have found to help in clearing the influenza virus in the respiratory tract (88). Levels of interferons, mucosal antibodies of lung and activity of NK cells, antigen presenting cells (APCs) are improved by probiotics (89). *Lactobacillus plantarum* DR7 strain has shown to have suppressing effect on the pro-inflammatory cytokines TNF-α, IFN-γ, enhances anti-inflammatory cytokines IL-10, IL-4 and also known to reduce plasma peroxidation levels as well as modulate immune system (90). It is reported that *Lactobacillus acidophilus* CMCC878 administration in mice infected with *Staphylococcus aureus*, and *Pseudomonas aeruginosa* decreased the damage in the lungs by reducing the bacterial load and reducing the inflammation (91). A clinical study has reported that administration of *Leuconostoc mesenteroides* 32-77:1, *Lactobacillus plantarum* 2,362, *L. paracasei ssp. paracasei* 19, *Pediococcus pentosaceus* 5-33:3 along with resistant starch, inulin prebiotics etc. reduced systemic inflammatory response syndrome and other infections (92). *Bifidobacterium longum* BB536 strain prevents infection from influenza and improves innate immunity (93). Though mechanism of their immunomodulating and anti-inflammatory effects in the lung are not clearly understood. In general, probiotics exert anti-inflammatory and immunomodulatory effects *via* modulation of the NF-κB, MAPK and pattern recognition receptors (PRR) pathways that decreases Th2 mediated responses and upregulates Th1 responses. Further, they have an ability to inhibit the attachment of bacterial LPS to CD14 receptor, hence decrease in the overall activation of

NF-κB and pro-inflammatory cytokines production (94, 95). Considering the role of probiotics in improving the host innate immune response as well as anti-inflammatory effects (87), and considering the fact that gut involvement and enterocytes (96) can be reservoirs of SARS-CoV-2 infection, probiotics can be repurposed as prophylactics as well as adjuvants to combat the pathogenesis of COVID-19.

LACTOFERRIN

Lactoferrin (Lf) is a naturally occurring and non-toxic glycoprotein that has been studied against a broad range of viruses, including SARS-CoV, which is closely related to SARS-CoV-2. Lf inhibits viral entry *via* binding to cell surface molecules or viral particles or both. It was also known to suppress virus replication as in the case of HIV. Therefore, it plays a crucial role in preventing the virus entry and replication (97). Studies have shown that it exerts immunomodulatory and antioxidant effects by inducing the T-cell activation, suppressing the levels of interleukins including IL-6, TNF-α, and downregulating the ferritin (98). Also it suppresses H_2O_2 -induced oxidative stress in human umbilical vein endothelial cells (99). Furthermore, zinc saturated Lf exerts a more potent antiviral effect (100). It is mainly used as a nutritional additive in infant formulas and clinical studies, with doses ranging from 100 mg to 4.5 g

TABLE 1 | Summary of food supplements and their major functional effects.

Serial number	Food supplements	Properties	Status of the clinical trial
1	Zinc (Zn) (Antiviral)	■ Protects against oxidative stress and inhibit TNF-α, IFN-γ, FasR and JAK-STAT signaling pathways. ■ Modulates the viral entry, fusion, replication, viral protein translation and virus budding of respiratory viruses.	Phase 1 and 2
2	Vitamin D (VD) (Immune-boosting anti-inflammatory)	■ Blocks NF-κB p65 activation *via* up-regulation of I-kappa-B-alpha (*I*κB-α). ■ Decreases the expression of the pro-inflammatory type 1 cytokines: IL-12, IL-16, IL-8, TNF-α and IFN-γ and increases type 2 cytokines IL-4, IL-5, IL-10. ■ Upregulates the levels of antioxidant NRF-2, facilitates balanced mitochondrial functions.	Phase 2
3	Vitamin C (VC) (Immune-boosting, antioxidant)	■ Decreases pro-inflammatory cytokines, TNF-α and IFN-γ and increases anti-inflammatory IL-10 production. ■ Reduces the duration and severity of upper respiratory infections (viral infections). ■ Scavenges ROS, prevents lipid peroxidation, and protein alkylation and thus protect cells from oxidative stress induced cellular damage.	Phase 2
4	Curcumin (Immune-boosting, antiviral, anti-inflammatory, and antioxidant)	■ Stimulates host interferon production to activate the host innate immunity. ■ Binds to S protein at RBD and ACE2 receptor and inhibits virus entry. ■ Inhibits NF-κB, cyclinD1, COX-2, TNF-α, and STAT signaling pathways. ■ Neutralizes free radicals and enhances the production of antioxidant enzymes.	Phase 1 and 2
5	Cinnamaldehyde (Anti-inflammatory)	■ Suppress the NF-κB, TLR4, and NLRP3 signaling pathways. ■ Downregulates the production of prostaglandins.	Phase 2
6	Allicin (Antiviral, anti-inflammatory)	■ Downregulates the proinflammatory cytokines and inhibits the nitric oxide synthase expression in macrophages. ■ Possess antiviral effect on broad spectrum of viruses of HSV family, parainfluenza virus and human rhinovirus.	Phase 1 and 2
7	Piperine (Anti-inflammatory and antioxidant)	■ Reduces the production of the IL-1β, IL-6, TNF-α, COX-2, nitric oxide synthase-2, and NF-κB. ■ Neutralizes free radicals, ROS, and hydroxyl radicals.	Phase 2
8	Selenium (Immune-boosting, and antioxidant)	■ Promotes the T cell proliferation, NK cell activity and innate cell function. ■ It downregulates the proinflammatory cytokines (IL-1β and IL-6). ■ Augments glutathione peroxidase and other antioxidant selenoenzymes activities.	Phase 2
9	Propolis (Immune-boosting, anti-inflammatory)	■ Exhibits the immunomodulatory effect through extracellular signal-regulated kinase 2 and MAPK pathways and by modulating the NFAT and NF-κB activation. ■ Inhibits various viruses such as dengue virus type 2, herpes simplex virus, human cytomegalovirus, influenza virus A1.	
10	Probiotics (Immune boosting, anti-inflammatory)	■ *Lactobacillus plantarum* DR7 suppress proinflammatory cytokines TNF-α, IFN-γ, enhances anti-inflammatory cytokines IL-10, IL-4. ■ *Lactobacillus acidophilus* CMCC878 reduces the bacterial load and inflammation in mice lungs infected with staphylococcus and pseudomonas. ■ *Leuconostoc mesenteroides* 32-77:1, *L. plantarum* 2,362, *L. paracasei ssp. paracasei* 19, *Pediococcus pentosaceus* 5-33:3 along with resistant starch, inulin etc reduce systemic inflammatory response syndrome and other infections. ■ *Bifidobacterium longum* BB536 prevents infection from influenza and improves innate immunity.	Phase 2
11	Lactoferrin (antiviral)	■ Downregulates the IL-6, TNF-α, and ferritin. ■ Inhibits the viral entry and suppress the viral replication.	Phase 1 and 2
12	Quercetin (antiviral)	■ Inhibits the production of the TNF-α, IL-8, IL-1α, COX, and LOX enzymes. ■ Possesses antiviral effects against both RNA (influenza and coronavirus) and DNA viruses (herpesvirus). ■ Act as ligand for the S protein of virus and ACE 2 and interferes in binding of virus to cells.	Phase 1 and 2

TABLE 2 | Registered clinical trials of food supplements (Source: ClinicalTrials.gov).

Serial number	Food supplements	Registered clinical trials for COVID-19
1	Zinc (Zn)	1. Evaluation of the Relationship Between Zinc Vitamin D and b12 Levels in the COVID-19 positive Pregnant Women (Pinar Yalcin Bahat Istanbul, Istanbul, Turkey). 2. Zinc with Chloroquine/Hydroxychloroquine in Treatment of COVID-19 (Tanta University hospital Tanta, Egypt). 3. A Study of Hydroxychloroquine, Vitamin C, Vitamin D, and Zinc for the Prevention of COVID-19 Infection (Progena Biome Ventura, California, United States).
2	Vitamin D (VD)	1. COVID-19 and Vitamin D in Nursing- home (Angers University Hospital Angers, France). 2. Vitamin D Supplementation in Patients with COVID-19 (Clinical Hospital of the School of Medicine, University of São Paulo São Paulo Brazil). 3. Vitamin D Testing and Treatment for COVID 19 (Arizona State University Tempe, Arizona, United States).
3	Vitamin C (VC)	1. Administration of Intravenous Vitamin C in the Novel Coronavirus Infection (COVID-19) and Decreased Oxygenation (Hunter Holmes Mcguire veteran Affairs Medical Center Richmond, Virginia, United States). 2. A Study of Hydroxychloroquine, Vitamin C, Vitamin D, and Zinc for the Prevention of COVID-19 Infection (ProgenaBiome Ventura, California, United States). 3. The Study of Quadruple Therapy Zinc, Quercetin, Bromelain and Vitamin C on the Clinical Outcomes of Patients Infected With COVID-19 (Ministry of Health. First health cluster, Riyadh, Riyadh, Saudi Arabia).
4	Curcumin	1. A phase II, Controlled Clinical Study Designed to Evaluate the Effect of ArtemiC in Patients Diagnosed With COVID-19 (Hillel Yaffe Medica Center Hedera, Haifa, Israel Nazareth Hospital EMMS Nazareth, North, Israel Rambam Health Care Campus Haifa, Israel).
5	Probiotics	1. Effect of Lactobacillus on the Microbiome of Household Contacts Exposed to COVID-19 (Duke University Durham, North Carolina, United States). 2. Oxygen-Ozone as Adjuvant Treatment in Early Control of COVID-19 Progression and Modulation of the gut Microbial Flora (Francesco Pugliese Rome, RM Italy). 3. Evaluation of the Probiotic *Lactobacillus Coryniformis* K8 on COVID-19 Prevention in Healthcare Workers (Raquel Rodrigues Blanque Granada, Spain). 4. Study to Evaluate the Effect of a Probiotic in COVID-19 (Hospital Universitario de Vinalopo Elache, Alicante, Spain, Hospital Universitario de Torrevieja Torreviveja, Alicante, Spain).
6	Quercetin	1. Effect of Quercetin on Prophylaxis and Treatment of COVID-19 (Kanuni Sultan Suleyman Training and Research Hospital Istanbul, Turkey). 2. The Study of Quadruple Therapy Zinc, Quercetin Bromelain and Vitamin C on the Clinical Outcomes of Patients Infected With COVID-19 (Ministry of Health. First Health cluster, Riyadh. Riyadh, Saudi Arabia). 3. Estrogen Patch for COVID-19 Symptoms (Stony Brook University Hospital, Stony Brook, New York. USA).

a day for various indications without apparent toxicities. and can be tried as a potential preventive and therapeutics against COVID-19 (98).

QUERCETIN

Quercetin is a well-known antioxidant with anti-inflammatory and antiviral bioactive. It inhibits TNF-α production in LPS-induced macrophages (101), IL-8 production in lung A549 cells (102), and mRNA levels of TNF-α and IL-1α in glial cells (103). It also limits the production of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes in rat liver epithelial cells (104). Studies have also shown that quercetin has antiviral effects on both RNA and DNA viruses. It inhibits the virus entry and viral-cell fusion (105) and reduces the expression of pro-inflammatory cytokines and lung inflammation induced by rhinovirus in mice (106). Further, quercetin metabolite (4',5-diacetyloxy-3,3',7-trimethoxyflavone) has been shown to inhibit the picornavirus replication by inhibiting the RNA replicase complex (107). Studies have also found that quercetin-3β-galactoside due to the presence of hydroxyl group, it binds to viral protease $3CL_{pro}$ and inhibits its proteolytic activity (108). In the context of SARS virus infection, supercomputer SUMMIT drug-docking screen and gene set enrichment analyses (GSEA) finds that quercetin, VD, and estradiol interferes the functioning of 85, 70 and 61% of the SARS-CoV-2 viral proteins in human cells, respectively. Based on these observations the study also predicts tripartite combination (quercetin/VD/estradiol) compared to bipartite (VD/quercetin) of may affect 73% human genes encoding SARS-CoV-2 targets implicating a robust mitigating agents against the COVID-19 (109). Further, increased ability of estradiol in affecting human genes encoding SARS-CoV-2 targets compared to testosterone suggests a plausible explanation of the apparently higher male mortality in this corona pandemic (109). In line with these observations, a randomized interventional clinical trial using estradiol or VD as a mitigating agent have been listed on the clinical trial (https://clinicaltrials.gov/ct2/show/NCT04359329).

Furthermore, as observed in prediction models that quercetin binds SARS-CoV-2 S-protein at its host receptor region or to the S-protein-human ACE2 interface interfering the virus entry into cells indicating its therapeutic potential (110). This prediction is consistent with the reports that both quercetin and a structurally similar luteolin inhibits the SARS-CoV virus infection (111). Additionally, other studies have also found that quercetin in combination with VC induces synergistic antiviral and immunomodulatory effects against COVID-19 (47). Taken together, various studies suggest that quercetin possesses potential anti-SARS-CoV-2 effects and can be repurposed as a preventive and therapeutic candidate to combat COVID-19.

CONCLUSIONS

Currently, there is one corona vaccine, Sputnik V, developed by the Gamaleya Research Institute, Moscow has been approved by the Ministry of Health, Russian Federation. It was fast-tracked for use as a corona vaccine, but experts have expressed concern about the vaccine's efficacy and safety since it has not yet been evaluated in phase 3 clinical trials. Presently, there are over 100 vaccines around the world in various stages of research and development. A few of them are in human clinical trials and are being tested rigorously for their safety, efficacy, and dosage standardization. Similarly, there are several drug candidates that have been identified and most are in various stages of research and development, whilst some of them have been repurposed and approved for emergency use in this pandemic. The notable ones approved for use in an emergency include hydroxychloroquine, favipiravir, remdesivir, tocilizumab, etc., Furthermore, no substantial research supports the use of specific food supplements as adjuvant therapy for the management of COVID-19 subjects. The plethora of existing literature provides the scientific evidence on immune-boosting, anti-inflammatory, antioxidant, and antiviral properties of several phytonutrients as summarized in **Table 1**. Initial studies find that some of these have been found to possess anti-SARS-CoV-2 effects and are being fast-tracked into clinical trials (**Table 2**). Repurposing of these nutrients in the right combination to achieve the functional synergy in the form of ready-to-eat food supplements may provide both prophylactic and adjuvant therapy against COVID-19.

AUTHOR CONTRIBUTIONS

MM, VP, RN, and PJ: drafted the article. PH and PVR: edited the article. All authors: contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Department of Biotechnology (DBT)-Ramalingaswami Fellowship (BT/HRD/35/02/2006), Department of Science and Technology-Science and Engineering Research Board (DST-SERB) (EMR/2016/007994), Govt. of India.

ACKNOWLEDGMENTS

We are thankful to the Director and to CSIR-CFTRI for providing facilities to carry out this study.

REFERENCES

1. Chan JFW, Yuan S, Kok KH, To KKW, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet.* (2020) 395:514–23. doi: 10.1016/S0140-6736(20)30154-9
2. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
3. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *N Engl J Med.* (2020) 382:1199–207. doi: 10.1056/NEJMoa2001316
4. Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, et al. COVID-19 infection: the perspectives on immune responses. *Cell Death Differ.* (2020) 27:1451–4. doi: 10.1038/s41418-020-0530-3
5. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARSCoV-2 cell entry depends on ace2 and tmprss2 and is blocked by a clinically proven protease inhibitor. *Cell.* (2020) 181:271–80.e8. doi: 10.1016/j.cell.2020.02.052
6. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARSCoV-2 on virus entry and its immune cross-reactivity with SARSCoV. *Nat Commun.* (2020) 11:1620. doi: 10.1038/s41467-020-15562-9
7. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol.* (2017) 39:529–39. doi: 10.1007/s00281-017-0629-x
8. Kindler E, Thiel V, Weber F. Interaction of SARS and MERS coronaviruses with the antiviral interferon response. *Adv Virus Res.* (2016) 96:219–43. doi: 10.1016/bs.aivir.2016.08.006
9. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal.* (2020) 10:102–8. doi: 10.1016/j.jpha.2020.03.001
10. Olagnier D, Farahani E, Thyrsted J, Cadanet JB, Herengt A, Idorn M, et al. Identification of SARSCoV2-mediated suppression of NRF2 signaling reveals a potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. *bioRxiv.* (2020). doi: 10.21203/rs.3.rs-31855/v1
11. Delgado-Roche L, Mesta F. Oxidative stress as key player in severe acute respiratory Syndrome Coronavirus (SARSCoV) infection. *Arch Med Res.* (2020) 51:384–7. doi: 10.1016/j.arcmed.2020.04.019
12. Ntyonga-Pono MP. COVID-19 infection and oxidative stress: an under-explored approach for prevention and treatment? *Pan Afr Med J.* (2020) 35:12. doi: 10.11604/pamj.2020.35.2.22877
13. Lin CW, Lin KH, Hsieh TH, Shiu SY, Li JY. Severe acute respiratory syndrome coronavirus 3C-like protease-induced apoptosis. *FEMS Immunol Med Microbiol.* (2006) 46:375–80. doi: 10.1111/j.1574-695X.2006.00045.x
14. Wu YH, Tseng CP, Cheng ML, Ho HY, Shih SR, Chiu DTY. Glucose-6-phosphate dehydrogenase deficiency enhances human coronavirus 229E infection. *J Infect Dis.* (2008) 197:812–6. doi: 10.1086/528377
15. Bell TJ, Brand OJ, Morgan DJ, Salek-Ardakani S, Jagger C, Fujimori T, et al. Defective lung function following influenza virus is due to prolonged, reversible hyaluronan synthesis. *Matrix Biol J Int Soc Matrix Biol.* (2019) 80:14–28. doi: 10.1016/j.matbio.2018.06.006
16. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA.* (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
17. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med.* (2020) 8:420–2. doi: 10.1016/S2213-2600(20)30076-X
18. Hällgren R, Samuelsson T, Laurent TC, Modig J. Accumulation of hyaluronan (hyaluronic acid) in the lung in adult respiratory distress syndrome. *Am Rev Respir Dis.* (1989) 139:682–7. doi: 10.1164/ajrccm/139.3.682
19. Read SA, Obeid S, Ahlenstiel C, Ahlenstiel G. The role of zinc in antiviral immunity. *Adv Nutr.* (2019) 10:696–710. doi: 10.1093/advances/nmz013
20. Biaggio VS, Pérez Chaca MV, Valdéz SR, Gómez NN, Gimenez MS. Alteration in the expression of inflammatory parameters as a result of oxidative stress produced by moderate zinc deficiency in rat lung. *Exp Lung Res.* (2010) 36:31–44. doi: 10.3109/01902140903061787

21. Bao S, Knoell DL. Zinc modulates cytokine-induced lung epithelial cell barrier permeability. *Am J Physiol Lung Cell Mol Physiol.* (2006) 291:L1132–41. doi: 10.1152/ajplung.00207.2006
22. Liu MJ, Bao S, Napolitano JR, Burris DL, Yu L, Tridandapani S, et al. Zinc regulates the acute phase response and serum amyloid a production in response to sepsis through JAKSTAT3 signaling. *PLoS ONE.* (2014) 9:e94934. doi: 10.1371/journal.pone.0094934
23. Ishida T. Review on the role of $Zn2^{+}$ ions in viral pathogenesis and the effect of $Zn2^{+}$ ions for host cell-virus growth inhibition. *Am J Biomed Sci Res.* (2019) 2:28–37. doi: 10.34297/AJBSR.2019.02.000566
24. Speth R, Carrera E, Jean-Baptiste M, Joachim A, Linares A. Concentration-dependent effects of zinc on angiotensin-converting enzyme-2 activity (1067.4). *FASEB J.* (2014) 28:1067.4. doi: 10.1096/fasebj.28.1_supplement.1067.4
25. te Velthuis AJW, van den Worm SHE, Sims AC, Baric RS, Snijder EJ, van Hemert MJ. $Zn2^{+}$ inhibits coronavirus and arterivirus RNA polymerase activity *in vitro* and zinc ionophores block the replication of these viruses in cell culture. *PLoS Pathog.* (2010) 6:e1001176. doi: 10.1371/journal.ppat.1001176
26. Hemilä H, Fitzgerald JT, Petrus EJ, Prasad A. Zinc acetate lozenges may improve the recovery rate of common cold patients: an individual patient data meta-analysis. *Open Forum Infect Dis.* (2017) 4:ofx059. doi: 10.1093/ofid/ofx059
27. Roth DE, Richard SA, Black RE. Zinc supplementation for the prevention of acute lower respiratory infection in children in developing countries: meta-analysis and meta-regression of randomized trials. *Int J Epidemiol.* (2010) 39:795–808. doi: 10.1093/ije/dyp391
28. Zhang L, Liu Y. Potential interventions for novel coronavirus in China: a systematic review. *J Med Virol.* (2020) 92:479–90. doi: 10.1002/jmv.25707
29. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. *Nutrients.* (2013) 5:2502–21. doi: 10.3390/nu5072502
30. Wimalawansa SJ. Vitamin D deficiency: effects on oxidative stress, epigenetics, gene regulation, and aging. *Biology.* (2019) 8:30. doi: 10.3390/biology8020030
31. Chen Y, Zhang J, Ge X, Du J, Deb DK, Li YC. Vitamin D receptor inhibits nuclear factor κB activation by interacting with IκB kinase β protein. *J Biol Chem.* (2013) 288:19450–8. doi: 10.1074/jbc.M113.467670
32. Lemire JM, Archer DC, Beck L, Spiegelberg HL. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. *J Nutr.* (1995) 125:1704S—8. doi: 10.1016/0960-0760(95)00106-A
33. Jeffery LE, Burke F, Mura M, Zheng Y, Qureshi OS, Hewison M, et al. 1, 25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol Baltim Md 1950.* (2009) 183:5458–67. doi: 10.4049/jimmunol.0803217
34. Monlezun DJ, Bittner EA, Christopher KB, Camargo CA, Quraishi SA. Vitamin D status and acute respiratory infection: cross sectional results from the United States National Health and Nutrition Examination Survey, 2001-2006. *Nutrients.* (2015) 7:1933–44. doi: 10.3390/nu7031933
35. Zdrenghea MT, Makrinioti H, Bagacean C, Bush A, Johnston SL, Stanciu LA. Vitamin D modulation of innate immune responses to respiratory viral infections. *Rev Med Virol.* (2017) 27. doi: 10.1002/rmv.1909
36. Abu-Mouch S, Fireman Z, Jarchovsky J, Zeina AR, Assy N. Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naïve patients. *World J Gastroenterol.* (2011) 17:5184–90. doi: 10.3748/wjg.v17.i47.5184
37. Ginde AA, Blatchford P, Breese K, Zarrabi L, Linnebur SA, Wallace JI, et al. High-dose monthly vitamin D for prevention of acute respiratory infection in older long-term care residents: a randomized clinical trial. *J Am Geriatr Soc.* (2017) 65:496–503. doi: 10.1111/jgs.14679
38. Behera MK, Shukla SK, Dixit VK, Nath P, Abhilash VB, Asati PK, et al. Effect of vitamin D supplementation on sustained virological response in genotype 1/4 chronic hepatitis C treatment-naïve patients from India. *Indian J Med Res.* (2018) 148:200–6. doi: 10.4103/ijmr.IJMR_1295_15
39. Nimer A, Mouch A. Vitamin D improves viral response in hepatitis C genotype 2-3 naïve patients. *World J Gastroenterol WJG.* (2012) 18:800–5. doi: 10.3748/wjg.v18.i8.800
40. Charan J, Goyal JP, Saxena D, Yadav P. Vitamin D for prevention of respiratory tract infections: a systematic review and meta-analysis. *J Pharmacol Pharmacother.* (2012) 3:300–3. doi: 10.4103/0976-500X.103685
41. Goncalves-Mendes N, Talvas J, Dualé C, Guttmann A, Corbin V, Marceau G, et al. Impact of vitamin D supplementation on influenza vaccine response and immune functions in deficient elderly persons: a randomized placebo-controlled trial. *Front Immunol.* (2019) 10:65. doi: 10.3389/fimmu.2019.00065
42. Carr AC, Maggini S. Vitamin C and immune function. *Nutrients.* (2017) 9:1211. doi: 10.3390/nu9111211
43. van Driel ML, Beller EM, Thielemans E, Deckx L, Price-Haywood E, Clark J, et al. Oral vitamin C supplements to prevent and treat acute upper respiratory tract infections. *Cochrane Database Syst Rev.* (2019) 2019:CD013292. doi: 10.1002/14651858.CD013292
44. Vázquez-Fresno R, Rosana ARR, Sajed T, Onookome-Okome T, Wishart NA, Wishart DS. Herbs and spices- biomarkers of intake based on human intervention studies – a systematic review. *Genes Nutr.* (2019) 14:18. doi: 10.1186/s12263-019-0636-8
45. USDA National Nutrient Database. *Foods highest in Vitamin C and Iron in Spices and Herbs*. (2008). Available online at: https://nutritiondata.self.com/foods-00210111900000000000-1w.html (accessed May 27, 2020).
46. Traber MG, Stevens JF. Vitamins C and E: beneficial effects from a mechanistic perspective. *Free Radic Biol Med.* (2011) 51:1000–13. doi: 10.1016/j.freeradbiomed.2011.05.017
47. Colunga Biancatelli RML, Berrill M, Catravas JD, Marik PE. Quercetin and vitamin C: an experimental, synergistic therapy for the prevention and treatment of SARSCoV-2 related disease (COVID-19). *Front Immunol.* (2020) 11:1451. doi: 10.3389/fimmu.2020.01451
48. Hunt C, Chakravorty NK, Annan G, Habibzadeh N, Schorah CJ. The clinical effects of vitamin C supplementation in elderly hospitalised patients with acute respiratory infections. *Int J Vitam Nutr Res.* (1994) 64:212–9.
49. Catanzaro M, Corsini E, Rosini M, Racchi M, Lanni C. Immunomodulators inspired by nature: a review on curcumin and echinacea. *Molecules.* (2018) 23:2778. doi: 10.3390/molecules23112778
50. Jin CY, Lee JD, Park C, Choi YH, Kim GY. Curcumin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated BV2 microglia. *Acta Pharmacol Sin.* (2007) 28:1645–51. doi: 10.1111/j.1745-7254.2007.00651.x
51. Cho JW, Lee KS, Kim CW. Curcumin attenuates the expression of IL-1beta, IL-6, and TNF-alpha as well as cyclin E in TNF-alpha-treated HaCaT cells; NF-kappaB and MAPKs as potential upstream targets. *Int J Mol Med.* (2007) 19:469–74. doi: 10.3892/ijmm.19.3.469
52. Ghosh S, Banerjee S, Sil PC. The beneficial role of curcumin on inflammation, diabetes and neurodegenerative disease: a recent update. *Food Chem Toxicol.* (2015) 83:111–24. doi: 10.1016/j.fct.2015.05.022
53. Chen TY, Chen DY, Wen HW, Ou JL, Chiou SS, Chen JM, et al. Inhibition of enveloped viruses infectivity by curcumin. *PLoS ONE.* (2013) 8:e62482. doi: 10.1371/journal.pone.0062482
54. Zahedipour F, Hosseini SA, Sathyapalan T, Majeed M, Jamialahmadi T, Al-Rasadi K, et al. Potential effects of curcumin in the treatment of COVID-19 infection. *Phytother Res PTR.* (2020). doi: 10.1002/ptr.6738
55. Ting D, Dong N, Fang L, Lu J, Bi J, Xiao S, et al. multisite inhibitors for enteric coronavirus: antiviral cationic carbon dots based on curcumin. *ACS Appl Nano Mater.* (2018) 1:5451–9. doi: 10.1021/acsanm.8b00779
56. Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S, Soetjipto S. Potential inhibitor of COVID-19 Main Protease (Mpro) from several medicinal plant compounds by molecular docking study. *Nat Prod Bioprospect.* (2020) 1–10. doi: 10.20944/preprints202003.0226.v1
57. Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. *Adv Exp Med Biol.* (2007) 595:105–25. doi: 10.1007/978-0-387-46401-5_3
58. Barclay LR, Vinqvist MR, Mukai K, Goto H, Hashimoto Y, Tokunaga A, et al. On the antioxidant mechanism of curcumin: classical methods are needed to determine antioxidant mechanism and activity. *Org Lett.* (2000) 2:2841–3. doi: 10.1021/ol000173t
59. Agarwal R, Goel SK, Behari JR. Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. *J Appl Toxicol.* (2010) 30:457–68. doi: 10.1002/jat.1517

60. Biswas SK, McClure D, Jimenez LA, Megson IL, Rahman I. Curcumin induces glutathione biosynthesis and inhibits NF-kappaB activation and interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. *Antioxid Redox Signal.* (2005) 7:32–41. doi: 10.1089/ars.2005.7.32
61. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. *Evid-Based Complement Altern Med.* (2014) 2014:642942. doi: 10.1155/2014/642942
62. Liao BC, Hsieh CW, Liu YC, Tzeng TT, Sun YW, Wung BS. Cinnamaldehyde inhibits the tumor necrosis factor-alpha-induced expression of cell adhesion molecules in endothelial cells by suppressing NF-kappaB activation: effects upon IkappaB and Nrf2. *Toxicol Appl Pharmacol.* (2008) 229:161–71. doi: 10.1016/j.taap.2008.01.021
63. Lee SC, Wang SY, Li CC, Liu CT. Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice. *J Food Drug Anal.* (2018) 26:211–20. doi: 10.1016/j.jfda.2017.03.006
64. Guo JY, Huo HR, Zhao BS, Liu HB, Li LF, Ma YY, et al. Cinnamaldehyde reduces IL-1beta-induced cyclooxygenase-2 activity in rat cerebral microvascular endothelial cells. *Eur J Pharmacol.* (2006) 537:174–80. doi: 10.1016/j.ejphar.2006.03.002
65. Lang A, Lahav M, Sakhnini E, Barshack I, Fidder HH, Avidan B, et al. Allicin inhibits spontaneous and TNF-alpha induced secretion of proinflammatory cytokines and chemokines from intestinal epithelial cells. *Clin Nutr Edinb Scotl.* (2004) 23:1199–208. doi: 10.1016/j.clnu.2004.03.011
66. Shin JH, Ryu JH, Kang MJ, Hwang CR, Han J, Kang D. Short-term heating reduces the anti-inflammatory effects of fresh raw garlic extracts on the LPS-induced production of NO and pro-inflammatory cytokines by downregulating allicin activity in RAW 264.7 macrophages. *Food Chem Toxicol.* (2013) 58:545–51. doi: 10.1016/j.fct.2013.04.002
67. Dirsch VM, Gerbes AL, Vollmar AM. Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kappaB. *Mol Pharmacol.* (1998) 53:402–7. doi: 10.1124/mol.53.3.402
68. Galabov AS. Virucidal agents in the eve of manorapid synergy. *GMS Krankenhaushyg Interdiszip.* (2007) 2:Doc18.
69. Weber ND, Andersen DO, North JA, Murray BK, Lawson LD, Hughes BG. *In vitro* virucidal effects of *Allium sativum* (garlic) extract and compounds. *Planta Med.* (1992) 58:417–23. doi: 10.1055/s-2006-961504
70. Siegers CP, Röbke A, Pentz R. Effects of garlic preparations on superoxide production by phorbol ester activated granulocytes. *Phytomedicine.* (1999) 6:13–6. doi: 10.1016/S0944-7113(99)80029-4
71. Bang JS, Oh DH, Choi HM, Sur BJ, Lim SJ, Kim JY, et al. Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1beta-stimulated fibroblast-like synoviocytes and in rat arthritis models. *Arthritis Res Ther.* (2009) 11:R49. doi: 10.1186/ar2662
72. Bae GS, Kim MS, Jung WS, Seo SW, Yun SW, Kim SG, et al. Inhibition of lipopolysaccharide-induced inflammatory responses by piperine. *Eur J Pharmacol.* (2010) 642:154–62. doi: 10.1016/j.ejphar.2010.05.026
73. Wang-Sheng C, Jie A, Jian-Jun L, Lan H, Zeng-Bao X, Chang-Qing L. Piperine attenuates lipopolysaccharide (LPS)-induced inflammatory responses in BV2 microglia. *Int Immunopharmacol.* (2017) 42:44–8. doi: 10.1016/j.intimp.2016.11.001
74. Chuchawankul S, Khorana N, Poovorawan Y. Piperine inhibits cytokine production by human peripheral blood mononuclear cells. *Genet Mol Res.* (2012) 11:617–27. doi: 10.4238/2012.March.14.5
75. Vaibhav K, Shrivastava P, Javed H, Khan A, Ahmed ME, Tabassum R, et al. Piperine suppresses cerebral ischemia-reperfusion-induced inflammation through the repression of COX-2, NOS-2, and NF-κB in middle cerebral artery occlusion rat model. *Mol Cell Biochem.* (2012) 367:73–84. doi: 10.1007/s11010-012-1321-z
76. Mittal R, Gupta RL. *In vitro* antioxidant activity of piperine. *Methods Find Exp Clin Pharmacol.* (2000) 22:271–4. doi: 10.1358/mf.2000.22.5.796644
77. Avery JC, Hoffmann PR. Selenium, selenoproteins, and immunity. *Nutrients.* (2018) 10:1203. doi: 10.3390/nu10091203
78. Mahmoodpoor A, Hamishehkar H, Shadvar K, Ostadi Z, Sanaie S, Saghaleini SH, et al. The effect of intravenous selenium on oxidative stress in critically Ill patients with acute respiratory distress syndrome. *Immunol Invest.* (2019) 48:147–59. doi: 10.1080/08820139.2018.1496098
79. Dhanjal NI kaur, Sharma S, Prabhu KS, Prakash NT. Selenium supplementation through Se-rich dietary matrices can upregulate the anti-inflammatory responses in lipopolysaccharide-stimulated murine macrophages. *Food Agric Immunol.* (2017) 28:1374–92. doi: 10.1080/09540105.2017.1343805
80. Stone CA, Kawai K, Kupka R, Fawzi WW. Role of Selenium in HIV infection. *Nutr Rev.* (2010) 68:671–81. doi: 10.1111/j.1753-4887.2010.00337.x
81. Dworkin BM. Selenium deficiency in HIV infection and the acquired immunodeficiency syndrome (AIDS). *Chem Biol Interact.* (1994) 91:181–6. doi: 10.1016/0009-2797(94)90038-8
82. Wolska K, Gorska A, Antosik K, Lugowska K. Immunomodulatory effects of propolis and its components on basic immune cell functions. *Indian J Pharm Sci.* (2019) 81:575–88. doi: 10.36468/pharmaceutical-sciences.548
83. Sforcin JM, Bankova V. Propolis: is there a potential for the development of new drugs? *J Ethnopharmacol.* (2011) 133:253–60. doi: 10.1016/j.jep.2010.10.032
84. Sforcin JM. Propolis and the immune system: a review. *J Ethnopharmacol.* (2007) 113:1–14. doi: 10.1016/j.jep.2007.05.012
85. Amoros M, Lurton E, Boustie J, Girre L, Sauvager F, Cormier M. Comparison of the anti-herpes simplex virus activities of propolis and 3-Methyl-but-2-enyl caffeate. *J Nat Prod.* (1994) 57:644–7. doi: 10.1021/np50107a013
86. Schreck Bird A, Gregory PJ, Jalloh MA, Risoldi Cochrane Z, Hein DJ. probiotics for the treatment of infantile colic: a systematic review. *J Pharm Pract.* (2017) 30:366–74. doi: 10.1177/0897190016634516
87. Zelaya H, Alvarez S, Kitazawa H, Villena J. Respiratory antiviral immunity and immunobiotics: beneficial effects on inflammation-coagulation interaction during influenza virus infection. *Front Immunol.* (2016) 7:633. doi: 10.3389/fimmu.2016.00633
88. Baud D, Dimopoulou Agri V, Gibson GR, Reid G, Giannoni E. Using probiotics to flatten the curve of coronavirus disease COVID-2019 pandemic. *Front Public Health.* (2020) 8:186. doi: 10.3389/fpubh.2020.00186
89. Mortaz E, Adcock IM, Folkerts G, Barnes PJ, Paul Vos A, Garssen J. Probiotics in the management of lung diseases. *Mediators Inflamm.* (2013) 2013:751068. doi: 10.1155/2013/751068
90. Chong HX, Yusoff NAA, Hor YY, Lew LC, Jaafar MH, Choi SB, et al. Lactobacillus plantarum DR7 improved upper respiratory tract infections *via* enhancing immune and inflammatory parameters: a randomized, double-blind, placebo-controlled study. *J Dairy Sci.* (2019) 102:4783–97. doi: 10.3168/jds.2018-16103
91. Shoaib A, Xin L, Xin Y. Oral administration of *Lactobacillus acidophilus* alleviates exacerbations in Pseudomonas aeruginosa and *Staphylococcus aureus* pulmonary infections. *Pak J Pharm Sci.* (2019) 32:1621–30.
92. Kotzampassi K, Giamarellos-Bourboulis EJ, Voudouris A, Kazamias P, Eleftheriadis E. Benefits of a synbiotic formula (Synbiotic 2000Forte) in critically Ill trauma patients: early results of a randomized controlled trial. *World J Surg.* (2006) 30:1848–55. doi: 10.1007/s00268-005-0653-1
93. Namba K, Hatano M, Yaeshima T, Takase M, Suzuki K. Effects of *Bifidobacterium longum* BB536 administration on influenza infection, influenza vaccine antibody titer, and cell-mediated immunity in the elderly. *Biosci Biotechnol Biochem.* (2010) 74:939–45. doi: 10.1271/bbb.90749
94. Sharma G, Im SH. Probiotics as a potential immunomodulating pharmabiotics in allergic diseases: current status and future prospects. *Allergy Asthma Immunol Res.* (2018) 10:575–90. doi: 10.4168/aair.2018.10.6.575
95. Hajavi J, Esmaeili SA, Varasteh AR, Vazini H, Atabati H, Mardani F, et al. The immunomodulatory role of probiotics in allergy therapy. *J Cell Physiol.* (2019) 234:2386–98. doi: 10.1002/jcp.27263
96. Lin L, Jiang X, Zhang Z, Huang S, Zhang Z, Fang Z, et al. Gastrointestinal symptoms of 95 cases with SARSCoV-2 infection. *Gut.* (2020) 69:997–1001. doi: 10.1136/gutjnl-2020-321013
97. Rosa L, Cutone A, Lepanto MS, Paesano R, Valenti P. Lactoferrin: a natural glycoprotein involved in iron and inflammatory homeostasis. *Int J Mol Sci.* (2017) 18:1985. doi: 10.3390/ijms18091985
98. Ishikado A, Imanaka H, Takeuchi T, Harada E, Makino T. Liposomalization of lactoferrin enhanced it's anti-inflammatory effects *via* oral administration. *Biol Pharm Bull.* (2005) 28:1717–21. doi: 10.1248/bpb.28.1717

99. Safaeian L, Javanmard SH, Mollanoori Y, Dana N. Cytoprotective and antioxidant effects of human lactoferrin against H2O2-induced oxidative stress in human umbilical vein endothelial cells. *Adv Biomed Res.* (2015) 4:188. doi: 10.4103/1735-5362.192488
100. Li S, Zhou H, Huang G, Liu N. Inhibition of HBV infection by bovine lactoferrin and iron-, zinc-saturated lactoferrin. *Med Microbiol Immunol (Berl).* (2009) 198:19–25. doi: 10.1007/s00430-008-0100-7
101. Manjeet KR, Ghosh B. Quercetin inhibits LPS-induced nitric oxide and tumor necrosis factor-alpha production in murine macrophages. *Int J Immunopharmacol.* (1999) 21:435–43. doi: 10.1016/S0192-0561(99)00024-7
102. Geraets L, Moonen HJJ, Brauers K, Wouters EFM, Bast A, Hageman GJ. Dietary flavones and flavonoles are inhibitors of poly(ADP-ribose)polymerase-1 in pulmonary epithelial cells. *J Nutr.* (2007) 137:2190–5. doi: 10.1093/jn/137.10.2190
103. Bureau G, Longpré F, Martinoli MG. Resveratrol and quercetin, two natural polyphenols, reduce apoptotic neuronal cell death induced by neuroinflammation. *J Neurosci Res.* (2008) 86:403–10. doi: 10.1002/jnr.21503
104. Lee KM, Hwang MK, Lee DE, Lee KW, Lee HJ. Protective effect of quercetin against arsenite-induced COX-2 expression by targeting PI3K in rat liver epithelial cells. *J Agric Food Chem.* (2010) 58:5815–20. doi: 10.1021/jf903698s
105. Wu W, Li R, Li X, He J, Jiang S, Liu S, et al. Quercetin as an antiviral agent inhibits influenza A Virus (IAV) entry. *Viruses.* (2015) 8:6. doi: 10.3390/v8010006
106. Ganesan S, Faris AN, Comstock AT, Wang Q, Nanua S, Hershenson MB, et al. Quercetin inhibits rhinovirus replication *in vitro* and *in vivo*. *Antiviral Res.* (2012) 94:258–71. doi: 10.1016/j.antiviral.2012.03.005
107. Antipicornavirus flavone Ro 09-0179. *Antimicrobial Agents and Chemotherapy*. Available online at: https://aac.asm.org/content/22/4/611 (accessed July 26, 2020).
108. Chen L, Li J, Luo C, Liu H, Xu W, Chen G, et al. Binding interaction of quercetin-3-β-galactoside and its synthetic derivatives with SARSCoV 3CLpro: structure–activity relationship studies reveal salient pharmacophore features. *Bioorg Med Chem.* (2006) 14:8295–306. doi: 10.1016/j.bmc.2006.09.014
109. Glinsky GV. Tripartite combination of candidate pandemic mitigation agents: vitamin D, quercetin, and estradiol manifest properties of medicinal agents for targeted mitigation of the COVID-19 pandemic defined by genomics-guided tracing of SARSCoV-2 targets in human cells. *Biomedicines.* (2020) 8:129. doi: 10.26434/chemrxiv.12052512.v7
110. Smith M, Smith JC. Repurposing therapeutics for COVID-19: supercomputer-based docking to the SARSCoV-2 viral spike protein and viral spike protein-human ACE2 interface. *ChemRxiv [Preprint].* (2020). doi: 10.26434/chemrxiv.11871402.v2
111. Yi L, Li Z, Yuan K, Qu X, Chen J, Wang G, et al. Small molecules blocking the entry of severe acute respiratory syndrome coronavirus into host cells. *J Virol.* (2004) 78:11334–9. doi: 10.1128/JVI.78.20.11334-11339.2004

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Mrityunjaya, Pavithra, Neelam, Janhavi, Halami and Ravindra. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

PERSPECTIVE
published: 08 October 2020
doi: 10.3389/fnut.2020.569542

Rethinking Urban and Food Policies to Improve Citizens Safety After COVID-19 Pandemic

Andrea Galimberti[1†], Hellas Cena[2,3†], Luca Campone[1], Emanuele Ferri[1], Mario Dell'Agli[4], Enrico Sangiovanni[4], Michael Belingheri[5], Michele Augusto Riva[5], Maurizio Casiraghi[1] and Massimo Labra[1]*

[1] Department of Biotechnology and Biosciences, University of Milano - Bicocca, Milan, Italy, [2] Laboratory of Dietetics and Clinical Nutrition, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy, [3] Clinical Nutrition and Dietetics Service, Unit of Internal Medicine and Endocrinology, Istituti Clinici Scientifici Maugeri Istituti di Ricovero e Cura a Carattere Scientifico, Pavia, Italy, [4] Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy, [5] School of Medicine and Surgery, University of Milano - Bicocca, Monza, Italy

The ongoing pandemic caused by the coronavirus disease 2019 (COVID-19) is literally changing the world. From December 2019 to date, more than 22 million cases have been reported worldwide and global health institutions are acting to slow down the virus transmission and are looking for possible prevention strategies in case of a new outbreak. As in other endemic or pandemic phenomena, the issues mostly covered by scientific and media attention are related to the diagnostic and therapeutic approach of COVID-19. However, a still neglected issue regards the adoption of a more systemic approach considering the close connection among the infection, the environment, and human behaviors, including the role of diet and urban management. To shed light on this issue, we brought together a faculty group involving experts in environment and biodiversity, food safety, human nutrition, and behavior, bioprospecting, as well as medical doctors having a deep knowledge of the complex historical relationship between humanity and vector-borne infections. Two main aspects emerged from the integrative overview of the current COVID-19 pandemic: (i) the scientific community should start sharing social actions and policy advocacy based on the assumption that human health strongly depends upon a sustainable exploitation of natural resources in populated areas; (ii) the specific strategic role of the cities in developing sustainable food systems and promoting healthy dietary patterns. Definitely, some priority issues should be addressed to achieve these goals, such as global efforts to increase food safety and security, which would benefit from urban and peri-urban agriculture enhancement, smallholder food producers support, and ecosystem services and local biodiversity maintenance.

Keywords: virus, spillover, food safety, micronutrients, post-normal science

OPEN ACCESS

Edited by:
Alexandros Tsoupras, University of Limerick, Ireland

Reviewed by:
Carla Ferreri, National Research Council (CNR), Italy
Marwan El Ghoch, Beirut Arab University, Lebanon
Maurizio Battino, University of Vigo, Spain

***Correspondence:**
Massimo Labra
massimo.labra@unimib.it

[†] These authors share first authorship

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *04 June 2020*
Accepted: *24 August 2020*
Published: *08 October 2020*

Citation:
Galimberti A, Cena H, Campone L, Ferri E, Dell'Agli M, Sangiovanni E, Belingheri M, Riva MA, Casiraghi M and Labra M (2020) Rethinking Urban and Food Policies to Improve Citizens Safety After COVID-19 Pandemic. Front. Nutr. 7:569542. doi: 10.3389/fnut.2020.569542

INTRODUCTION

The ongoing pandemic caused by the coronavirus disease 2019 (COVID-19) is literally changing the world (1, 2). From the first documented human patient in Wuhan (Hubei, People's Republic of China) in December 2019, on August 2020, more than 22 million cases have been reported worldwide, of which more than six million still active (1% in serious or critical conditions) and

almost 800 k deaths. WHO and other authorities soon realized that it was no longer possible to contain the virus spread, but only to slow down its transmission and try, at least, to reduce "pressure" on national health systems. As in other endemic or pandemic outbreaks, the issues mostly covered by scientific and media attention are related to the diagnostic and therapeutic approach of COVID-19 contagion. However, greater consideration should be given to a systemic approach considering the close connection between this disease, the environment and human behaviors, in a framework of building a safer, more sustainable and healthier world (3). How is it possible that a virus from a Chinese market has spread to other continents so quickly, penetrating the heart of cities and killing the weakest citizens?

To shed light on this issue, we brought together a faculty group involving experts in environment and biodiversity, food safety, human nutrition, and behavior, biological activity of natural products as well as medical doctors having a deep knowledge of the complex historical relationship between humanity and vector-borne infections.

We believe that unlike the pandemics of the past, the factors triggering the current spread of COVID-19 outbreak, should be analyzed not only by scientists and politicians but also by societal stakeholders. Many European countries most afflicted with COVID-19 have started thinking ahead are now facing with the "phase two" of the COVID-19 situation by operating the recovery of industrial and social activities keeping, at the same time, the infection spread as low as possible. In such a context, the linear science, which analyzes those biological variables dealing with the pathogen and its infectivity to find possible solutions [e.g., a vaccine or a therapy (4)], clashes with the "Post-Normal Science" (PSN) approach, for which, societal values (e.g., the right to freedom, economic needs, and relational aspects) claim their importance (5). PNS is designed to deal with situations of uncertain facts, values in dispute, high stake and urgent decisions [(6); **Figure 1**]. PNS should be operated together with Responsible Research and Innovation (RRI) tools (https://www.rri-tools.eu/about-rri) that offer a broad set of strategies to address global challenges throughout the analysis of "real-world complexities' considering new scientific knowledge and technologies, but also the needs of different stakeholder categories. The contrast between these approaches to science is exerting some pressure on governments that even proposed solving the problem of COVID-19 with autonomous strategies or hypothesized to reach a sort of herd immunity by sacrificing an entire generation of older people (8, 9). This situation demonstrates the lack of organization by the modern society to address complex global issues.

Our contribution aims at supporting the adoption of a PNS response to COVID-19 pandemic, also considering the geopolitical and social aspects which caused the dramatic susceptibility to such infection. For this reason, we aimed at better addressing two main concepts regarding COVID-19 "effect" on cities and citizen-led community responses, to prevent future pandemic events.

PANDEMICS PREVENTION

The first point to discuss is the unexpected permeability of cities to this virus and hypothesize how the spillover from wildlife of such a kind of pathogens can reach urban centers. This evaluation is of primary concern to better define suitable prevention strategies for limiting or blocking the current one and other future pandemic spreads. What characterizes COVID-19 contagion, is that it fully took advantage of globalization facilities, enabling rapid spread of the virus across the world.

Generally, accurate controls are performed on goods transported worldwide but in case of novel pathogens (e.g., the COVID-19) diagnostic options are very limited. Moreover, when human mobility is a major factor in the spread of infectious diseases, we should acknowledge that screening measures adopted at airports or customs should be implemented (10). Temperature screening alone may not be very effective as it may miss travelers incubating the disease or concealing fever during travel, or it may yield false positives (e.g., having fever of a different cause), therefore it should be accompanied by other health messages, questionnaires and data collection (11).

A different scenario occurs for the trading of food commodities for which strict regulations about quality and safety impose the adoption of analytical tools and innovation technologies to prevent the spread of foodborne pathogens or other contaminants (12, 13). In most cases, food quality evaluation is based on bioindicators, both at chemical and microbiological level (14, 15). Most diagnostic techniques allow to characterize the internal microbiome and virome (16–19). Considering COVID-19, what can be said with certainty is that such controls did not occur in the Wuhan market where the virus started its incredible global spread. This situation further remarks the concept that pandemics are strictly linked to insufficient or absent food safety assessment and disease prevention protocols (e.g., as happening every time Ebola Viruses outbreak in Central Africa). It is necessary to remark that the food regulations across countries vary and the quality standards of the same food items produced in different countries are not the same (20, 21). The current COVID-19 pandemic, perhaps more than others, highlights that it is necessary to align food security protocols on a global scale since country specific inadequacies may cause serious global consequences. Therefore, we believe that to prevent future pandemic outbreaks it is more and more important to consider issues arising from the establishment of supranational risk-governance systems. These typically operate in a framework of compromise between the local governance and local producers needs and the global safety for human health. Safety is a priority for all the stakeholders associated with the entire food supply-chain. Moreover, after the COVID-19 emergency, it is desirable that citizens enhance its awareness toward the topic of food control and the concept of food safety will acquire a stronger social meaning based on "shared values, beliefs and norms that affect human mindset and behavior" (22). Consumers' behavior and choices will help modify food supply-chains safety to prevent zoonoses and reduce other risks for humans.

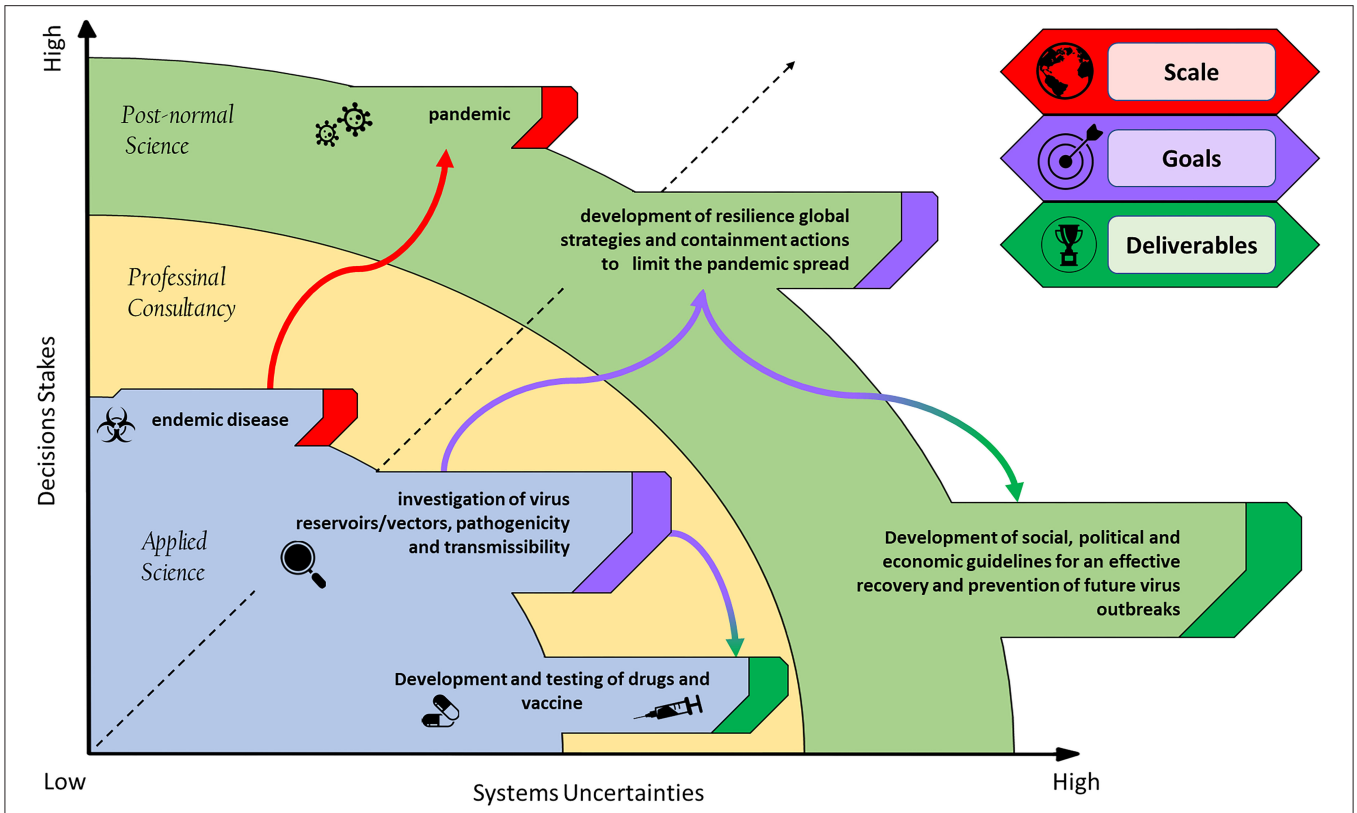


FIGURE 1 | Schematization of a virus pandemic outbreak: scale, goals, and deliverables in a post-normal science context. The post-normal science diagram [taken from (7)] provides a framework to map systems uncertainty against decision stakes.

Global citizenship is based on rights to be actively involved in debates, responsibility, shared decisions (following careful risk evaluation), and actions to control and implement shared strategies. With regard to COVID-19, we know that a bat species and/or the Malayan Pangolin have been found to be likely reservoir hosts for the virus; however, the definitive identity of any intermediate host that might have facilitated spillover to humans is still unknown (23, 24). Overall, the identification of the vector has a relatively important value. The central point is that the unceasing exploitation of wildlife and habitat has dramatically increased the risk of exposure to zoonotic diseases, as already and sadly demonstrated for example by HIV, Ebola and H5N1 (25). But how calculating the risk of these phenomena? Are stakeholders and citizens aware of the risks? These elements are fundamental for a PNS discussion that is necessary to drive the path of food safety.

It is time to realize that food safety cannot rely only on the production chain but potential risks to human health and the environment should be considered as well. The increasing advances in scientific and technological tools have now been adopted to assess such risks, thus opening a new era of "prediction" rather than "reaction" to reduce pathogen contamination and foodborne outbreaks (26). For example, the current trends in food safety research rely on the application of (i) genomic analyses for foodborne pathogen identification and traceability, (ii) Geographic Information Systems (GIS) to prevent and predict the spatial spread of pathogens outbreak, (iii) tools adapted from landscape ecology (species distribution and niche modeling) and Social Network Analysis for predicting patterns of disease outbreaks, as well as guidance for interventions, and (iv) meta-analysis tools to confer an overall summary of available study findings, providing generalizable estimates and generating strategic highlights to be used by policy-makers and decision makers (26).

Overall, risk prevention remains the key factor. The history of pandemics teaches us that almost all recent human pandemics and most of the emerging infectious diseases originated from animals (mainly in wildlife). It is known that species more resistant to human pressure are likely to become the new competent hosts of vector-borne diseases and then to become the most probable spillover agents toward human hosts (27, 28). Furthermore, we must remember that biodiversity perturbation and its trivialization is the main trigger of virus spillover events (29), as probably happened for COVID-19 (**Figure 2**).

Given these assumptions, the international food policies concerning food safety should consider biodiversity and ecological interventions to prevent zoonotic spillover events. This would be especially urgent in rural areas, where farming and livestocking often overlap with wildlife species ranges and it has been documented that livestock species usually act as

Virus (Family)	Year	Principal Reservoir hosts	Spillover category	Introduction pathways*
Andes virus (Bunyaviridae)	1995	Mammalia: Rodentia		Ingestion of water or food (contaminated by), or direct contact with rodent feces or other fluids.
Bundibugyo ebolavirus (Filoviridae)	2007	unknown		Contact with meat or fluids of the infected host.
Crimean Congo hemorrhagic fever virus (Bunyaviridae)	1944	Mammalia: Rodentia, Lagomorpha and other 7 orders		Hematophagous insect vector; contact with meat or fluids of the infected host.
Guanarito virus (Arenaviridae)	1989	Mammalia: Rodentia		Ingestion of water or food (contaminated by), or direct contact with rodent feces or other fluids.
Hepatitis E (Hepeviridae)	1955	Mammalia: Artiodactyla, Rodentia and other 10 orders		Ingestion of contaminated water or food.
Influenza A (Orthomyxoviridae)	around 1850	Mammalia and Aves (six orders)		Direct contact with with faeces, or fluids of infected animals.
Influenza B (Orthomyxoviridae)	?	Mammalia: Carnivora		_
Junin virus (Arenaviridae)	1958	Mammalia: Rodentia		Ingestion of water or food (contaminated by), or direct contact with rodent feces or other fluids.
Lassa virus (Arenaviridae)	1950s	Mammalia: Rodentia		Ingestion of water or food (contaminated by), or direct contact with rodent feces or other fluids.
Lujo virus (Arenaviridae)	2008	Mammalia: Rodentia		Ingestion of water or food (contaminated by), or direct contact with rodent feces or other fluids.
Machupo virus (Arenaviridae)	1962	Mammalia: Rodentia		Ingestion of water or food (contaminated by), or direct contact with rodent feces or other fluids.
Marburg virus (Filoviridae)	1960s	Mammalia: Chiroptera, Primates		Direct contact with with faeces, or fluids of infected animals.
Monkeypox virus (Poxviridae)	1958	Mammalia: Chiroptera, Primates		Contact with meat or fluids of the infected animals, or bite.
Nipah virus (Paramyxoviridae)	1998	Mammalia: Chiroptera, Artiodactyla		Ingestion/contact of infected swine meat and fruit or water contaminated by infected bats.
Rotavirus A (Reoviridae)	1950s	Mammalia and Aves (10 orders)		Direct contact with with faeces, or fluids of infected animals.
SARS coronavirus (Coronaviridae)	2002	Mammalia: Chiroptera and other four orders		Direct contact with with faeces, or fluids of infected animals.
Sudan ebolavirus (Filoviridae)	1976	unknown	U	Direct contact with with faeces, or fluids of infected animals.
Whitewater Arroyo virus (Arenaviridae)	1990s	Mammalia: Rodentia		Ingestion of water or food (contaminated by), or direct contact with rodent feces or other fluids.
Zaire ebola virus (Filoviridae)	1976	Mammalia: Chiroptera (suspected)		Direct contact with with faeces, or fluids of infected animals.

FIGURE 2 | List of the principal pandemic and emerging zoonotic viruses showing human-to-human transmission after the spillover is occurred. Summarized host and spillover interface data are provided as in (29) (mouse: directly from wildlife; swine: directly from domestic animals; mosquito: transmission by vector involving wildlife or domestic host respectively; U: unknown). Detailed references for each listed virus are provided in (29).

intermediate hosts of spillover events (e.g., influenza A and SARS coronavirus, **Figure 1**). For this reason, it is also time to rethink urban areas by projecting proximity buffer zones to prevent direct contact between agricultural/zootechnic activities and natural habitats. Finally, the conservation of natural biodiversity and its related species interactions are essential conditions to reduce the risk of spillover events (27). On the whole, a cooperative work (RRI-driven) involving human-health agencies, agricultural authorities, farmers, and natural resource managing institutions, could be essential to promote the global ecological management to avoid the spread of a new putative pandemic "COVID-20" or other risky vector-borne pathogens that may adversely affect human health, the environment and economy.

CITIZEN FORTIFICATION

Our second consideration regards the fragility of citizens, especially the weakest ones such as the elderly, and their sensitivity to diseases.

It is now clear that these social categories are the most susceptible to severe COVID-19 outcomes, particularly if they already suffer from multiple pathologies. Diabetes is the most common comorbidity observed in infected deceased patients in Italy, after hypertension (30). Furthermore, recent data showed a high prevalence of obesity (26%) and overweight (41%) in 928 Italian patients, median age 65 years, from 76 different Italian ICUs, confirming evidence available so far in the literature supporting impaired immune response to viral infections (31). Therefore, beyond the infectious capacity of this virus, it is important to focus on those elements of modern society which could increase citizens' vulnerability, including diet, lifestyle and environmental factors, strictly linked to morbidity and mortality for all Non-Communicable Diseases (NCDs) (32, 33). Although this concept is well-established, today, the global average consumption of healthy foods is substantially lower than the reference dietary intake, whereas overconsumption of highly processed, energy dense, and nutrient-poor foods is increasing (34).

The Mediterranean diet is considered by UNESCO as one of the "Intangible Cultural Heritage of Humanity" with multiple health benefits, including fortification of immune defenses. However, epidemiological data on COVID-19 would seem to contradict this belief since Mediterranean countries (e.g., Italy and Spain) have the highest number of confirmed COVID-19 cases in the world. Five years after the EXPO 2015, dedicated to the theme "Feeding the Planet, Energy for life," the city of Milan, which hosted the event, is under siege by COVID-19 pandemic. Recent dietary changes within the Mediterranean basin, with a decreased consumption of plant foods, increased consumption of fast meals and junk food, and negative health consequences such as rise in obesity rates and in NCDs incidence (e.g., diabetes, cardiovascular diseases, and cancer) are partially responsible of this burden (35).

The modern-day change in food choices is the results of lifestyle standardization, enhanced technologies in food production and processing and limited time for culinary activities. This caused for example the progressive erosion of Mediterranean food cultures (36, 37). Moreover, environmental emergence, such as water scarcity in most Mediterranean countries and land wasting also has deleterious consequences on Mediterranean food production. Global climate changes have also produced the failure of several crops, fisheries, and livestock productions, and the declining of Mediterranean biodiversity and agrobiodiversity does not allow the selection of new varieties and resistant breeds. So, once again the erosion of the environment and biodiversity is closely connected to health risks (38). How to react to these social and environmental changes showing serious health consequences? The sustainability of the food supply chain is certainly essential (34). Kinnunen et al. showed that less than one-third of the world's population can meet their food demand within a 100-km radius (39). We should also change our view of food and diet which should no longer, or rather not only, considered an energy source but a reservoir of bioactive molecules beneficial to human health. Greater consumption of health-promoting foods and limited intake of unhealthier options are intrinsic to the eating habits of certain regional diets such as the Mediterranean diet (32). Healthy dietary patterns positively influence health and promotes the prevention of common non-communicable diseases (NCDs), strengthening host community defenses (32, 36). This concept assumes a particular importance since the over 65 years old citizens could be more at risk of being infected by COVID-19, not only for intrinsic conditions due to natural aging processes and comorbidities development, but also for inadequate nutritional status and related inadequate intake of macronutrients (e.g., proteins and healthy fatty acids, like omega-3), micronutrients (e.g., vitamins A, B6, B12, C, D, E, and folate), trace elements (e.g., zinc, iron, selenium, magnesium, and copper) and phytochemicals which are pillars in preventing many chronic degenerative diseases and supporting the immune system (40).

This would seem to be true even for younger patients with metabolic and cardiovascular diseases, showing severe Acute Respiratory Distress Syndrome (ARDS) caused by COVID-19 (41). These considerations are also well-known for past pandemics. A recent retrospective data analysis from the 1918 pandemic flu, showed that nutrition played a consistent role in the severity of the disease and was related to mortality also in younger age groups (42). More recently, chronic malnutrition has been correlated to high morbidity and mortality during the 2009 influenza pandemic (43). Similarly, malnourished children appear to be at increased risk for viral pneumonia (44).

In dietary recommendation, fat quality has to be addressed (45), since evidence shows the need to achieve a balance between dietary intake of omega-6 and omega-3 for optimal nutrition (46), especially in those subjects more vulnerable to malnutrition and "silent inflammation" which disposes to a greater propensity to viral infections (47, 48). Certainly, the presence of an unbalance between pro- and anti-inflammatory lipid mediators has been reported in literature (49) and it is important to remark that science has already given solutions (50) which are not adopted in new diagnostic policies for primary and secondary prevention.

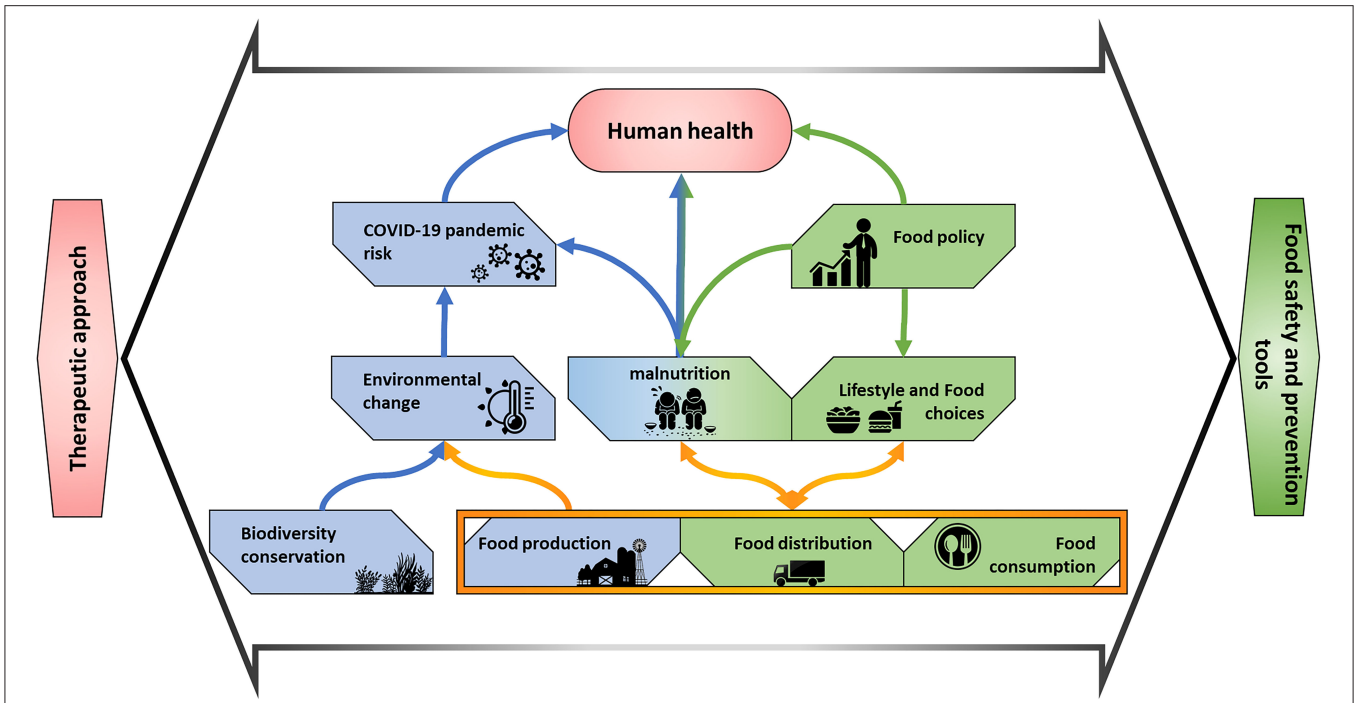


FIGURE 3 | Network of interactions including food supply-chain elements and their effects (i) on environment and biodiversity that might lead to increased risk of spillover and (ii) on human health and wellbeing that affect infection risk prevention and therapy response.

Moreover, food supplements such as vitamins C and D might also be considered to help both innate and adaptive immune cells (51–54). Studies on human coronaviruses (HCoVs), including severe acute respiratory syndrome coronavirus (SARS-CoV), have highlighted that secondary metabolites of some plant species seem to inhibit virus proteins, cellular infection, and intracellular replication (55). Extracts of spontaneous plants such as root tubers from *Rheum officinale* Baill. (rhubarb), root tubers or vines from *Polygonum multiflorum* Thunb., (Polygonaceae) showed an inhibitory activity against the interaction of SARS-CoV S protein with ACE2 (56). Procyanidins and other secondary metabolites extracted from Cinnamomi cortex (*Cinnamomum cassia* J. Presl) can reduce the virus infection by interfering with endocytosis (57). The extract of *Cimicifuga* rhizoma, *Meliae cortex*, *Coptidis* rhizoma, *Phellodendron* cortex, and *Sophora subprostrata* radix showed an ability to inhibit of RNA-dependent RNA polymerase and/or proteases crucial for coronavirus RNA replication (58). Finally, the antiviral activity of licorice (*Glycyrrhiza glabra* L.), containing glycyrrhizin, inhibits replication, absorption and penetration of the SARS-CoV acting on the early steps of the replicative cycle (59). In this field, traditional Chinese medicine is working actively to identify dedicated compounds to specifically contrast COVID-19 infection (60). This practice relying on biodiversity and known as "bioprospecting," may be a good strategy to find compounds having a positive effect on human health as well as to find new raw materials to produce novel and fortified foods for modern citizens.

PANDEMICS AND THE INTERACTION WITH ENVIRONMENTAL AND FOOD POLICY

Recently, Di Marco et al. (27) suggested that the risk of Emerging Infectious Diseases (EIDs) is a key aspect for developing suitable policy strategies at the global scale. Within this framework, the conservation of biodiversity and food production are two additional pillars that should be considered to prevent drastic environmental changes and the risks of zoonoses, and virus spread. In **Figure 3** we aimed at further remarking this concept including additional factors strictly related to COVID-19 pandemic.

From a nutritional point of view, addressing subclinical micronutrient deficiencies is one of the first steps that must be considered to improve resistance to infectious diseases like COVID-19 (and other pathogens) (61). It is therefore recommended that micronutrient testing, such as vitamin D measurement, should be applied in the annual check up of selected individuals at high risk of deficiency (62).

A nutritional approach, ensuring a long-term sustainability, is essential to improve micronutrient status by increasing the availability and consumption of micronutrient-rich foods (63). Besides lifestyle changes, including diet has been shown to positively affect metabolic and cardiovascular diseases, which are the most frequent comorbidities associated to severe COVID-19 disease. The spread of inappropriate eating habits and inactivity in Western societies, particularly among the younger,

"comfortably off" generations, has led to the development of chronic degenerative diseases defined as "comfort" diseases (64) early in life, leading to an increase in premature deaths for NCDs.

Food is readily available in developed countries but there is an evident split-up between scientific evidence, food choices, and dietary patterns of consumers. This, over time has favored the spread of the obesity epidemic and other diet-related diseases.

It is time to acknowledge that environmental factors exert a major influence on dietary behavior, primarily by facilitating meals consumption away from home and by minimizing time dedicated to meal preparation and consumption and secondly, making food of poor nutritional quality available on the market and appealing for appearance, taste and price. This burden is exerted by market rules that affect behavior and food choices with scarce public awareness of the potential negative impact on health. It is necessary that science, technology, education, legislation, and community policies combine to create the urban structures and environment required to encourage healthy lifestyle including dietary choices, not just for few, but for everyone (65).

More efforts must be addressed to reduce exposure to ambient air pollution, strongly associated with population density (66), promoting chronic inflammatory state and affecting resilience to infectious diseases not last COVID-19 (67).

Finally, Western medicine generally tends to identify pharmacological molecules that act on specific disease mechanisms; however, human body complexity and individual answers are sometimes underestimated. Thus, it could happen that infected patients die more due to comorbidities associated to infection with COVID-19 than for COVID-19 per se. The time has come to apply a systems biology approach where drugs, foods and lifestyle work in synergy to promote patient healing and prevent further infections, gaining a holistic approach to community health (68) to support the further personalization of health and social care (69).

For these reasons, in our scheme, we stressed the impact of environment and food system of therapeutic approaches.

This integrative framework demands both an increased attitude of sharing by the scientific and technical community as well as a social participation since we believe that, as previously anticipated, food safety and human health should be regarded more as a social issue.

CONCLUSIONS

Our final suggestion is to start a frank and open scientific discussion on COVID-19 issues and future risks for new pandemic outbreaks, continuing the legacy of EXPO 2015, declared in the Milan Urban Food Policy Pact, signed by more than 200 cities in the world (65). In this document, the strategic role of the cities in developing sustainable food systems and promoting healthy diets is stated, yet acknowledging all the differences in their natural and policy endowments, including economic background and cultural innovation, managing vast public resources, infrastructure, investments, and expertise. These issues will be fundamental especially for those countries that are coming out of COVID-19 lockdown restrictions and where it is more expected that political and social contrast will emerge if different stakeholders needs and opinions will not be analyzed and considered for planning the recovery after the pandemic event.

We would like to undermine and integrate this overview with few take-home messages that arise from the teachings of this dramatic situation we are experiencing firsthand:

(i) Food safety is a global issue. The unsafety of local food markets, like the Wuhan's one, can exert severe and global impact.
(ii) Smallholder food producers play a key role in feeding cities, by helping to maintain resilient, equitable, culturally appropriate food systems, and promote sustainable diets. In cities with a high percentage of elderly, local food production should be tailored for specific targets to maintain adequate nutritional status, including fortification of immune system. Similarly, in developing countries, smallholder farms should improve the production of local crops rich in macro and micronutrients to improve food security and health of local populations (70).
(iii) Acknowledgment that urban and peri-urban agriculture may offer opportunities to protect and integrate biodiversity into urban landscapes and food systems, thereby contributing to synergies across food security, ecosystem services, and human wellbeing. This is very important to prevent the spillover of viruses but also to offer better efficacy of new drugs synthesized to fight future diseases.

A unified approach to nutritional screening and assessment is recommended guiding toward integrated panels of biomarkers to investigate the nutritional status and predict future health outcomes of the individual and moving from stratified to personalized to precision nutrition (71).

The whole scientific community should start sharing directions, social actions and policy advocacy recognizing that the health of people is closely connected to the health of biodiversity and ecosystems where they live. In this context, the "One health" approach (https://www.cdc.gov/onehealth/basics/index.html) represent a good starting point for successful public health interventions by fostering collaboration across all sectors, taking the right steps to promote health not for the few but for the whole planet.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

This opinion paper was conceived by ML, AG, and HC. All authors contributed to its execution and write up with ML leading on the manuscript structure, biodiversity-related aspects

were contributed AG and MC, nutrition aspects by HC, botanic and phytochemistry aspects by MD, ES, EF, and LC, and medical aspects by MR and MB.

ACKNOWLEDGMENTS

The authors are grateful to Davide Magnani for artworks support.

REFERENCES

1. Heymann DL, Shindo N. COVID-19: what is next for public health? *Lancet.* (2020) 395:542–5. doi: 10.1016/S0140-6736(20)30374-3
2. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun.* (2020) 109:102433. doi: 10.1016/j.jaut.2020.102433
3. European Commission. *The Junction of Health, Environment and the Bioeconomy: Foresight and Implications for European Research and Innovation Policies.* (2016). Available online at: https://op.europa.eu/en/publication-detail/-/publication/375971b3-ba8a-11e5-8d3c-01aa75ed71a1 (accessed April 7, 2020).
4. Sharpe HR, Gilbride C, Allen E, Belij-Rammerstorfer S, Bissett C, Ewer K, et al. The early landscape of coronavirus disease 2019 vaccine development in the UK and rest of the world. *Immunology.* (2020) 160:223–32. doi: 10.1111/imm.13222
5. Nicola M, Alsafi Z, Sohrabi C, Kerwan A, Al-Jabir A, Iosifidis C, et al. The socio-economic implications of the coronavirus and COVID-19 pandemic: a review. *Int J Surg.* (2020) 78:185–93. doi: 10.1016/j.ijsu.2020.04.018
6. Funtowicz SO, Ravetz JR. Science for the post normal age. In: Westra L, Lemons J, editors. *Perspectives on Ecological Integrity.* Dordrecht: Springer (1995). p. 146–61. doi: 10.1007/978-94-011-0451-7_10
7. Funtowicz SO, Ravetz JR. Post-normal science. *International Society for Ecological Economics.* Online Encyclopedia of Ecological Economics (2003).
8. Horton R. COVID-19 and the NHS– "a national scandal". *Lancet.* (2020) 395:1022. doi: 10.1016/S0140-6736(20)30727-3
9. Altmann DM, Douek DC, Boyton RJ. What policy makers need to know about COVID-19 protective immunity. *Lancet.* (2020) 395:1527–29. doi: 10.1016/S0140-6736(20)30985-5
10. Gaber W, Goetsch U, Diel R, Doerr HW, Gottschalk R. Screening for infectious diseases at international airports: the Frankfurt model. *Aviat Space Environ Med.* (2009) 80:595–600. doi: 10.3357/ASEM.2360.2009
11. World Health Organization. *Key Considerations for Repatriation Quarantine of Travellers in Relation to the Outbreak of Novel Coronavirus 2019-nCoV.* (2020). Available online at: https://www.who.int/ith/Repatriation_Quarantine_nCoV-key-considerations_HQ-final11Feb.pdf (accessed August 1, 2020).
12. Galimberti A, Casiraghi M, Bruni I, Guzzetti L, Cortis P, Berterame N, et al. From DNA barcoding to personalized nutrition: the evolution of food traceability. *Curr Opin Food Sci.* (2019) 21:41–8. doi: 10.1016/j.cofs.2019.07.008
13. Behnke K, Janssen MFWHA. Boundary conditions for traceability in food supply chains using blockchain technology. *Int J Inf Manage.* (2020) 52:101969. doi: 10.1016/j.ijinfomgt.2019.05.025
14. Kourkoutas Y, Chorianopoulos N, Nisiotou A, Valdramidis VP, Karatzas KA. Application of innovative technologies for improved food quality and safety. *BioMed Res Int.* (2016) 2016:9160375. doi: 10.1155/2016/9160375
15. Campone L, Rizzo S, Piccinelli AL, Celano R, Pagano I, Russo M, et al. Determination of mycotoxins in beer by multi heart-cutting two-dimensional liquid chromatography tandem mass spectrometry method. *Food Chem.* (2020) 318:126496. doi: 10.1016/j.foodchem.2020.126496
16. Rosario K, Breitbart M. Exploring the viral world through metagenomics. *Curr Opin Virol.* (2011) 1:289–97. doi: 10.1016/j.coviro.2011.06.004
17. Ercolini D. High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. *Appl Environ Microbiol.* (2013) 79:3148–55. doi: 10.1128/AEM.00256-13
18. Galimberti A, Bruno A, Mezzasalma V, De Mattia F, Bruni I, Labra M. Emerging DNA-based technologies to characterize food ecosystems. *Food Res Int.* (2015) 69:424–33. doi: 10.1016/j.foodres.2015.01.017
19. Nieuwenhuijse DF, Koopmans MP. Metagenomic sequencing for surveillance of food-and waterborne viral diseases. *Front Microbiol.* (2017) 8:230. doi: 10.3389/fmicb.2017.00230
20. Ezzatpanah H, Lelieveld H. Scientific trends of global food laws and regulations. *Qual Assur Saf Crop.* (2019) 11:589–91. doi: 10.3920/QAS2019.x002
21. Lo Piano S, Robinson M. Nutrition and public health economic evaluations under the lenses of post normal science. *Futures.* (2019) 112:102436. doi: 10.1016/j.futures.2019.06.008
22. Global Food Safety Initiative. *A Culture of Food Safety*. A position paper from the Global Food Safety Initiative (GFSI) (2018). Available online at: https://mygfsi.com/wp-content/uploads/2019/09/GFSI-Food-Safety-Culture-Full.pdf (accessed August 1, 2020).
23. Lam TTY, Jia N, Zhang YW, Shum MHH, Jiang JF, Zhu HC, et al. Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins. *Nature.* (2020) 583:282–5. doi: 10.1038/s41586-020-2169-0
24. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* (2020) 579:270–3. doi: 10.1038/s41586-020-2012-7
25. Mendoza H, Rubio AV, García-Peña GE, Suzán G, Simonetti JA. Does land-use change increase the abundance of zoonotic reservoirs? Rodents say yes. *Eur J Wildl Res.* (2020) 66:6. doi: 10.1007/s10344-019-1344-9
26. Wang S, Weller D, Falardeau J, Strawn LK, Mardones FO, Adell AD, et al. Food safety trends: from globalization of whole genome sequencing to application of new tools to prevent foodborne diseases. *Trends Food Sci Technol.* (2016) 57:188–98. doi: 10.1016/j.tifs.2016.09.016
27. Di Marco M, Baker ML, Daszak P, De Barro P, Eskew EA, Godde CM, et al. Sustainable development must account for pandemic risk. *Proc Natl Acad Sci USA.* (2020) 117:3888–92. doi: 10.1073/pnas.2001655117
28. Zhang G, Li B, Yoo D, Qin T, Zhang X, Jia Y, et al. Animal coronaviruses and SARS-CoV-2. *Transbound Emerg Dis.* (2020) 1–14. doi: 10.1111/tbed.13791
29. Johnson CK, Hitchens PL, Evans TS, Goldstein T, Thomas K, Clements A, et al. Spillover and pandemic properties of zoonotic viruses with high host plasticity. *Sci Rep.* (2015) 5:14830. doi: 10.1038/srep14830
30. ISS. *Characteristics of COVID-19 Patients Dying in Italy.* Report based on available data (2020). Available online at: https://www.epicentro.iss.it/en/coronavirus/bollettino/Report-COVID-2019_23_april_2020.pdf (accessed August 1, 2020).
31. Cena H, Chieppa M. Coronavirus disease (COVID-19 - SARS-CoV-2) and nutrition, is the Italian infection suggesting a connection? *Front Immunol.* (2020) 11:944. doi: 10.3389/fimmu.2020.00944
32. Cena H, Calder PC. Defining a healthy diet: evidence for the role of contemporary dietary patterns in health and disease. *Nutrients.* (2020) 12:334. doi: 10.3390/nu12020334
33. Gaddi AV, Capello F, Andrisano V, Aspriello SD, Bertolotti M, Bonsanto F, et al. Humankind versus virus: are we winning the battle but losing the war? *Med J Nutrition Metab.* (2020) 13:1–5. doi: 10.3233/MNM-200415
34. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet.* (2019) 393:447–92. doi: 10.1016/S0140-6736(18)31788-4
35. Belahsen R. Nutrition transition and food sustainability. *Proc Nutr Soc.* (2014) 73:385–88. doi: 10.1017/S0029665114000135
36. Lăcătu?u CM, Grigorescu ED, Floria M, Onofriescu A, Mihai BM. The Mediterranean Diet: from an environment-driven food culture to an emerging medical prescription. *Int J Environ Res Public Health.* (2019) 16:942. doi: 10.3390/ijerph16060942
37. Barnaba L, Intorre F, Azzini E, Ciarapica D, Venneria E, Foddai MS, et al. Evaluation of the adherence to the Mediterranean diet and its association with clinical and biological markers in an Italian population. *Nutrition.* (2020) 2020:110813. doi: 10.1016/j.nut.2020.110813
38. Tilman D, Clark M. Global diets link environmental sustainability and human health. *Nature.* (2014) 515:518–522. doi: 10.1038/nature13959

39. Kinnunen P, Guillaume JH, Taka M, D'Odorico P, Siebert S, Puma MJ, et al. Local food crop production can fulfil demand for less than one-third of the population. *Nature Food.* (2020) 1:229–37. doi: 10.1038/s43016-020-0060-7
40. Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients.* (2020) 12:1181. doi: 10.3390/nu12041181
41. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA.* (2020) 2019:25–8. doi: 10.1001/jama.2020.2648
42. Short KR, Kedzierska K, van de Sandt CE. Back to the future: lessons learned from the 1918 influenza pandemic. *Front Cell Infect Microbiol.* (2018) 8:343. doi: 10.3389/fcimb.2018.00343
43. Reyes L, Arvelo W, Estevez A, Gray J, Moir JC, Gordillo B, et al. Population-based surveillance for 2009 pandemic influenza A (H1N1) virus in Guatemala, 2009. *Influenza Other Respir Viruses*. (2010) 4:129–40. doi: 10.1111/j.1750-2659.2010.00138.x
44. Maruyama T, Fujisawa T, Suga S, Nakamura H, Nagao M, Taniguchi KT, et al. Outcomes and prognostic features of patients with influenza requiring hospitalization and receiving early antiviral therapy: a prospective multicenter cohort study. *Chest.* (2016) 149:526–34. doi: 10.1378/chest.14-2768
45. Murray CJ. Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study. *Lancet.* (2019) 393:1958–72. doi: 10.1016/S0140-6736(19)30041-8
46. Marangoni F, Agostoni C, Borghi C, Catapano AL, Cena H, Ghiselli A, et al. Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects. *Atherosclerosis.* (2020) 292:90–8. doi: 10.1016/j.atherosclerosis.2019.11.018
47. de Jong AJ, Kloppenburg M, Toes RE, Ioan-Facsinay A. Fatty acids, lipid mediators, and T-cell function. *Front Immunol.* (2014) 5:483. doi: 10.3389/fimmu.2014.00483
48. Kumar NG, Contaifer D, Madurantakam P, Carbone S, Price ET, Van Tassell B, et al. Dietary bioactive fatty acids as modulators of immune function: implications on human health. *Nutrients.* (2019) 11:2974. doi: 10.3390/nu11122974
49. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature.* (2014) 510:92–101. doi: 10.1038/nature13479
50. Ferreri C, Masi A, Sansone A, Giacometti G, Larocca AV, Menounou G, et al. Fatty acids in membranes as homeostatic, metabolic and nutritional biomarkers: recent advancements in analytics and diagnostics. *Diagnostics.* (2017) 7:1. doi: 10.3390/diagnostics7010001
51. Greiller CL, Martineau AR. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients.* (2015) 7:4240–70. doi: 10.3390/nu7064240
52. Hemila H, Chalker E. Vitamin C can shorten the length of stay in the ICU: a meta-analysis. *Nutrients.* (2019) 11:1–30. doi: 10.3390/nu11040708
53. Biesalski HK. Vitamin D deficiency and co-morbidities in COVID-19 patients – a fatal relationship? *Nfs J.* (2020) 20:10–21. doi: 10.1016/j.nfs.2020.06.001
54. Simonson W. Vitamin C and coronavirus. *Geriatr Nurs.* (2020) 41:331–2. doi: 10.1016/j.gerinurse.2020.05.002
55. Zhou Y, Hou Y, Shen J, Huang Y, Martin W, Cheng F. Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. *Cell Disc.* (2020) 6:1–18. doi: 10.1038/s41421-020-0153-3
56. Ho TY, Wu SL, Chen JC, Li CC, Hsiang CY. Emodin blocks the SARS coronavirus spike protein and angiotensin-converting enzyme 2 interaction. *Antivir Res.* (2007) 74:92–101. doi: 10.1016/j.antiviral.2006.04.014
57. Zhuang M, Jiang H, Suzuki Y, Li X, Xiao P, Tanaka T, et al. Procyanidins and butanol extract of Cinnamomi Cortex inhibit SARS-CoV infection. *Antiviral Res.* (2009) 82:73–81. doi: 10.1016/j.antiviral.2009.02.001
58. Kim HY, Shin HS, Park H, Kim YC, Yun YG, Park S, et al. *In vitro* inhibition of coronavirus replications by the traditionally used medicinal herbal extracts, Cimicifuga rhizoma, Meliae cortex, Coptidis rhizoma, and Phellodendron cortex. *J Clin Virol.* (2008) 41:122–8. doi: 10.1016/j.jcv.2007.10.011
59. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of sars-associated coronavirus. *Lancet.* (2003) 361:2045–2046. doi: 10.1016/S0140-6736(03)13615-X
60. Zhang DH, Wu KL, Zhang X, Deng SQ, Peng B. *In silico* screening of Chinese herbal medicines with the potential to directly inhibit 2019 novel coronavirus. *J Integr Med.* (2020) 18:152–58. doi: 10.1016/j.joim.2020.02.005
61. Cena H, Maffoni S, Braschi V, Brazzo S, Pallavicini C, Vietti I, et al. Position paper of the Italian Association of Medical Specialists in Dietetics and Clinical Nutrition (ANSISA) on nutritional management of patients with COVID-19 disease. *Med J Nutrition Metab.* (2020) 13:113–7. doi: 10.3233/MNM-200425
62. Pilz S, Zittermann A, Trummer C, Theiler-Schwetz V, Lerchbaum E, Keppel MH, et al. Vitamin D testing and treatment: a narrative review of current evidence. *Endocr Connect.* (2019) 8:R27–43. doi: 10.1530/EC-18-0432
63. Thompson B, Amoroso L. editors. *Improving Diets and Nutrition: Food-Based Approaches*. Rome: CABI (2014). doi: 10.1079/9781780642994.0000
64. Allegri C, Turconi G, Cena H. Dietary attitudes and diseases of comfort. *Eat Weight Disord.* (2011) 16:226?35. doi: 10.1007/BF03327465
65. *Milan Urban Food Policy Pact.* (2015). Available online at: http://www.milanurbanfoodpolicypact.org/text/ (accessed April 7, 2020).
66. Chen B, Kan H. Air pollution and population health: a global challenge. *Environ Health Prev.* (2008) 13:94–101. doi: 10.1007/s12199-007-0018-5
67. Hoang U, Jones NR. *Is there an Association Between Exposure to Air Pollution and Severity of COVID-19 Infection?* Oxford COVID-19 Evidence Service Team Centre for Evidence-Based Medicine (2020). Available online at: https://www.cebm.net/covid-19/is-there-an-association-between-exposure-to-air-pollution-and-severity-of-covid-19-infection/ (accessed August 1, 2020).
68. Greene JA, Loscalzo J. Putting the patient back together-social medicine, network medicine, and the limits of reductionism. *N Engl J Med.* (2017) 377:2493–9. doi: 10.1056/NEJMms1706744
69. Miller C, Wilton C. *Developing the Power of Strong, Inclusive Communities.* A framework for Health and Wellbeing Boards Office for Public Management (2014). Available online at: https://www.thinklocalactpersonal.org.uk/_assets/Resources/BCC/Report/TLAP_Developing_the_Power_Brochure_FINAL.pdf (accessed August 1, 2020).
70. Conti MV, Campanaro A, Coccetti P, De Giuseppe R, Galimberti A, Labra M, et al. Potential role of neglected and underutilized plant species in improving women's empowerment and nutrition in areas of sub-Saharan Africa. *Nutr Rev.* (2019) 77:817–28. doi: 10.1093/nutrit/nuz038
71. Ordovas Jose M, Ferguson Lynnette R, Tai E Shyong Mathers John C. Personalised nutrition and health *BMJ.* (2018) 361:bmj.k2173. doi: 10.1136/bmj.k2173

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer ML declared a past co-authorship with one of the author MR to the handling editor.

Copyright © 2020 Galimberti, Cena, Campone, Ferri, Dell'Agli, Sangiovanni, Belingheri, Riva, Casiraghi and Labra. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

REVIEW
published: 22 October 2020
doi: 10.3389/fnut.2020.580504

Clinical Impact Potential of Supplemental Nutrients as Adjuncts of Therapy in High-Risk COVID-19 for Obese Patients

Emre Sahin[1], *Cemal Orhan*[1], *Fatih M. Uckun*[2,3] *and Kazim Sahin*[1]*

[1] Department of Nutrition, School of Veterinary Medicine, Firat University, Elazig, Turkey, [2] COVID-19 Task Force, Reven Pharmaceuticals, Golden, CO, United States, [3] Department of Developmental Therapeutics, Immunology and Integrative Medicine, Ares Pharmaceuticals, St. Paul, MN, United States

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19) in China at the end of 2019 caused a major global pandemic and continues to be an unresolved global health crisis. The supportive care interventions for reducing the severity of symptoms along with participation in clinical trials of investigational treatments are the mainstay of COVID-19 management because there is no effective standard therapy for COVID-19. The comorbidity of COVID-19 rises in obese patients. Micronutrients may boost the host immunity against viral infections, including COVID-19. In this review, we discuss the clinical impact potential of supplemental nutrients as adjuncts of therapy in high-risk COVID-19 for obese patients.

Keywords: SARS-CoV-2, inflammation, cytokines, micronutrients, nutrition

OPEN ACCESS

Edited by:
Alexandros Tsoupras, University of Limerick, Ireland

Reviewed by:
Alex Kojo Anderson, University of Georgia, United States
Carla Ferreri, National Research Council (CNR), Italy

***Correspondence:**
Kazim Sahin
nsahinkm@yahoo.com

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *06 July 2020*
Accepted: *10 September 2020*
Published: *22 October 2020*

Citation:
Sahin E, Orhan C, Uckun FM and Sahin K (2020) Clinical Impact Potential of Supplemental Nutrients as Adjuncts of Therapy in High-Risk COVID-19 for Obese Patients. Front. Nutr. 7:580504. doi: 10.3389/fnut.2020.580504

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19) has been declared a pandemic by the World Health Organization with more than 13.378.800 million confirmed cases and more than 580.000 deaths worldwide (1). Immunocompromised risk groups of the populations have high mortality rates because of the insufficient host immunity (2, 3). SARS-CoV-2 damages the respiratory tract and causes acute lung injury (ALI) (2, 4, 5). ALI triggers an inflammatory response while stimulating the immune system. This inflammatory immune response is associated with a cytokine storm that may result in a potentially fatal acute respiratory distress syndrome (ARDS) characterized by increased production of reactive oxygen species (ROS) as well as pro-inflammatory cytokines and chemokines (6). The cytokine storm may disrupt an effective anti-viral immune response and cause severe lymphocytopenia as well as T-cell exhaustion in affected COVID-19 patients (7, 8).

The nutritional status of the human body plays a pivotal role in developing an effective and appropriately balanced immune response to pathogenic viruses (9). Recent studies confirmed the importance of host nutritional status in surviving the COVID-19 challenge (6, 10, 11). The protein-energy malnutrition (PEM) causes an imbalanced immune response to viral pathogens that can result in infiltration of the lungs by inflammatory cells and the development of pneumonitis following viral infection (12). The comorbidities of COVID-19 patients are correlated with the severity of PEM and contribute to a higher risk of ARDS and increased case mortality rate (13). In COVID-19, the decreased serum albumin (14) and prealbumin (15) levels have prognostic value. Low serum prealbumin levels serve as a surrogate marker for malnutrition and a poor

prognostic factor (16). We believe that the nutritional status of all COVID-19 patients should be carefully evaluated, and consideration given to patient-tailored special diet programs that ensure an adequate and balanced intake of proteins, calories, and micronutrients (17). Adequate daily protein, especially whey and soy, intake have beneficial effects on the antioxidant defense system and host immunity (18). Among the various sources of protein, the whey protein has been recently recommended as a well-balanced and easy to digest amino acid and protein source with anabolic (19), anti-inflammatory (20), and immunomodulatory properties (21) as well as antiviral effects (22). Besides being an energy-rich part of the daily diet for balanced caloric intake, dietary fats, including fish oil and vegetable oils, provide a source for essential fatty acids as well as fat-soluble vitamins affecting metabolism and immunity. The essential fatty acid alpha-linolenic acid (ALA, 18:3n-3, omega-3), and the semi-essential fatty acids eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (23, 24), can be useful in supporting immune defense and the treatment of inflammatory diseases caused by both viruses and bacteria (25). In addition, essential micronutrients including vitamins and minerals, play an important role for the functional integrity and responsiveness of our immune system. Some of the vitamins (A, pyridoxine, cobalamin, folate, C, D, and E) and trace minerals such as Zn, Cu, Se, and Fe takes the crucial role to maintain and support the immune system (6). Balanced nutrition and intake of nutrients in appropriate amounts and composition may reduce the levels of pro-inflammatory cytokines and their side effects in COVID-19 patients (10).

Obesity has a rising prevalence, and it is considered a clinically significant risk factor for metabolic diseases as well as infections (26, 27). The consumption of "poor quality" foods often results in a nutritional deficiency in obese persons despite the higher than average amounts of food consumed (25). Such a nutritional deficiency may increase the severity of COVID-19 with increased morbidity and mortality (28). In this review, we discuss the possible role of micronutrients in the pathophysiology and survival outcome of COVID-19. We also review the current knowledge about the emerging role of supplemental nutrients as adjuncts to the supportive care for COVID-19, especially in obese patients.

OBESITY AND COVID-19

Obesity has detrimental effects on pulmonary function. Functional residual capacity and expiratory reserve volume are negatively affected by obesity as a consequence of the airway closure by fat accumulation in the mediastinal, thoracic, and abdominal cavity (29). Along with the rising body fat content, excessive secretion of adipokines, and cytokines from adipose tissue are thought to both compromises the immune system responsiveness to infections and cause systemic inflammation (27). The levels of free fatty acid (FFA), and lipopolysaccharide (LPS) released by gut bacteria increase during obesity which triggers activation of the (i) Toll-like receptor 4 (TLR4) pathway, (ii) adipose tissue macrophages (30) as well as (iii) nuclear factor-kappa β (NF-κβ) pathway (31). M1 phenotype macrophages that initiate and regulate inflammatory reactions through interferon-gamma (IFN-γ), TLR4, LPS, and FFA stimulation become the primary immune system elements located in the adipose tissue (32). Many inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), IL-6, IL-12, and IL-18 are secreted by M1 macrophages. Therefore, T helper cell 1 (Th1) to Th2 ratio, (26) and Th17 increase in obesity, which results in an immune imbalance (33). M1 macrophages also play an essential role in the development of tissue-level insulin resistance in obesity. This is owing in part to the elevated levels of inflammatory factors that impair the c-Jun N-Terminal Protein Kinase 1 and Iκβ kinase/NF-κβ cascades which regulate phosphorylation of insulin receptor substrates (IRS1 and IRS2) (34) (**Figure 1**). Besides the insulin resistance, the beta-cell function of the pancreas may be decreased during longterm obesity due to the continuous FFA exposure that activates the NF-κβ signaling pathway (35). In addition, venous thromboembolism is encountered more frequently by obese individuals due to the prothrombotic effects of low-grade chronic inflammation (36–38). The prothrombotic effects of inflammation are triggered by platelet activation (39), increased activity of coagulant factors (factor VIIa, VIII, IX, X, fibrinogen, and von Willebrand factor), stimulation adhesion molecules (P-selectin), and downregulation of endogenous anticoagulant factors (antithrombin, and protein C) (40). In addition, the plasminogen activator inhibitor-1 (PAI-1), a prothrombotic adipokine, also contributes to augmented venous thromboembolism of obesity (41). Coagulopathy, including DIC, is one of the main causes of mortality in COVID-19 (42).

Because of the overlapping systemic inflammation of obesity and systemic inflammation triggered by viral sepsis in COVID-19, obese patients with COVID-19 experience greater severity of pulmonary and metabolic complications as well as multi-organ dysfunction (43–45). Therefore, several micronutrients may have clinically meaningful beneficial effects in obese COVID-19 patients (46) (**Figure 2**). In COVID-19 patients many evidence demonstrates the metabolic link between inflammatory state and cytokine storm that mainly responsible for respiratory symptoms (45).

Several studies have shown a relationship between high BMI and severity of COVID-19. Ho et al. (47) reported that the risk of critical illness in COVID-19 increases by 44% for overweight people and almost doubles for obese patients. Likewise, an observational study using electronic health records indicated that obesity is an important contributing factor for case mortality in COVID-19 (48). Because obese patients have an impaired immune system, they may have inadequate cellular immune responses to pathogens and this acquired immunodeficiency increases their susceptibility to infections (49). In addition to the reduced T-effector cell function, the unbalanced production of immunomodulatory endocrine hormones also contribute to poor host immunity of obese persons against infections (50). Balanced nutrition and micronutrients help prevent the

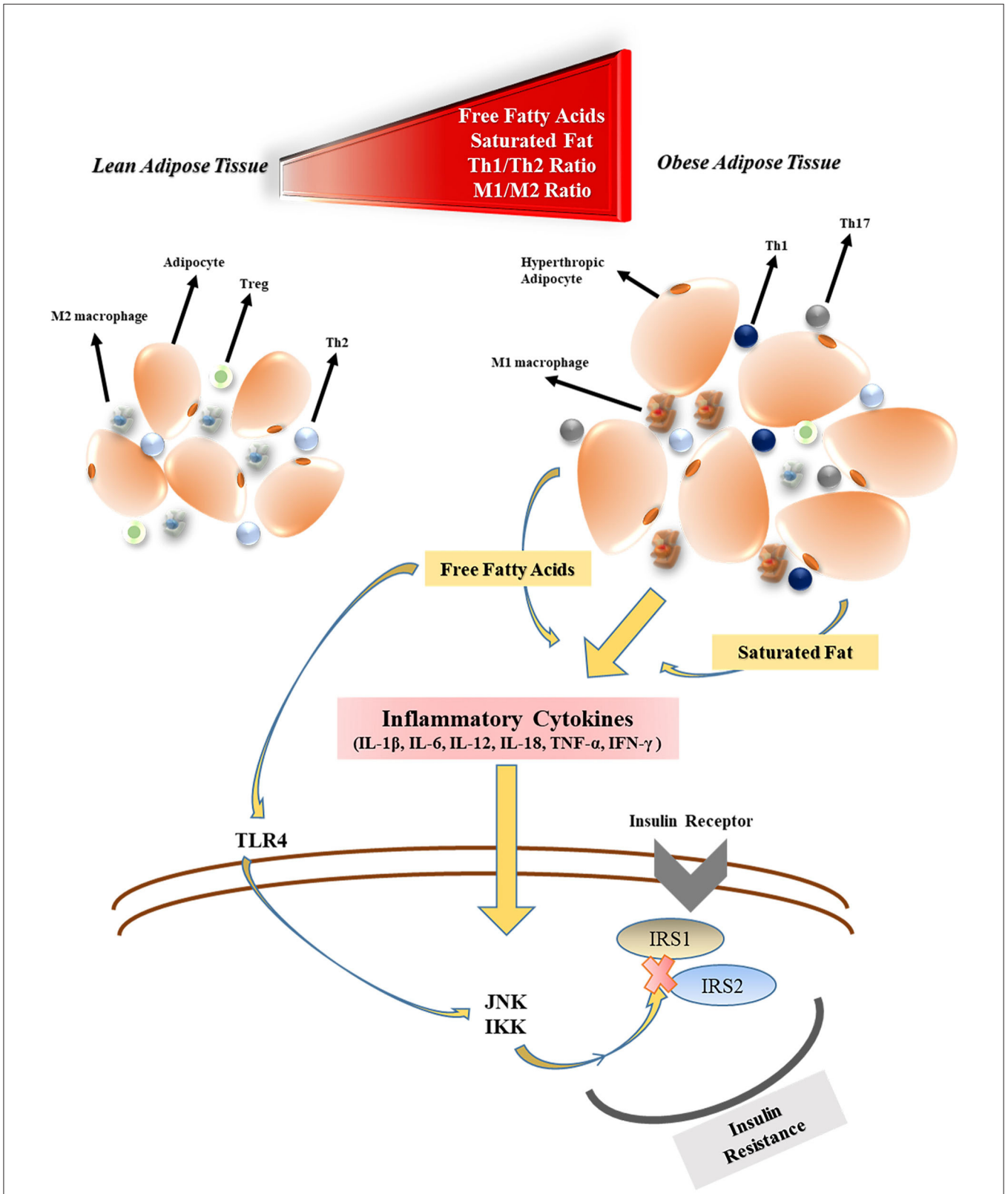


FIGURE 1 | The effects of obesity on inflammatory cytokine production and insulin resistance. Treg, regulatory T cell; Th2, T helper cell 2; M1, type 1 macrophages; FFA, free fatty acid; TLR4, toll-like receptor-4; JNK1, c-Jun N-Terminal Protein Kinase 1; IKK, Iκβ kinase; IRS, insulin receptor substrate.

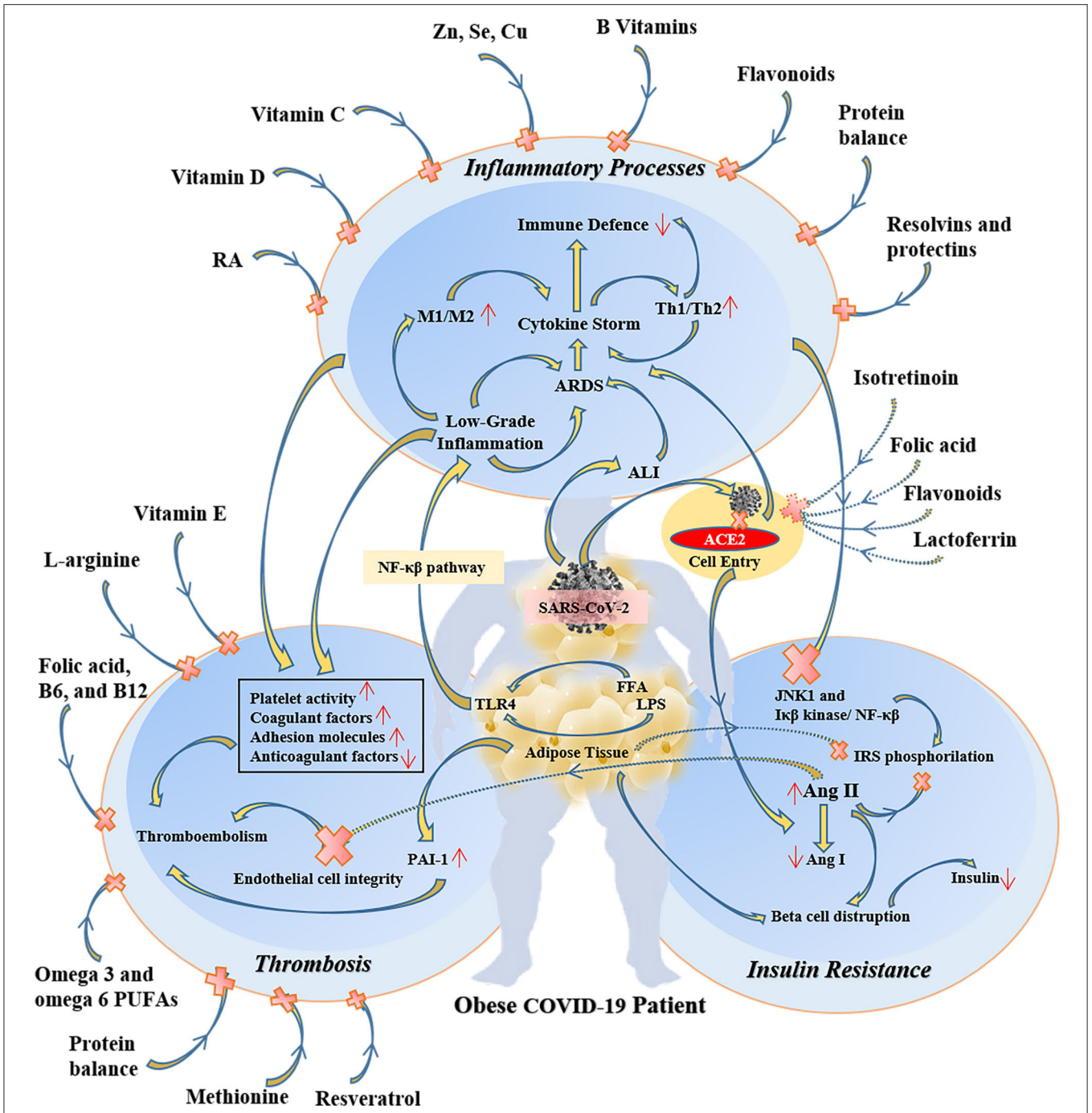


FIGURE 2 | The protective and therapeutic effects of some specific micronutrients on inflammatory, thrombotic, and insulin resistance pathways in obese COVID-19 patients. RA, retinoic acid; M1, type 1 macrophages; Th1, T helper cell 1; ARDS, acute respiratory distress syndrome; ALI, acute lung injury; ACE2, angiotensin-converting enzyme 2; NF-κβ, nuclear factor-kappa β; LPS, lipopolysaccharide; FFA, free fatty acid; TLR4, toll-like receptor-4; JNK1, c-Jun N-Terminal Protein Kinase 1; IRS, insulin receptor substrate; Ang, angiotensin; PAI-1, plasminogen activator inhibitor-1.

unfavorable outcomes observed in both obesity and COVID-19 (**Table 1**) (51–78).

The complex interplay between obesity and COVID-19 is explained by several mechanisms. SARS-CoV-2 uses the angiotensin-converting enzyme-2 (ACE2) for cell entry, and the amount of this transmembrane enzyme is found larger amounts in obese individuals (79). It is probably due to higher ACE2 expression in adipocytes of people with obesity (80). Therefore, adipose tissue of obese individuals can be a potential target for SARS-CoV-2 before spreading to other organs (81). Assuming

TABLE 1 | The possible effects of some micronutrients on common symptoms of COVID-19 and obesity.

Micronutrients	Key Mechanism of Action	Outcomes	References
Vitamin A	• Inhibition of M1 macrophage • Downregulation of IFN-γ • Promotion of Treg via inhibiting Th17 shifting • Inhibition of ACE2 by isotretinoin • Epithelial cell repairing properties	• Inflammatory status ↓ • Probably insulin resistance ↓ • Probably viral replication ↓ • Lung damage prevention	(51–55)
Vitamin C	• Protection of the respiratory system • Promotion of antioxidation and anti-inflammation properties • ROS scavenging activity • Inhibition of NF-κβ pathway	• Inflammatory status ↓ • Immunity ↑	(56)
Vitamin D	• Reduction of the risk of contracting respiratory infections • Regulation of Th1/Th2 balance	Immunity ↑	(57)
Vitamin E	• ROS scavenging activity • Inhibition of acute neutrophil inflammation in lung	• Inflammatory status ↓ • Lung damage prevention	(58, 59)
B vitamins	• Protection the respiratory system • Reduction of the risks of infection and re-infection • Reduction of inflammatory cytokine production • Regulation of the CD4/CD8 ratio and natural killer cell activity by vitamin B12 • Inhibition of ACE2 by folic acid • Prevention of hyperhomocysteinemia by folic acid, B6, and B12	• Inflammatory status↓ • Lung damage prevention • Immunity ↑ • Probably insulin resistance ↓ • Probably viral replication ↓ • Prevents thromboembolism	(60–64)
Selenium	• Antioxidative and anti-inflammatory properties in high-risk adults • Regulation of M1/M2 macrophage • Cofactor for glutathione peroxidase • Oxidizing capacity • Anticoagulant	• Inflammatory status ↓ • Antiviral activity ↑ • Prevents thromboembolism	(65, 66)
Zinc	• Antioxidative and anti-inflammatory properties in high-risk adults • Reduction of inflammatory cytokine production • Regulation of Th1/Th2 balance • Inhibition of ACE2 • Exert antiviral effect	• Inflammatory status↓ • Immunity ↑ • Probably insulin resistance ↓ • Probably viral replication ↓	(67–73)
Copper	• Regulation of Th1/Th2 balance • Reduction of inflammatory cytokine production • Oxidizing activity	• Inflammatory status↓ • Antiviral activity ↑	(74–76)
Magnesium	• Reduction of inflammatory cytokine production • Regulation of M1/M2 macrophage • Inhibits PARP	• Inflammatory status↓ • Prevents thromboembolism • Lung damage prevention	(77, 78)

that SARS-CoV/CoV-2 affects the pulmonary lipofibroblast transcriptional program that leads to pulmonary fibrosis, the use of peroxisome proliferator-activated receptor-gamma (PPARγ) agonists could be an option to reduce the risk of pulmonary fibrosis. This strategy may show strong anti-fibrotic effects that disrupt myofibroblast differentiation and transforming growth factor-beta (TGF-β) signaling. PPARy induction could lead to an effective reduction of the problem and organ fibrotic disease, including pulmonary fibrosis (81) by decreasing the fat mass of adiponectin (82).

The Spike glycoprotein (S), a structural protein of SARS-CoV-2, is responsible for binding to the host cell. Hoffmann et al. (83) reported that S protein is primed serine protease and recognized by the cell receptor. Liu et al. (84) showed that the S protein of SARS-CoV-2 had two trimers that bind to the ACE2 heterodimer. Dipeptidyl peptidase 4 (DPP4) is a ubiquitous membrane-bound aminopeptidase that circulates in the plasma has multifunctional roles in metabolism, immunity, and the endocrine system. DPP4 regulates glucose homeostasis and inflammation differently through its immunomodulatory properties. Bassendine et al. (85) reported that obesity and metabolic syndrome strongly affect the severity of COVID-19 by modulating the DPP4 expression.

In COVID-19, loss of the ACE2 enzyme that converts angiotensin-II to angiotensin-I may lead to insulin resistance (86) and endothelial cell dysfunction (87). Although the lung tissue is the primary target of SARS-CoV-2, ACE2 protein expression levels in adipose tissue (80) and pancreas (88) are higher than in the lung. High-level ACE2 expression in the pancreas may predispose to viral pancreatitis (88) and subsequently diabetes as a complication. Therefore, obese individuals may be at higher risk for metabolic complications of COVID-19.

MICRONUTRIENTS AND COVID-19 PATIENTS WITH OBESITY

The insufficiency of micronutrients including vitamin A, vitamin D, vitamin E, vitamin B1, vitamin B6, vitamin B12, vitamin C, Fe, Zn, and Se, called "latent hunger" causes

important health problems globally. Correa-Rodríguez et al. (89) observed significant reductions in vitamin C, vitamin A, and Se intake in overweight or obese young adults. Therefore, the supplementation of micronutrients may support the body's natural defense system by enhancing immunity, epithelial barriers, cellular immunity, and antibody production (90).

Vitamins

Vitamin A

Vitamin A is accepted as an anti-inflammatory or anti-infective micronutrient owing to its immunomodulatory, and epithelial cell repairing functions (55). Vitamin A has been shown to reduce the severity of viral pneumonia caused by an avian coronavirus (91), measles (92), influenza A, rotavirus, and Newcastle disease virus (93). Therefore, retinoids could potentially inhibit the replication of SARS-CoV-2 and thereby reduce the severity of COVID-19 (94). Furthermore, the vitamin A derivate named isotretinoin (54) may interfere with the cellular uptake of SARS-CoV-2 and its lung-directed pathogenicity by inhibiting the ACE2 (95). Administration of all-trans retinoic acid in a hypoxia/reoxygenation model increased the mRNA expression of ACE2 and down-regulated the mRNA expression of ACE1 and TGF-β1 in renal tubular epithelial cells (96). In individuals with low vitamin A levels, histopathological changes have been detected in pulmonary epithelia and lung parenchyma, along with an increased risk of pulmonary dysfunction and respiratory disease (97). Normal serum retinol levels may mask the severity of vitamin A deficiency in obesity (98). The continuous consumption of the western diet reduces tissue vitamin A levels (99). Vitamin A deficiency and increased expression of leptin, enhance the levels of pro-inflammatory cytokines that contribute to the systemic inflammation in obesity (100). Penkert et al. (98) reported that vitamin A supplementation protects against a respiratory virus infection by controlling respiratory virus clearance, decreasing inflammatory cytokines in the blood, and altering the lung immune capacity in obese C57BL/6 mice. In addition, high doses of oral vitamin A supplementation has been shown to reduce obesity by upregulating brown adipose tissue-uncoupling protein1 (BAT-UCP1) expression in the WNIN/Ob rat model (101). Considering the effects of COVID-19 on lung function and protective properties of vitamin A in the organism, vitamin A is expected to have a beneficial effect in obese COVID-19 patients.

Vitamin C

Vitamin C has been used to boost the antiviral host immune defense (56), reduce or prevent the symptoms of the common cold and other respiratory infections caused by viruses (102, 103). Vitamin C regulates the immune responses in the early stage of influenza infection through increasing the levels of type I interferons (IFN-α and IFN-β) (104) having critical functions to attenuate viral pathogenesis (105). Vitamin C is an effective intracellular antioxidant for biomolecules and has significant ROS scavenging activity that results in the inhibition of the inflammatory NF-κβ signal transduction pathway (56). In addition, the phagocytic activity of neutrophils and macrophages is regulated by their vitamin C content (106). Supplemental vitamin C may decrease the severity of obesity and its co-morbidities by regulating lipid accumulation, inhibiting lipolysis that reduces systematic FFA efflux, and glucocorticoid production, reducing ROS activity and interfering adipocyte macrophages, thus decrease pro-inflammatory adipokines (leptin) and cytokines (107). In a meta-analysis of eight randomized clinical trials in 3,135 children aged 3 months to 18 years, vitamin C administration decreased to the duration of upper respiratory tract infection by 1.6 days. In the same study, it was reported that children 6 years of age benefit from more effective vitamin C administration associated with Echinacea (108). In a randomized, double-blind, placebo-controlled, phase I trial, ascorbic acid infusion rapidly increased plasma ascorbic acid concentration and reduced the pro-inflammatory biomarkers C-reactive protein (CRP) and procalcitonin levels, prevented an increase in thrombomodulin levels consistent with reduced vascular damage, and caused reductions in sequential organ failure assessment scores (109). In addition, a time-delayed infusion protocol of both ascorbic acid and dehydroascorbic acid attenuated pro-inflammatory, procoagulant states that induce lung vascular damage and significantly prolonged survival (110). In obesity, low vitamin C status correlates with inflammatory reactions and vascular dysfunction (111), and a dose of 1 g/day vitamin C treatment for 8 weeks could reduce CRP and IL-6 levels in both hypertensive and diabetic obese patients (112). In higher doses, vitamin C can act as an oxidizing agent (106). The oxidizing properties of vitamin C are boosted with the presence of iron, increasing its antiviral activity via the Fenton reaction that results in the production of hydrogen peroxide and hydroxyl radicals (113). The different doses of vitamin C supplementation (125 and 250 mg/kg) reduced mitochondrial antiviral signaling, interferon-regulating factor 3, and steroid hydroxylase in mice exposed to restraint stress and H1N1-induced pneumonia (114). Recently, Peng (115) started a randomized controlled vitamin C infusion trial that aims to attenuate the respiratory symptoms of COVID-19 infection. High-dose vitamin C might be an effective choice in the early treatment of COVID-19 (116). Consumption of citrus fruits and vegetables containing vitamin C has been proposed as a low-cost strategy to support the immune system during the COVID-19 pandemic (11).

Vitamin D

There are multiple variables such as age, body mass index, skin color, and genetic variants that can affect the vitamin D stores of the body (117). Low serum 25-hydroxyvitamin D [25(OH)D] concentrations have been reported in obese humans and an inverse relationship between BMI and serum 25(OH)D has been reported in obese humans (118). Lin et al. (119) were observed that vitamin D deficiency [25(OH)D $<$20 ng/mL] and insufficiency [20 $<$25(OH)D $<$30 ng/mL] in 52 obese (mean BMI 37.6 $\pm$ 6.4 kg/m^2) were at 73 and 22% prevalence. Vitamin D metabolizing enzyme expression (Cyp2r1, Cyp27a1, and Cyp2j3) was affected by high fat diet-induced obesity, which may partially explain the mechanisms of the modified vitamin D endocrine system related to obesity (120). Adequate daily intake of vitamin D is thought to curb viral infections (121, 122). Seasonal viral infections affecting the respiratory tract as well as COVID-19 may

be facilitated by vitamin D deficiency (123, 124). The serum levels of 25(OH)D, the circulating metabolite of vitamin D, are inversely correlated with pulmonary inflammation (125) and directly correlated with the pulmonary function (120) as well as host immune response (126) during respiratory virus infection. 1,25-dihydroxyvitamin D (1,25D), an active metabolite of vitamin D, has pleiotropic effects on immune system elements (57, 127–129) and may reduce the production of pro-inflammatory cytokines that have been implicated in the pathophysiology of COVID-19 associated ARDS (IFN-γ, TNF-α, IL-1, IL-6, IL-2, IL-12, and IL-17) (**Figure 3**) (130). Administration of vitamin D stimulated binding of the SARS-CoV-2 cell entry receptor ACE2 to angiotensin-II receptor type 1, decreasing the number of virus particles that could attach to ACE2 and enter the cell (131, 132). Therefore, vitamin D supplementation could potentially reduce the incidence of severe COVID-19 (124). In randomized controlled trials of vitamin D for prevention of respiratory tract infection (Of 1,137 citations retrieved, 11 placebo-controlled studies of 5,660 patients), vitamin D showed a protective effect against respiratory tract infection, with daily dosing appearing to be the most effective strategy (133). That being said, a clinical study conducted by Hastie et al. (134) indicated that the relation between COVID-19 and serum vitamin D levels was not significant. Additionally, the intake of high doses of vitamin D may have harmful effects on COVID-19 patients (124).

Vitamin E

Vitamin E supplementation generally reduces the severity of infectious diseases, owing to antioxidant and immunomodulatory effects (135). Tocopherols (α and γ), natural vitamin E isomers, exhibits ROS scavenging activity (58) and can block acute neutrophil inflammation in the lung (59). Dietary vitamin E deficiency may increase IL-1 induced lung leak in rats (136), and probably provokes acute hyperoxic lung injury related to IL-6 and 8-iso-prostaglandin $F_{2\alpha}$ stimulated inflammation in mice (137). Oral vitamin E intake is positively associated with lung health (138). In a randomized clinical trial performed by Agler et al. (139) proved that a dose of 600 IU vitamin E (every other day) reduced the risk of chronic lung disease. No studies have been done in the case of the link between vitamin E and COVID-19 patients with obesity. Meydani et al. (140) found that a daily intake of 200 IU vitamin E has not any effect on lower respiratory infection. In the same study, the incidence of upper respiratory infections such as common cold found lower in older people.

After ACE2 mediated cell entry, coronaviruses firstly activate aryl hydrocarbon receptors (AhRs) without indoleamine 2,3-dioxygenase (IDO1) stimulation (141). Activated AhRs initiates the production of inflammation factors (IL-1β, IL-6, and TNF-α), induces tissue factor (TF) and PAI-1 mediated thromboembolism (AhR-TF/PAI-1 pathway) (142), and lead multiple organ fibrosis via Cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1)/IL-22 signaling pathway with signal transducer and activator of transcription 3 (STAT3) (143). The produced inflammatory cytokines trigger IDO1 that metabolizes tryptophan to kynurenine (AhR stimulator). Consequently, these signaling factors lead to the IDO1-AhR-IDO1 loop (**Figure 3**) (141). AhR signaling pathway stimulation also modulates obesity via disrupting fat metabolism (144). The degree of AhR activation raises depend on the severity of obesity due to enhanced inflammatory factors, including TLR2/4- NF-κβ mediated (145). Likewise, elevated dietary fat intake increases the level of serum (low-density-lipoprotein) LDL (146) that responsible for the AhR activation (147). SARS-CoV-2 induced IDO1-AhR-IDO1 loop might be exponentially increased in obese COVID-19 patients, and probably inhibited by vitamin D for AhR (148), and vitamin E for IDO1 (149). Therefore, using both vitamin D and E together most likely reduces the clinical symptoms in COVID-19 infection and obesity-associated complications.

B Vitamins

The prevention or treatment of lung damage is critical for the survival outcome of COVID-19 patients. Vitamin B3, a claimed protector of lungs, can promote the healing of tissue damage in the lungs (60, 61), most likely owing to its ability to inhibit the poly ADP ribose polymerase (PARP) (61). Because the increased activity of PARP elevates the inflammatory cytokines that contribute to the cytokine storm, vitamin B3 therapy may reduce cytokine storm in COVID-19 (6). The pyridine-nucleoside form of vitamin B3 called nicotinamide riboside functions as a precursor to nicotinamide adenine dinucleotide (NAD^+), prevented ALI/ARDS and heart injury, and improved the survival of mice after the LPS challenge or sepsis caused by intraperitoneal injection of feces (150). In LPS-challenged rats, the administration of nicotinamide prevented the decrease in mitochondrial respiration and intracellular NAD^+ levels in macrophages (151). In obesity, adipose tissue nicotinamide phosphoribosyltransferase (NAMPT) expression reduces, and NAD^+ biosynthesis impairs. The reduction in NAD^+ levels causes decreasing adiponectin and increasing FFA production (152). The dietary supplementation of NAD^+ precursors alleviates inflammation, improves insulin sensitivity, and reduces body weight gain in obesity (153).

Vitamin B6 levels and the severity of inflammatory reactions are inversely correlated (154, 155). The utilization of vitamin B6 rapidly increases under inflammatory conditions, and COVID-19 patients probably may have vitamin B6 deficiency (156). Vitamin B6 downregulated the pulmonary inflammation by inhibiting macrophage activation, as reduced production of IL-1β, IL-6, and TNF-α in macrophages challenged with LPS of mice (155). In a rat model of systemic inflammation, orally administered 500 mg/kg riboflavin and 600 mg/kg thiamine increased the anti-inflammatory activity of dexamethasone, along with reducing TNF-α and IL-6 production (157). The relationship between dexamethasone and thiamine caused more inhibition of IL-6 production compared to dexamethasone-induced. Multivitamin supplementation within 48 h of hospital admission, including thiamine, riboflavin, and niacin was associated with lower overall mortality in patients with Ebola Virus Disease (158).

The folic acid and its derivates such as tetrahydrofolic acid and 5-methyl tetrahydrofolate may interfere with the cell entry of SARS-CoV 2 (63) via inhibition of furin protein that has essential for COVID-19 progression (159). In addition to the

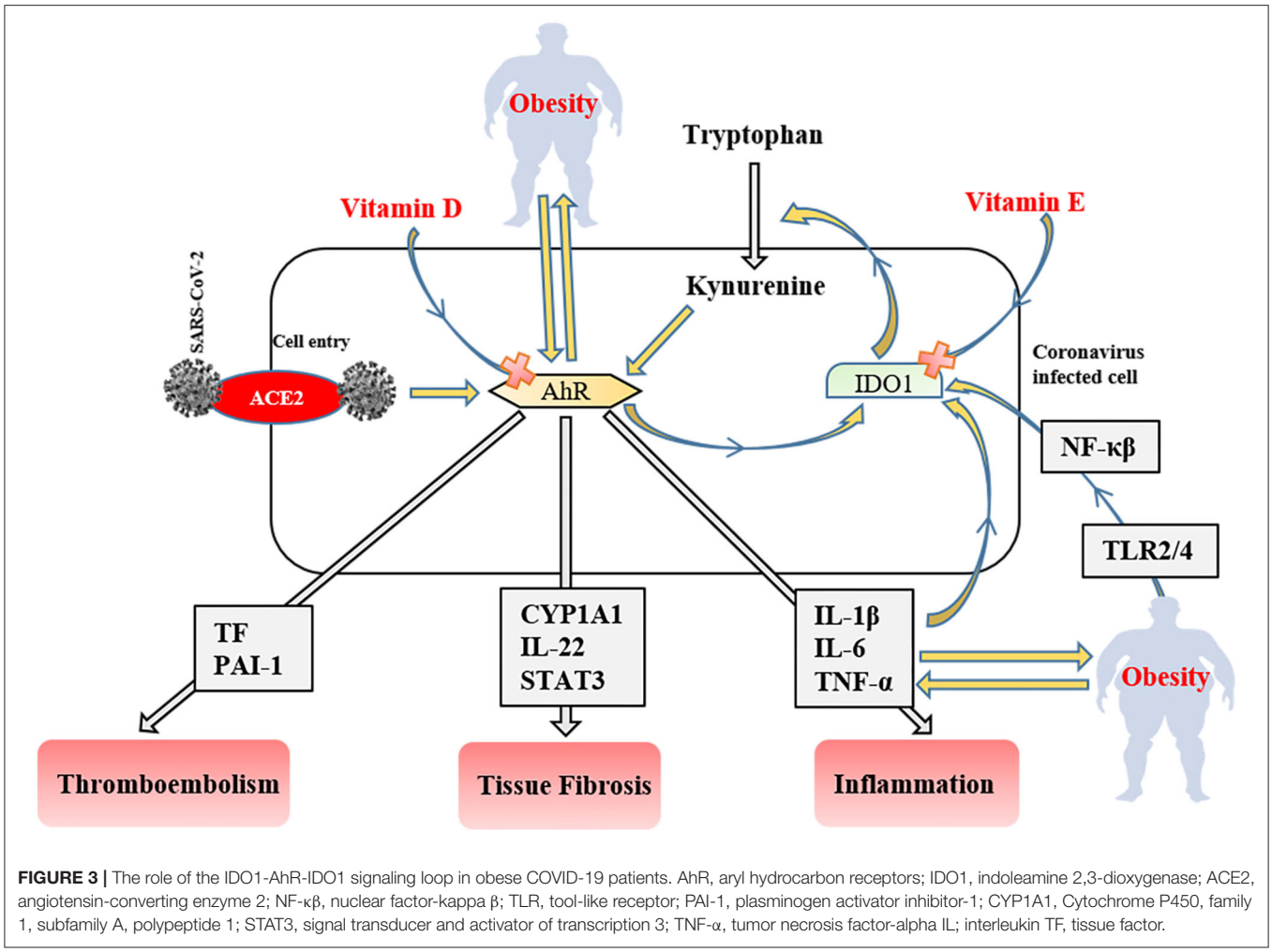


FIGURE 3 | The role of the IDO1-AhR-IDO1 signaling loop in obese COVID-19 patients. AhR, aryl hydrocarbon receptors; IDO1, indoleamine 2,3-dioxygenase; ACE2, angiotensin-converting enzyme 2; NF-κβ, nuclear factor-kappa β; TLR, tool-like receptor; PAI-1, plasminogen activator inhibitor-1; CYP1A1, Cytochrome P450, family 1, subfamily A, polypeptide 1; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-alpha IL; interleukin TF, tissue factor.

ACE2 protein, S-glycoprotein, some of the proteases (M^{pro} and PL^{pro}), RNA dependent RNA polymerase, and Nsp15 promote the cellular entry of SARS-CoV 2 (63, 160). In a virtual screening study among the 106 nutraceuticals, the folic acid and folic acid derivates were identified as potential agents that could have potential in post-exposure prophylaxis (63).

The deficiency of some B vitamins (folic acid, B6, and B12) and dietary essential amino acid methionine results in hyperhomocysteinemia that leads to venous thromboembolism (64). In obesity, the high cardiovascular risk that is related to hyperhomocysteinemia correlates with insufficient nutritional status of folate and vitamin B12. The decreased levels of plasma folate and vitamin B12 are accepted as a predictor of vascular dysfunction (161). A high dose of vitamin B6 administration may reduce the TNF-α, IL-6, and D-dimer levels and improves endothelial integrity along with preventing coagulopathy in COVID-19 patients (156).

Minerals

Magnesium

Magnesium can alleviate inflammatory disorders, including obesity (162) and respiratory infections (78). The M1 type macrophages producing NF-κβ depended on pro-inflammatory mediators shifts to M2 type macrophages after Mg treatment and stimulates the anti-inflammatory cytokine secretion (77). The low dietary Mg intake inversely was associated with endothelial cell dysfunction and biomarkers of systemic inflammation (163). Different forms of Mg could be used against various lung diseases (164). Li et al. (78) reported that $MgSO_4$ administration reduced the PARP-1 and apoptosis-inducing factor levels in LPS induced ALI mice. Recently Tan et al. (165) observed that the administration of oral vitamin D, Mg, and vitamin B12 combination reduces the clinical deterioration in COVID-19 patients. Mg nutrition may be an effective strategy for the treatment and prevention of COVID-19 infection (166, 167). Moreover, many diseases, such as obesity that cause Mg deficiency (162), probably exacerbate the clinical symptoms of COVID-19.

Selenium

Selenium is considered an important antioxidant trace mineral. The severity and mortality of viral infections were inversely correlated with serum Se levels in several studies (168–170). Virulence and pathogenesis levels of viruses can be increased

due to the weakened immune system after the long term intake of inadequate Se containing diets (171). The M1 type macrophages increase with Se deficiency or low Se intake (65, 172). However, high amounts of Se intake lead to shifts Th2 phenotype to the Th1 phenotype (173). Zhang et al. (174) recently reported that Chinese persons with lower hair Se content had more severe COVID-19 infections. Se supplementation may therefore have clinical utility in COVID-19 pending further confirmation of the prognostic role of Se for the survival outcome of COVID-19 patients. Fakhrolmobasheri et al. (175) reported that Se could prevent cell death caused by viral replication. In patients with ARDS, sodium selenite (1 mg for 3 days and 1 mg/d for a further 6 days) supplementation replenished Se levels and Se concentrations were positively correlated with antioxidant activity. Serum concentrations of IL-1β and IL-6 were inversely associated with serum Se concentrations. Nevertheless, there was no effect on overall survival, mechanical ventilation time, and length of stay in intensive care (176). In LPS-stressed RAW264.7 cells, LPS increased mRNA profiles of inflammatory genes, while short-time Se pretreatment reduced the LPS-induced upregulation of cyclooxygenase-2, intercellular adhesion molecule−1, IL-1β, IL-6, IL-10, nitric oxide synthase, and monocyte chemoattractant protein-1 and further increased expression of IFN-β and TNF-α (177). In the same study, LPS decreased mRNA levels of selenoprotein encoding genes, whereas increased mRNA levels of thioredoxin reductases (TXNRD1and TXNRD3) in cells. Se deficiency or overexposure impairs the selenoprotein synthesis (glutathione peroxidase and TXNRDs) that cause adipocyte dysfunction leading to various metabolic disorders. The expression of these selenoproteins is decreased in obese individuals due to their lower Se status (178). In COVID-19, selenoprotein expression may also be reduced by inflammatory factors and suppressed immune status (179). Therefore, dietary Se supplementation may help alleviate the respiratory and inflammatory clinical symptoms in obese patients suffering from COVID-19.

Zinc

Zinc is another important trace mineral to improve the immune functions against viral infections (180, 181). In risk groups for Zn deficiency, including aging, immune deficiency, obesity, diabetes, and atherosclerosis, low Zn status may relate to severe COVID-19 risk (182). Zn can exert its antiviral effect by suppressing viral replication, improvement of mucociliary clearance and increasing immune responses, prevention of lung injury, and regulation of antiviral and antibacterial immunity (73). Zn can provide low-cost and effective adjunctive therapy for some viral diseases, including respiratory infections (183). For example, Mossad et al. (184) reported that the duration of common cold symptoms was shortened from 7.6 to 4.4 days with zinc gluconate (containing 13.3 mg elemental Zn). *In vitro* studies showed that Zn exhibits antiviral activity by inhibiting the SARS-CoV RNA polymerase (73). Indirect data suggest that Zn may decrease the activity of ACE2. The anti-inflammatory activity of Zn depends on NF-κB signaling pathway inhibition and modulation of Treg function, which may help reduce the risk of cytokine storm in COVID-19 (73). In addition, Zn has been revealed to be vital for respiratory epithelium, owing to antioxidant and anti-inflammatory activity (185), and also the regulation of tightly binding proteins zonula occludens-1 and Claudin-1, thereby enhancing barrier functions (186). In an *in-vitro* study was demonstrated that Zn administration (10 μm Zn preincubation) inhibited respiratory syncytial virus replication by more than 1.000-fold reduction (187). Antiviral agent chloroquine, a Zn ionosphere that is used in the treatment of COVID-19, increases the Zn transport into the cells (73, 188, 189). It has recently been proposed that the severity of COVID-19 infection could be reduced with an adequate daily intake of Zn (183). Recently, Finzi (190) reported that high dose oral supplementation of Zn salts (zinc citrate, zinc gluconate, or zinc acetate) reduced the respiratory clinical symptoms of COVID-19 patients (190). In line with this information, it was suggested that Zn could be one of the most promising micronutrients for COVID-19 prevention or treatment (191, 192). The effects of Zn on obesity and respiratory viral infections may help to treatment of COVID-19 in both obese and overweight patients.

Copper

Copper (Cu), a trace mineral, has an important role in host immunity against viruses, regulating inflammatory responses, and boosting the immunity of the host in many infections (193–198). Elevating the Cu levels in the lung tissue has been suggested as a strategy for treating or preventing pulmonary inflammation (199). The appropriate dietary Cu intake within normal daily limits probably increases the number of phagocytic cells, the activity of Natural killer cells, the proliferation of Th cells (200), and more importantly the Th1-stimulated production of IL-2, but not TNF-α (75). In this context, Cu is may require to maintain the balance of the Th1/Th2 profile (74). The increased pro-inflammatory cytokine TNF-α causes decreasing Cu levels in the lungs during lung infections (199). Raha et al. (201) hypothesized that Cu supplementation could protect the high-risk COVID-19 patient populations with Cu deficiency from developing ARDS.

In addition, the raising of the ROS concentration may be used to exhibit the antiviral action by Cu (76). The Cu-peroxide complexes could enhance the effectiveness of this action (202). Since ROS production properties of Cu containing surfaces, SARS-CoV and SARS-CoV-2 viruses are sensitive to Cu alloys (203, 204). However, Cu supplementation may also increase the risk of sepsis and ARDS and should not be attempted outside a well-controlled clinical trial.

OTHER IMPORTANT NUTRIENTS IN COVID-19

Flavonoids

Plant-derived flavonoids having anti-inflammatory, antioxidant, and antimicrobial activities (205) also have anti-obesity and anti-diabetic potential (206). In obesity and other inflammatory disorders, dietary flavonoids could inhibit inflammatory cytokine production, leptin secretion, insulin resistance, and improve immune responses (207). Polyphenols inhibit NF-κB and activator protein-1 activates nuclear factor erythroid 2–related

factor 2 (Nrf2) and improves lipid profiles via enhancing HDL-cholesterol, while the reduction in LDL-cholesterol. Therefore, the intake of high-polyphenol diets shows various antioxidant, anti-inflammatory, and dyslipidemia-reducing effects (182). Vernarelli and Lambert (208) reported that dietary flavonoid consumption was inversely correlated with the severity of obesity and serum CRP levels. The inhibition of inflammatory cytokines by flavonoids (205) in the context of pulmonary infection (209, 210) may prevent the development of or reduce the negative consequences of the cytokine storm in COVID-19. Additionally, the coagulopathy associated with COVID-19 may be alleviated by flavonoids through the reduction of endothelial TF availability (211). Both *in vitro* and *in vivo* studies indicate that flavonoids exhibit antiviral activity against respiratory tract viruses including SARS-CoV and influenza (212). In a meta-analysis performed by Somerville et al. (213), flavonoids have been shown to potentially reduce the incidence of upper respiratory tract infections caused by viruses. *In silico* virtual computational screening studies have been demonstrated that natural compounds like flavonoids may inhibit SARS-CoV-2 by binding to S proteins that have an affinity to ACE2 (214). Also, Adem et al. (215) demonstrated that flavonoids may inhibit M^{pro} used by SARS-CoV-2 for viral replication. Especially, quercetin and catechins have antiviral activity on SARS-CoV (216), and probably on SARS-CoV 2 (217, 218). In addition, curcumin (219, 220) indomethacin and resveratrol have been proposed as potential supportive care supplements against COVID-19 (221).

Lactoferrin

Lactoferrin, which shows antimicrobial activity, has anti-inflammatory and immunomodulatory activities (222). Due to its antiviral activity, many viruses, including SARS-CoV (223), could be killed by lactoferrin (224–226). The reported antiviral action of lactoferrin against SARS and COVID-19 most probably stems from blocking the activity of ACE2 and Heparan Sulfate Proteoglycan, which are required for cell entry of SARS-CoV and SARS-CoV 2 (227). Additionally, a clinical study performed by Serrano et al. (228) indicated that bovine liposomal lactoferrin using combined with vitamin C and Zn attenuated symptoms of COVID-19 infection. Likewise, lactoferrin can alleviate obesity by inhibiting leptin production and controlling LPS releasing from gut microbiota (229). In this context, the leptin reducing functions of Zn and vitamin C (230) when combined with lactoferrin may be beneficial in the treatment of COVID-19 in obese individuals.

Essential Fatty Acids

Dietary polyunsaturated fatty acids (PUFAs) and their metabolites exert protective effective effects during systemic inflammation (231). Supplementation of n-3 PUFAs reduces the systemic inflammation of non-diabetic obese patients (232). EPA and DHA may inhibit the inflammatory NF-κB and TLR signaling pathways (233). These long-chain fatty acids also decrease the M1/M2 macrophage ratio in adipose tissues, thereby reducing the inflammatory state and decreasing the insulin resistance (234). Omega-3 PUFAs and its lipid derivatives such as resolvins, and protectins (protectin D1), when used at appropriate dose levels and according to rational administration schedules, could be potentially useful in reducing the pro-inflammatory cytokine production that leads to cytokine storm in COVID-19 (235). Importantly, omega-3 and omega-6 PUFAs have been shown to reduce platelet aggregation and may therefore prevent thrombosis (236) and reduce the risk of thromboembolic complications in COVID-19 patients that have been associated with a poor survival outcome (237).

CONCLUSION

A deficiency of micronutrients due to malnutrition has the potential to increase the severity of viral infections. Many essential nutrients like vitamins, minerals, amino acids, and fatty acids are important for the pleiotropic functions of our immune system. Balanced nutrition and intake of nutrients in appropriate amounts and composition may reduce the levels of pro-inflammatory cytokines and their side effects in COVID-19 patients. More importantly, obese COVID-19 patients are more susceptible to inflammation, lung diseases, coagulopathy, and probably insulin resistance than lean patients. Some *in vitro* and *in silico* studies suggested that specific nutrients might exhibit protective effects against COVID-19 infection. However, further studies are needed to improve our current knowledge about the emerging role of supplemental nutrients as adjuncts to the supportive care for obese COVID-19 patients.

AUTHOR CONTRIBUTIONS

KS and ES conceived the study, participated in its design and coordination, and drafted and authored the manuscript. CO and FU participated in the study design, interpretation of the data, and helped to draft manuscript revisions. All authors have read and approved the final manuscript.

FUNDING

This research was funded by the Turkish Academy of Science (Ankara, Turkey).

REFERENCES

1. World Health Organization. *WHO Coronavirus Disease (COVID-19) Dashboard*, (2020) Available online at: https://covid19.who.int/(accessed July 17, 2020).
2. Wu D, Wu T, Liu Q, Yang Z. The SARS-CoV-2 outbreak: what we know. *Int J Infect Dis.* (2020) 94:44–8. doi: 10.1016/j.ijid.2020.03.004
3. Jordan RE, Adab P, Cheng KK. Covid-19: risk factors for severe disease and death. *BMJ.* (2020) 368:m1198. doi: 10.1136/bmj.m1198

4. Weiss SR, Leibowitz JL. Chapter 4 - coronavirus pathogenesis. In: Maramorosch K, Shatkin AJ, Murphy FA, editors. *Advances in Virus Research*. *81*. Cambridge: Academic Press (2011) p. 85–164.
5. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. *Trends Microbiol.* (2016) 24:490–502. doi: 10.1016/j.tim.2016.03.003
6. Calder PC. Nutrition, immunity and COVID-19. *BMJ Nutr Prev Health.* (2020) 3:85. doi: 10.1136/bmjnph-2020-000085
7. Pedersen SF, Ho Y-C. SARS-CoV-2: a storm is raging. *J Clin Invest.* (2020) 130:2202–5. doi: 10.1172/JCI137647
8. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
9. Chandra RK, Sarchielli P. Nutritional status and immune responses. *Clin Lab Med.* (1993) 13:455–61. doi: 10.1016/S0272-2712(18)30448-7
10. Cena H, Chieppa M. Coronavirus disease (COVID-19–SARS-CoV-2) and nutrition: is infection in Italy suggesting a connection? *Front Immunol.* (2020) 11:944. doi: 10.3389/fimmu.2020.00944
11. Kalantar-Zadeh K, Moore LW. Impact of nutrition and diet on COVID-19 infection and implications for kidney health and kidney disease management. *J Renal Nutr.* (2020) 30:179–81. doi: 10.1053/j.jrn.2020.03.006
12. Taylor AK, Cao W, Vora KP, Cruz JDL, Shieh W-J, Zaki SR, et al. Protein energy malnutrition decreases immunity and increases susceptibility to influenza infection in mice. *J Infect Dis.* (2013) 207:501–10. doi: 10.1093/infdis/jis527
13. Galli F, Reglero G, Bartolini D, Visioli F. Better prepare for the next one. Lifestyle lessons from the COVID-19 pandemic. *Pharma Nutr.* (2020) 12:100193. doi: 10.1016/j.phanu.2020.100193
14. Liu W, Tao Z-W, Wang L, Yuan M-L, Liu K, Zhou L, et al. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. *Chin Med J.* (2020) 133:1032–8. doi: 10.1097/CM9.0000000000000775
15. Wu C, Chen X, Cai Y, Xia Ja, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 Pneumonia in Wuhan, China. *JAMA Int Med.* (2020) 180:1–11. doi: 10.1001/jamainternmed.2020.0994
16. Keller U. Nutritional laboratory markers in malnutrition. *J Clin Med.* (2019) 8:775. doi: 10.3390/jcm8060775
17. Caccialanza R, Laviano A, Lobascio F, Montagna E, Bruno R, Ludovisi S, et al. Early nutritional supplementation in non-critically ill patients hospitalized for the 2019 novel coronavirus disease (COVID-19): rationale and feasibility of a shared pragmatic protocol. *Nutrition.* (2020) 74:110835. doi: 10.1016/j.nut.2020.110835
18. Draganidis D, Karagounis LG, Athanailidis I, Chatzinikolaou A, Jamurtas AZ, Fatouros IG. Inflammaging and skeletal muscle: can protein intake make a difference? *J Nutr.* (2016) 146:1940–52. doi: 10.3945/jn.116.230912
19. Katsanos CS, Chinkes DL, Paddon-Jones D, Zhang X-j, Aarsland A, Wolfe RR. Whey protein ingestion in elderly persons results in greater muscle protein accrual than ingestion of its constituent essential amino acid content. *Nutr Res.* (2008) 28:651–8. doi: 10.1016/j.nutres.2008.06.007
20. Hashemilar M, Khalili M, Rezaeimanesh N, Sadeghi Hokmabadi E, Rasulzade S, Shamshirgaran SM, et al. Effect of whey protein supplementation on inflammatory and antioxidant markers, and clinical prognosis in acute ischemic stroke (TNS Trial): a randomized, double blind, controlled, clinical trial. *Adv Pharm Bull.* (2020) 10:135–40. doi: 10.15171/apb.2020.018
21. Kiewiet BGM, Faas MM, De Vos P. Immunomodulatory protein hydrolysates and their application. *Nutrients.* (2018) 10:904. doi: 10.3390/nu10070904
22. Ng TB, Cheung RCF, Wong JH, Wang Y, Ip DTM, Wan DCC, et al. Antiviral activities of whey proteins. *Appl Microbiol Biotechnol.* (2015) 99:6997–7008. doi: 10.1007/s00253-015-6818-4
23. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol.* (2013) 75:645–62. doi: 10.1111/j.1365-2125.2012.04374.x
24. Baker EJ, Miles EA, Burdge GC, Yaqoob P, Calder PC. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Prog Lipid Res.* (2016) 64:30–56. doi: 10.1016/j.plipres.2016.07.002
25. Husson M-O, Ley D, Portal C, Gottrand M, Hueso T, Desseyn J-L, et al. Modulation of host defence against bacterial and viral infections by omega-3 polyunsaturated fatty acids. *J Infect.* (2016) 73:523–35. doi: 10.1016/j.jinf.2016.10.001
26. Viardot A, Heilbronn LK, Samocha-Bonet D, Mackay F, Campbell LV, Samaras K. Obesity is associated with activated and insulin resistant immune cells. *Diabetes Metab Res Rev.* (2012) 28:447–54. doi: 10.1002/dmrr.2302
27. Huttunen R, Syrjänen J. Obesity and the risk and outcome of infection. *Int J Obes.* (2013) 37:333–40. doi: 10.1038/ijo.2012.62
28. Sattar N, McInnes IB, McMurray JJV. Obesity is a risk factor for severe COVID-19 infection. *Circulation.* (2020) 142:4–6. doi: 10.1161/CIRCULATIONAHA.120.047659
29. Dixon AE, Peters U. The effect of obesity on lung function. *Expert Rev Respir Med.* (2018) 12:755–67. doi: 10.1080/17476348.2018.1506331
30. Saberi M, Woods N-B, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, et al. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab.* (2009) 10:419–29. doi: 10.1016/j.cmet.2009.09.006
31. Vitseva OI, Tanriverdi K, Tchkonia TT, Kirkland JL, McDonnell ME, Apovian CM, et al. Inducible toll-like receptor and NF-kappaB regulatory pathway expression in human adipose tissue. *Obesity.* (2008) 16:932–7. doi: 10.1038/oby.2008.25
32. Thomas D, Apovian C. Macrophage functions in lean and obese adipose tissue. *Metabolism.* (2017) 72:120–43. doi: 10.1016/j.metabol.2017.04.005
33. Karczewski J, Sledzińska E, Baturo A, Jończyk I, Maleszko A, Samborski P, et al. Obesity and inflammation. *Eur Cytok Netw.* (2018) 29:83–94. doi: 10.1684/ecn.2018.0415
34. Chen L, Chen R, Wang H, Liang F. Mechanisms linking inflammation to insulin resistance. *Int J Endocrinol.* (2015) 2015:508409. doi: 10.1155/2015/508409
35. Cerf ME. Beta cell dysfunction and insulin resistance. *Front Endocrinol.* (2013) 4:37. doi: 10.3389/fendo.2013.00037
36. Darvall KAL, Sam RC, Silverman SH, Bradbury AW, Adam DJ. Obesity and thrombosis. *Eur J Vasc Endovasc Surg.* (2007) 33:223–33. doi: 10.1016/j.ejvs.2006.10.006
37. Samad F, Ruf W. Inflammation, obesity, and thrombosis. *Blood.* (2013) 122:3415–22. doi: 10.1182/blood-2013-05-427708
38. Czuprynska J, Arya R. Chapter 27 - obesity and venous thromboembolism. In: Mahmood TA, Arulkumaran S, Chervenak FA, editors. *Obesity and Gynecology.* 2nd ed. Amsterdam: Elsevier (2020). p. 245–53.
39. Freedman JE, Larson MG, Tanriverdi K, O'Donnell CJ, Morin K, Hakanson AS, et al. Relation of platelet and leukocyte inflammatory transcripts to body mass index in the Framingham heart study. *Circulation.* (2010) 122:119–29. doi: 10.1161/CIRCULATIONAHA.109.928192
40. Blokhin IO, Lentz SR. Mechanisms of thrombosis in obesity. *Curr Opin Hematol.* (2013) 20:437–44. doi: 10.1097/MOH.0b013e3283634443
41. Samad F, Loskutoff DJ. Tissue distribution and regulation of plasminogen activator inhibitor-1 in obese mice. *Mol Med.* (1996) 2:568–82. doi: 10.1007/BF03401641
42. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol.* (2020) 75:2950–73. doi: 10.1016/j.jacc.2020.04.031
43. Kass DA, Duggal P, Cingolani O. Obesity could shift severe COVID-19 disease to younger ages. *Lancet.* (2020) 395:1544–5. doi: 10.1016/S0140-6736(20)31024-2
44. Stefan N, Birkenfeld AL, Schulze MB, Ludwig DS. Obesity and impaired metabolic health in patients with COVID-19. *Nat Rev Endocrinol.* (2020) 16:341–2. doi: 10.1038/s41574-020-0364-6
45. Petrakis D, Marginǎ D, Tsarouhas K, Tekos F, Stan M, Nikitovic D, et al. Obesity - a risk factor for increased COVID-19 prevalence, severity and lethality (Review). *Mol Med Rep.* (2020) 22:9–19. doi: 10.3892/mmr.2020.11127
46. Zabetakis I, Lordan R, Norton C, Tsoupras A. COVID-19: the inflammation link and the role of nutrition in potential mitigation. *Nutrients.* (2020) 12:1466. doi: 10.3390/nu12051466

47. Ho FK, Celis-Morales CA, Gray SR, Katikireddi SV, Niedzwiedz CL, Hastie C, et al. Modifiable and non-modifiable risk factors for COVID-19: results from UK Biobank. *medRxiv*. (2020). doi: 10.1101/2020.04.28.20083295
48. Williamson E, Walker AJ, Bhaskaran KJ, Bacon S, Bates C, Morton CE, et al. OpenSAFELY: factors associated with COVID-19-related hospital death in the linked electronic health records of 17 million adult NHS patients. *medRxiv*. (2020). doi: 10.1101/2020.05.06.20092999
49. Hussain A, Mahawar K, Xia Z, Yang W, El-Hasani S. Obesity and mortality of COVID-19. Meta-analysis. *Obes Res Clin Pract.* (2020) 14:295–300. doi: 10.1016/j.orcp.2020.07.002
50. Rojas-Osornio SA, Cruz-Hernández TR, Drago-Serrano ME, Campos-Rodríguez R. Immunity to influenza: impact of obesity. *Obes Res Clin Pract*. (2019) 13:419–29. doi: 10.1016/j.orcp.2019.05.003
51. Vellozo NS, Pereira-Marques ST, Cabral-Piccin MP, Filardy AA, Ribeiro-Gomes FL, Rigoni TS, et al. All-trans retinoic acid promotes an M1- to M2-phenotype shift and inhibits macrophage-mediated immunity to leishmania major. *Front Immunol.* (2017) 8:1560. doi: 10.3389/fimmu.2017.01560
52. Chang H-K, Hou W-S. Retinoic acid modulates interferon-γ production by hepatic natural killer T cells via phosphatase 2A and the extracellular signal-regulated kinase pathway. *J Interferon Cytok Res.* (2015) 35:200–12. doi: 10.1089/jir.2014.0098
53. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science*. (2007) 317:256–60. doi: 10.1126/science.1145697
54. Sinha S, Cheng K, Aldape K, Schiff E, Ruppin E. Systematic cell line-based identification of drugs modifying ACE2 expression. *Preprints*. (2020). doi: 10.20944/preprints202003.0446.v1
55. Huang Z, Liu Y, Qi G, Brand D, Zheng SG. Role of vitamin A in the immune system. *J Clin Med.* (2018) 7:258. doi: 10.3390/jcm7090258
56. Carr AC, Maggini S. Vitamin C and immune function. *Nutrients.* (2017) 9:1211. doi: 10.3390/nu9111211
57. Matheu V, Bäck O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. *J Allergy Clin Immunol.* (2003) 112:585–92. doi: 10.1016/S0091-6749(03)01855-4
58. Nishio K, Horie M, Akazawa Y, Shichiri M, Iwahashi H, Hagihara Y, et al. Attenuation of lipopolysaccharide (LPS)-induced cytotoxicity by tocopherols and tocotrienols. *Redox Biol.* (2013) 1:97–103. doi: 10.1016/j.redox.2012.10.002
59. Hernandez ML, Wagner JG, Kala A, Mills K, Wells HB, Alexis NE, et al. Vitamin E, γ-tocopherol, reduces airway neutrophil recruitment after inhaled endotoxin challenge in rats and in healthy volunteers. *Free Radic Biol Med.* (2013) 60:56–62. doi: 10.1016/j.freeradbiomed.2013.02.001
60. Nagai A, Matsumiya H, Hayashi M, Yasui S, Okamoto H, Konno K. Effects of nicotinamide and niacin on bleomycin-induced acute injury and subsequent fibrosis in hamster lungs. *Exp Lung Res.* (1994) 20:263–81. doi: 10.3109/01902149409064387
61. Gharote MA. Role of poly (ADP) ribose polymerase-1 inhibition by nicotinamide as a possible additive treatment to modulate host immune response and prevention of cytokine storm in COVID-19. *Indian J Med Sci.* (2020) 72:25–8. doi: 10.25259/IJMS_29_2020
62. Tamura J, Kubota K, Murakami H, Sawamura M, Matsushima T, Tamura T, et al. Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. *Clin Exp Immunol.* (1999) 116:28–32. doi: 10.1046/j.1365-2249.1999.00870.x
63. Kumar V, Jena M. *In silico* virtual screening-based study of nutraceuticals predicts the therapeutic potentials of folic acid and its derivatives against COVID-19. *Res Square*. (2020). doi: 10.21203/rs.3.rs-31775/v1
64. Hirmerová J. Homocysteine and venous thromboembolism—Is there any link? *Cor et Vasa.* (2013) 55:e248–58. doi: 10.1016/j.crvasa.2013.01.007
65. Nelson SM, Lei X, Prabhu KS. Selenium levels affect the IL-4–induced expression of alternative activation markers in murine macrophages. *J Nutr.* (2011) 141:1754–61. doi: 10.3945/jn.111.141176
66. Kieliszek M, Lipinski B. Selenium supplementation in the prevention of coronavirus infections (COVID-19). *Med Hypotheses.* (2020) 143:109878. doi: 10.1016/j.mehy.2020.109878
67. Morgan CI, Ledford JR, Zhou P, Page K. Zinc supplementation alters airway inflammation and airway hyperresponsiveness to a common allergen. *J Inflamm.* (2011) 8:36. doi: 10.1186/1476-9255-8-36
68. Lang CJ, Hansen M, Roscioli E, Jones J, Murgia C, Leigh Ackland M, et al. Dietary zinc mediates inflammation and protects against wasting and metabolic derangement caused by sustained cigarette smoke exposure in mice. *Biometals*. (2011) 24:23–39. doi: 10.1007/s10534-010-9370-9
69. Prasad AS, Bao B, Beck FWJ, Sarkar FH. Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-κB. *Nutrition.* (2011) 27:816–23. doi: 10.1016/j.nut.2010.08.010
70. Beck FW, Prasad AS, Kaplan J, Fitzgerald JT, Brewer GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. *Am J Physiol Endocrinol Metab.* (1997) 272:E1002–7. doi: 10.1152/ajpendo.1997.272.6.E1002
71. Prasad AS. Effects of zinc deficiency on Th1 and Th2 cytokine shifts. *J Infect Dis.* (2000) 182:S62–8. doi: 10.1086/315916
72. Speth R, Carrera E, Jean-Baptiste M, Joachim A, Linares A. Concentration-dependent effects of zinc on angiotensin-converting enzyme-2 activity (1067.4). *FASEB J.* (2014) 28:1067.4. doi: 10.1096/fasebj.28.1_supplement.1067.4
73. Skalny AV, Rink L, Ajsuvakova OP, Aschner M, Gritsenko VA, Alekseenko SI, et al. Zinc and respiratory tract infections: perspectives for COVID-19 (Review). *Int J Mol Med.* (2020) 46:17–26. doi: 10.3892/ijmm.2020.4575
74. Adamcakova-Dodd A, Monick MM, Powers LS, Gibson-Corley KN, Thorne PS. Effects of prenatal inhalation exposure to copper nanoparticles on murine dams and offspring. *Particle Fibre Toxicol.* (2015) 12:30. doi: 10.1186/s12989-015-0105-5
75. Muñoz C, López M, Olivares M, Pizarro F, Arredondo M, Araya M. Differential response of interleukin-2 production to chronic copper supplementation in healthy humans. *Eur Cytok Netw.* (2005) 16:261–5.
76. Gaetke LM, Chow-Johnson HS, Chow CK. Copper: toxicological relevance and mechanisms. *Arch Toxicol.* (2014) 88:1929–38. doi: 10.1007/s00204-014-1355-y
77. Li X, Li L, Tao L, Zheng H, Sun M, Chen Y, et al. Magnesium sulfate prophylaxis attenuates the postpartum effects of preeclampsia by promoting M2 macrophage polarization. *Hypertens Res.* (2020) doi: 10.1038/s41440-020-0511-4. [Epub ahead of print].
78. Li W, Wu X, Yu J, Ma C, Zhuang P, Zeng J, et al. Magnesium sulfate attenuates lipopolysaccharides-induced acute lung injury in mice. *Chin J Physiol.* (2019) 62:203–9. doi: 10.4103/CJP.CJP_48_19
79. Van de Graaf RC, Hofstra L. Obesity and covid-19: the role of the food industry. *BMJ.* (2020) 370:m2813. doi: 10.1136/bmj.m2813
80. Jia X, Yin C, Lu S, Chen Y, Liu Q, Bai J, et al. Two things about COVID-19 might need attention. *Preprints*. (2020). doi: 10.20944/preprints202002.0315.v1
81. Kruglikov IL, Scherer PE. The role of adipocytes and adipocyte-like cells in the severity of COVID-19 infections. *Obesity.* (2020) 28:1187–90. doi: 10.1002/oby.22856
82. Mendy A, Gopal R, Alcorn JF, Forno E. Reduced mortality from lower respiratory tract disease in adult diabetic patients treated with metformin. *Respirology.* (2019) 24:646–51. doi: 10.1111/resp.13486
83. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* (2020) 181:271–80.e8. doi: 10.1016/j.cell.2020.02.052
84. Liu Z, Xiao X, Wei X, Li J, Yang J, Tan H, et al. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. *J Med Virol.* (2020) 92:595–601. doi: 10.1002/jmv.25726
85. Bassendine MF, Bridge SH, McCaughan GW, Gorrell MD. COVID-19 and comorbidities: a role for dipeptidyl peptidase 4 (DPP4) in disease severity? *J Diabetes.* (2020) 12:649–58. doi: 10.1111/1753-0407.13052
86. Patel VB, Mori J, McLean BA, Basu R, Das SK, Ramprasath T, et al. ACE2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes.* (2016) 65:85. doi: 10.2337/db15-0399
87. Cure E, Cumhur Cure M. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may be harmful in patients with diabetes

during COVID-19 pandemic. *Diabetes Metab Syndr Clin Res Rev.* (2020) 14:349–50. doi: 10.1016/j.dsx.2020.04.019
88. Liu F, Long X, Zhang B, Zhang W, Chen X, Zhang Z. ACE2 expression in pancreas may cause pancreatic damage after SARS-CoV-2 infection. *Clin Gastroenterol Hepatol.* (2020) 18:2128–30.e2. doi: 10.1101/2020.02.28.20029181
89. Correa-Rodríguez M, Luis Gómez-Urquiza J, Medina-Martínez I, González-Jiménez E, Schmidt-RioValle J, Rueda-Medina B. Low intakes of vitamins C and A are associated with obesity in early adulthood. *Int J Vitamin Nutr Res.* (2020) 1–10. doi: 10.1024/0300-9831/a000661
90. Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. *Ann Nutr Metab.* (2007) 51:301–23. doi: 10.1159/000107673
91. West CE, Sijtsma SR, Kouwenhoven B, Rombout JHWM, van der Zijpp AJ. Epithelia-damaging virus infections affect vitamin a status in chickens. *J Nutr.* (1992) 122:333–9. doi: 10.1093/jn/122.2.333
92. Zhang L, Liu Y. Potential interventions for novel coronavirus in China: a systematic review. *J Med Virol.* (2020) 92:479–90. doi: 10.1002/jmv.25707
93. Ross AC, Stephensen CB. Vitamin A and retinoids in antiviral responses. *FASEB J.* (1996) 10:979–85. doi: 10.1096/fasebj.10.9.8801180
94. Trasino SE. A role for retinoids in the treatment of covid-19? *Clin Exp Pharmacol Physiol.* (2020) 47:1765–7. doi: 10.1111/1440-1681.13354
95. Li G, He X, Zhang L, Ran Q, Wang J, Xiong A, et al. Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19. *J Autoimmunity.* (2020) 2020:102463. doi: 10.1016/j.jaut.2020.102463
96. Zhou TB, Ou C, Rong L, Drummen GP. Effect of all-trans retinoic acid treatment on prohibitin and renin-angiotensin-aldosterone system expression in hypoxia-induced renal tubular epithelial cell injury. *J Renin Angiotensin Aldosterone Syst.* (2014) 15:243–9. doi: 10.1177/1470320314542727
97. Timoneda J, Rodríguez-Fernández L, Zaragozá R, Marín MP, Cabezuelo MT, Torres L, et al. Vitamin A deficiency and the lung. *Nutrients.* (2018) 10:1132. doi: 10.3390/nu10091132
98. Penkert RR, Cortez V, Karlsson EA, Livingston B, Surman SL, Li Y, et al. Vitamin A corrects tissue deficits in diet-induced obese mice and reduces influenza infection after vaccination and challenge. *Obesity.* (2020) 28:1631–6. doi: 10.1002/oby.22929
99. Trasino SE, Tang X-H, Jessurun J, Gudas LJ. Obesity leads to tissue, but not serum vitamin A deficiency. *Sci Rep.* (2015) 5:15893. doi: 10.1038/srep15893
100. Garcia OP. Effect of vitamin A deficiency on the immune response in obesity. *Proc Nutr Soc.* (2012) 71:290–7. doi: 10.1017/S0029665112000079
101. Jeyakumar SM, Vajreswari A, Giridharan NV. Chronic dietary vitamin A supplementation regulates obesity in an obese mutant WNIN/Ob rat model. *Obesity.* (2006) 14:52–9. doi: 10.1038/oby.2006.7
102. Hemilä H. Vitamin C and infections. *Nutrients.* (2017) 9:339. doi: 10.3390/nu9040339
103. Hemilä H. Vitamin C supplementation and respiratory infections: a systematic review. *Military Med.* (2004) 169:920–5. doi: 10.7205/MILMED.169.11.920
104. Kim Y, Kim H, Bae S, Choi J, Lim SY, Lee N, et al. Vitamin C is an essential factor on the anti-viral immune responses through the production of interferon-α/β at the initial stage of influenza A virus (H3N2) infection. *Immune Netw.* (2013) 13:70–4. doi: 10.4110/in.2013.13.2.70
105. Trinchieri G. Type I interferon: friend or foe? *J Exp Med.* (2010) 207:2053–63. doi: 10.1084/jem.20101664
106. Marik PE. Hydrocortisone, ascorbic acid and Thiamine (HAT Therapy) for the treatment of sepsis. Focus on ascorbic acid. *Nutrients.* (2018) 10:1762. doi: 10.3390/nu10111762
107. Garcia-Diaz DF, Lopez-Legarrea P, Quintero P, Martinez JA. Vitamin C in the treatment and/or prevention of obesity. *J Nutr Sci Vitaminol.* (2014) 60:367–79. doi: 10.3177/jnsv.60.367
108. Vorilhon P, Arpajou B, Vaillant Roussel H, Merlin É, Pereira B, Cabaillot A. Efficacy of vitamin C for the prevention and treatment of upper respiratory tract infection. A meta-analysis in children. *Eur J Clin Pharmacol.* (2019) 75:303–11. doi: 10.1007/s00228-018-2601-7
109. Fowler AA, 3rd, Syed AA, Knowlson S, Sculthorpe R, Farthing D, deWilde C, et al. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J Transl Med.* (2014) 12:32. doi: 10.1186/1479-5876-12-32
110. Fisher BJ, Seropian IM, Kraskauskas D, Thakkar JN, Voelkel NF, Fowler AA, et al. Ascorbic acid attenuates lipopolysaccharide-induced acute lung injury. *Crit Care Med.* (2011) 39:1454–60. doi: 10.1097/CCM.0b013e3182120cb8
111. Mah E, Matos MD, Kawiecki D, Ballard K, Guo Y, Volek JS, et al. Vitamin C status is related to proinflammatory responses and impaired vascular endothelial function in healthy, college-aged lean and obese men. *J Am Diet Assoc.* (2011) 111:737–43. doi: 10.1016/j.jada.2011.02.003
112. Ellulu MS, Rahmat A, Patimah I, Khaza'ai H, Abed Y. Effect of vitamin C on inflammation and metabolic markers in hypertensive and/or diabetic obese adults: a randomized controlled trial. *Drug Des Devel Ther.* (2015) 9:3405–12. doi: 10.2147/DDDT.S83144
113. Furuya A, Uozaki M, Yamasaki H, Arakawa T, Arita M, Koyama AH. Antiviral effects of ascorbic and dehydroascorbic acids *in vitro*. *Int J Mol Med.* (2008) 22:541–5. doi: 10.3892/ijmm_00000053
114. Cai Y, Li Y-F, Tang L-P, Tsoi B, Chen M, Chen H, et al. A new mechanism of vitamin C effects on A/FM/1/47(H1N1) virus-induced pneumonia in restraint-stressed mice. *Biomed Res Int.* (2015) 2015:675149. doi: 10.1155/2015/675149
115. Peng Z. *Vitamin C Infusion for the Treatment of Severe 2019-nCoV Infected Pneumonia*. (2020). Available online at: https://clinicaltrials.gov/ct2/show/NCT04264533 (accessed June 1, 2020).
116. Erol A. *High-Dose Intravenous Vitamin C Treatment for COVID-19.* (2020). Available online at: https://osf.io/p7ex8/ (accessed June 5, 2020).
117. Ginde AA, Mansbach JM, Camargo CA Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National health and nutrition examination survey. *Arch Intern Med.* (2009) 169:384–90. doi: 10.1001/archinternmed.2008.560
118. Park JM, Park CY, Han SN. High fat diet-Induced obesity alters vitamin D metabolizing enzyme expression in mice. *BioFactors.* (2015) 41:175–82. doi: 10.1002/biof.1211
119. Lin C-H, Liao W-L, Wu C-C, Shaw H-M, Hsu W-L, Lu Y-C, et al. Nutritional status of obese Taiwanese before bariatric-metabolic surgery and their serum 25-hydroxyvitamin D concentrations for maximal suppression of parathyroid hormone. *Obes Surg.* (2020) 10:3940–6. doi: 10.1007/s11695-020-04759-y
120. Craveiro V, Cabral M, Araujo J, Falcao H, Guimaraes JT, Ramos E. Association of serum 25-hydroxyvitamin D concentration with pulmonary function in young adults. *Nutrients.* (2018) 10:1728. doi: 10.3390/nu10111728
121. Greiller CL, Martineau AR. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients.* (2015) 7:4240–70. doi: 10.3390/nu7064240
122. Gruber-Bzura BM. Vitamin D and influenza-prevention or therapy? *Int J Mol Sci.* (2018) 19:2419. doi: 10.3390/ijms19082419
123. Kohlmeier M. Avoidance of vitamin D deficiency to slow the COVID-19 pandemic. *BMJ Nutr Prev Health.* (2020) 3:bmjnph-2020-000096. doi: 10.1136/bmjnph-2020-000096
124. Lanham-New SA, Webb AR, Cashman KD, Buttriss JL, Fallowfield JL, Masud T, et al. Vitamin D and SARS-CoV-2 virus/COVID-19 disease. *BMJ Nutr Prev Health.* (2020) 3:bmjnph-2020-000089. doi: 10.1136/bmjnph-2020-000089
125. Hughes DA, Norton R. Vitamin D and respiratory health. *Clin Exp Immunol.* (2009) 158:20–5. doi: 10.1111/j.1365-2249.2009.04001.x
126. Zdrenghea MT, Makrinioti H, Bagacean C, Bush A, Johnston SL, Stanciu LA. Vitamin D modulation of innate immune responses to respiratory viral infections. *Rev Med Virol.* (2017) 27:e1909. doi: 10.1002/rmv.1909
127. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J ImmunoL.* (2007) 179:1634–47. doi: 10.4049/jimmunol.179.3.1634
128. Cantorna MT, Snyder L, Lin Y-D, Yang L. Vitamin D and 1,25(OH)2D regulation of T cells. *Nutrients.* (2015) 7:3011–21. doi: 10.3390/nu7043011
129. Almerighi C, Sinistro A, Cavazza A, Ciaprini C, Rocchi G, Bergamini A. 1Alpha,25-dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in human monocytes. *Cytokine.* (2009) 45:190–7. doi: 10.1016/j.cyto.2008.12.009
130. Aranow C. Vitamin D and the immune system. *J Investig Med.* (2011) 59:881–6. doi: 10.2310/JIM.0b013e31821b8755
131. Glaab E, Ruepp A, Montrone C, Fobo G, Ostaszewski M. *The Role of the Interaction Between the SARS-CoV-2 Spike Protein and the Renin-Angiotensin*

Pathway, in Particular Human ACE2 in Pulmonary Blood Pressure Regulation. (2020). Available online at: https://fairdomhub.org/models/709 (accessed June 25, 2020).
132. Rafiullah M. Can a combination of AT1R antagonist and Vitamin D treat the lung complication of COVID-19? *Am J Med Sci*. (2020) 360:338–41. doi: 10.1016/j.amjms.2020.07.018
133. Bergman P, Lindh AU, Björkhem-Bergman L, Lindh JD. Vitamin D and respiratory tract infections: a systematic review and meta-analysis of randomized controlled trials. *PLoS ONE*. (2013) 8:e65835. doi: 10.1371/journal.pone.0065835
134. Hastie CE, Mackay DF, Ho F, Celis-Morales CA, Katikireddi SV, Niedzwiedz CL, et al. Vitamin D concentrations and COVID-19 infection in UK Biobank. *Diabetes Metab Syndr Clin Res Rev*. (2020) 14:561–5. doi: 10.1016/j.dsx.2020.04.050
135. Moriguchi S, Muraga M. Vitamin E and immunity. *Vitamins Hormones*. (2000) 59:305–36. doi: 10.1016/S0083-6729(00)59011-6
136. Hybertson BM, Leff JA, Beehler CJ, Barry PC, Repine JE. Effect of vitamin E deficiency and supercritical fluid aerosolized vitamin E supplementation on interleukin-1-induced oxidative lung injury in rats. *Free Radic Biol Med*. (1995) 18:537–42. doi: 10.1016/0891-5849(94)00180-R
137. Yamaoka S, Kim H-S, Ogihara T, Oue S, Takitani K, Yoshida Y, et al. Severe Vitamin E deficiency exacerbates acute hyperoxic lung injury associated with increased oxidative stress and inflammation. *Free Radic Res*. (2008) 42:602–12. doi: 10.1080/10715760802189864
138. Hanson C, Lyden E, Furtado J, Campos H, Sparrow D, Vokonas P, et al. Serum tocopherol levels and vitamin E intake are associated with lung function in the normative aging study. *Clin Nutr*. (2016) 35:169–74. doi: 10.1016/j.clnu.2015.01.020
139. Agler AH, Kurth T, Gaziano JM, Buring JE, Cassano PA. Randomised vitamin E supplementation and risk of chronic lung disease in the Women's Health Study. *Thorax*. (2011) 66:320–5. doi: 10.1136/thx.2010.155028
140. Meydani SN, Leka LS, Fine BC, Dallal GE, Keusch GT, Singh MF, et al. Vitamin E and respiratory tract infections in elderly nursing home residents: a randomized controlled trial. *JAMA*. (2004) 292:828–36. doi: 10.1001/jama.292.7.828
141. Turski WA, Wnorowski A, Turski GN, Turski CA, Turski L. AhR and IDO1 in pathogenesis of Covid-19 and the "systemic AhR activation syndrome:" translational review and therapeutic perspectives. *Restor Neurol Neurosci*. (2020) 38, 343–54. doi: 10.3233/RNN-201042
142. Belghasem M, Roth D, Richards S, Napolene MA, Walker J, Yin W, et al. Metabolites in a mouse cancer model enhance venous thrombogenicity through the aryl hydrocarbon receptor–tissue factor axis. *Blood*. (2019) 134:2399–413. doi: 10.1182/blood.2019001675
143. Weidenbusch M, Rodler S, Song S, Romoli S, Marschner Julian A, Kraft F, et al. Gene expression profiling of the Notch-AhR-IL22 axis at homeostasis and in response to tissue injury. *Biosci Rep*. (2017) 37:BSR20170099. doi: 10.1042/BSR20170099
144. Kerley-Hamilton JS, Trask HW, Ridley CJA, Dufour E, Ringelberg CS, Nurinova N, et al. Obesity is mediated by differential aryl hydrocarbon receptor signaling in mice fed a Western diet. *Environ Health Perspect*. (2012) 120:1252–9. doi: 10.1289/ehp.1205003
145. Moyer BJ, Rojas IY, Kerley-Hamilton JS, Hazlett HF, Nemani KV, Trask HW, et al. Inhibition of the aryl hydrocarbon receptor prevents Western diet-induced obesity. Model for AHR activation by kynurenine via oxidized-LDL, TLR2/4, TGFβ, and IDO1. *Toxicol Appl Pharmacol*. (2016) 300:13–24. doi: 10.1016/j.taap.2016.03.011
146. Kulanuwat S, Tungtrongchitr R, Billington D, Davies IG. Prevalence of plasma small dense LDL is increased in obesity in a Thai population. *Lipids Health Dis*. (2015) 14:30. doi: 10.1186/s12944-015-0034-1
147. McMillan BJ, Bradfield CA. The aryl hydrocarbon receptor is activated by modified low-density lipoprotein. *Proc Natl Acad Sci USA*. (2007) 104:1412–7. doi: 10.1073/pnas.0607296104
148. Takami M, Fujimaki K, Nishimura MI, Iwashima M. Cutting edge: AhR is a molecular target of calcitriol in human T cells. *J Immunol*. (2015) 195:2520. doi: 10.4049/jimmunol.1500344
149. Stapelberg M, Zobalova R, Nguyen MN, Walker T, Stantic M, Goodwin J, et al. Indoleamine-2,3-dioxygenase elevated in tumor-initiating cells is suppressed by mitocans. *Free Radic Biol Med*. (2014) 67:41–50. doi: 10.1016/j.freeradbiomed.2013.10.003
150. Hong G, Zheng D, Zhang L, Ni R, Wang G, Fan G-C, et al. Administration of nicotinamide riboside prevents oxidative stress and organ injury in sepsis. *Free Radic Biol Med*. (2018) 123:125–37. doi: 10.1016/j.freeradbiomed.2018.05.073
151. Zingarelli B, Salzman AL, Szabó C. Protective effects of nicotinamide against nitric oxide-mediated delayed vascular failure in endotoxic shock: potential involvement of polyADP ribosyl synthetase. *Shock*. (1996) 5:258–64. doi: 10.1097/00024382-199604000-00005
152. Yamaguchi S, Yoshino J. Adipose tissue NAD(+) biology in obesity and insulin resistance: from mechanism to therapy. *Bioessays*. (2017) 39:10.1002/bies.201600227. doi: 10.1002/bies.201600227
153. Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, et al. The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. *Cell Metab*. (2012) 15:838–47. doi: 10.1016/j.cmet.2012.04.022
154. Fimognari FL, Di Simone S, Corsonello A, Pastorelli R, Sampietro F, Loffredo L, et al. Poor vitamin B6 status: a novel potential thrombotic factor in chronic obstructive pulmonary disease. *Nutr Metab Cardiovasc Dis*. (2010) 20:e17–8. doi: 10.1016/j.numecd.2010.06.008
155. Shan M-R, Zhou S-N, Fu C-N, Song J-W, Wang X-Q, Bai W-W, et al. Vitamin B6 inhibits macrophage activation to prevent lipopolysaccharide-induced acute pneumonia in mice. *J Cell Mol Med*. (2020) 24:3139–48. doi: 10.1111/jcmm.14983
156. Desbarats J. Pyridoxal 5'-phosphate to mitigate immune dysregulation and coagulopathy in COVID-19. *Preprints*. (2020). doi: 10.20944/preprints202005.0144.v1
157. Menezes RR, Godin AM, Rodrigues FF, Coura GME, Melo ISF, Brito AMS, et al. Thiamine and riboflavin inhibit production of cytokines and increase the anti-inflammatory activity of a corticosteroid in a chronic model of inflammation induced by complete Freund's adjuvant. *Pharmacol Rep*. (2017) 69:1036–43. doi: 10.1016/j.pharep.2017.04.011
158. Yam D, Aluisio AR, Perera SM, Peters JL, Cho DK, Kennedy SB, et al. Association between multivitamin supplementation and mortality among patients with Ebola virus disease: an international multisite cohort study. *Afr J Emerg Med*. (2020) 10:23–9. d 0.1016/j.afjem.2019.11.001
159. Sheybani Z, Dokoohaki MH, Negahdaripour M, Dehdashti M, Zolghadr H, Moghadami M, et al. The role of folic acid in the management of respiratory disease caused by COVID-19. *ChemRxiv*. (2020). doi: 10.26434/chemrxiv.12034980
160. Serseg T, Benarous K, Yousfi M. Hispidin and lepidine E: two natural compounds and folic acid as potential inhibitors of 2019-novel coronavirus main protease (2019-nCoVMpro), molecular docking and SAR study. *Curr Comput Aided Drug Des*. (2020) 16:1–11. doi: 10.2174/1573409916666200422075440
161. Haloul M, Vinjamuri SJ, Naquiallah D, Mirza MI, Qureshi M, Hassan C, et al. Hyperhomocysteinemia and low folate and vitamin B12 are associated with vascular dysfunction and impaired nitric oxide sensitivity in morbidly obese patients. *Nutrients*. (2020) 12:2014. doi: 10.3390/nu12072014
162. Nielsen FH. Magnesium, inflammation, and obesity in chronic disease. *Nutr Rev*. (2010) 68:333–40. doi: 10.1111/j.1753-4887.2010.00293.x
163. Costello RB, Elin RJ, Rosanoff A, Wallace TC, Guerrero-Romero F, Hruby A, et al. Perspective: the case for an evidence-based reference interval for serum magnesium: the time has come. *Adv Nutr*. (2016) 7:977–93. doi: 10.3945/an.116.012765
164. Mathew R, Altura BM. The role of magnesium in lung diseases: asthma, allergy and pulmonary hypertension. *Magnes Trace Elem*. (1991) 10:220–8.
165. Tan CW, Ho LP, Kalimuddin S, Cherng BPZ, Teh YE, Thien SY, et al. A cohort study to evaluate the effect of combination Vitamin D, Magnesium and Vitamin B12 (DMB) on progression to severe outcome in older COVID-19 patients. *medRxiv*. (2020) 2020.06.01.20112334. doi: 10.1101/2020.06.01.20112334
166. Wallace TC. Combating COVID-19 and building immune resilience: a potential role for magnesium nutrition? *J Am Coll Nutr*. (2020) 10:1–9. doi: 10.1080/07315724.2020.1785971
167. Parichehr P, Guitti P. Magnesium sulfate: a potential adjuvant treatment on COVID-19. *Adv J Emerg Med*. (2020) doi: 10.22114/ajem.v0i0.508

168. Rayman MP. Selenium and human health. *Lancet.* (2012) 379:1256–68. doi: 10.1016/S0140-6736(11)61452-9
169. Hoffmann PR, Berry MJ. The influence of selenium on immune responses. *Mol Nutr Food Res.* (2008) 52:1273–80. doi: 10.1002/mnfr.200700330
170. Baum MK, Shor-Posner G, Lai S, Zhang G, Lai H, Fletcher MA, et al. High risk of HIV-related mortality is associated with selenium deficiency. *J Acqu Immune Def Syndr Hum Retrovirol.* (1997) 15:370–4. doi: 10.1097/00042560-199708150-00007
171. Beck MA, Levander OA, Handy J. Selenium deficiency and viral infection. *J Nutr.* (2003) 133:1463–7S. doi: 10.1093/jn/133.5.1463S
172. Steinbrenner H, Al-Quraishy S, Dkhil MA, Wunderlich F, Sies H. Dietary selenium in adjuvant therapy of viral and bacterial infections. *Adv Nutr.* (2015) 6:73–82. doi: 10.3945/an.114.007575
173. Hoffmann FW, Hashimoto AC, Shafer LA, Dow S, Berry MJ, Hoffmann PR. Dietary selenium modulates activation and differentiation of CD4+ T cells in mice through a mechanism involving cellular free thiols. *J Nutr.* (2010) 140:1155–61. doi: 10.3945/jn.109.120725
174. Zhang J, Taylor EW, Bennett K, Saad R, Rayman MP. Association between regional selenium status and reported outcome of COVID-19 cases in China. *Am J Clin Nutr.* (2020) 111:1297–9. doi: 10.1093/ajcn/nqaa095
175. Fakhrolmobasheri M, Nasr-Esfahany Z, Khanahmad H, Zeinalian M. Selenium supplementation can relieve the clinical complications of COVID-19 and other similar viral infections. *Int J Vitam Nutr Res.* (2020). doi: 10.1024/0300-9831/a000663. [Epub ahead of print].
176. Mahmoodpoor A, Hamishehkar H, Shadvar K, Ostadi Z, Sanaie S, Saghaleini SH, et al. The effect of intravenous selenium on oxidative stress in critically Ill patients with acute respiratory distress syndrome. *Immunol Invest.* (2019) 48:147–59. doi: 10.1080/08820139.2018.1496098
177. Wang L, Jing J, Yan H, Tang J, Jia G, Liu G, et al. Selenium pretreatment alleviated LPS-induced immunological stress via upregulation of several selenoprotein encoding genes in murine RAW264.7 cells. *Biol Trace Elem Res.* (2018) 186:505–13. doi: 10.1007/s12011-018-1333-y
178. Tinkov AA, Ajsuvakova OP, Filippini T, Zhou JC, Lei XG, Gatiatulina ER, et al. Selenium and selenoproteins in adipose tissue physiology and obesity. *Biomolecules.* (2020) 10:658. doi: 10.3390/biom10040658
179. Moghaddam A, Heller RA, Sun Q, Seelig J, Cherkezov A, Seibert L, et al. Selenium deficiency is associated with mortality risk from COVID-19. *Nutrients.* (2020) 12:2098. doi: 10.3390/nu12072098
180. Prasad AS. Zinc: role in immunity, oxidative stress and chronic inflammation. *Curr Opin Clini Nutr Metab Care.* (2009) 12:646–52. doi: 10.1097/MCO.0b013e3283312956
181. Koch J, Neal EA, Schlott MJ, Garcia-Shelton YL, Chan MF, Weaver KE, et al. Zinc levels and infections in hospitalized patients with AIDS. *Nutrition.* (1996) 12:515–8. doi: 10.1016/S0899-9007(96)00173-6
182. Iddir M, Brito A, Dingeo G, Fernandez Del Campo SS, Samouda H, La Frano MR, et al. Strengthening the immune system and reducing inflammation and oxidative stress through diet and nutrition: considerations during the COVID-19 crisis. *Nutrients.* (2020) 12:1562. doi: 10.3390/nu12061562
183. Razzaque M. COVID-19 pandemic: can maintaining optimal zinc balance enhance host resistance? *Preprints.* (2020). doi: 10.20944/preprints202004.0006.v1
184. Mossad SB, Macknin ML, Medendorp SV, Mason P. Zinc gluconate lozenges for treating the common cold. A randomized, double-blind, placebo-controlled study. *Ann Int Med.* (1996) 125:81–8. doi: 10.7326/0003-4819-125-2-199607150-00001
185. Truong-Tran AQ, Carter J, Ruffin R, Zalewski PD. New insights into the role of zinc in the respiratory epithelium. *Immunol Cell Biol.* (2001) 79:170–7. doi: 10.1046/j.1440-1711.2001.00986.x
186. Roscioli E, Jersmann HP, Lester S, Badiei A, Fon A, Zalewski P, et al. Zinc deficiency as a codeterminant for airway epithelial barrier dysfunction in an *ex vivo* model of COPD. *Int J Chron Obstruct Pulmon Dis.* (2017) 12:3503–10. doi: 10.2147/COPD.S149589
187. Suara RO, Crowe JE Jr. Effect of zinc salts on respiratory syncytial virus replication. *Antimicrob Agents Chemother.* (2004) 48:783–90. doi: 10.1128/AAC.48.3.783-790.2004
188. Xue J, Moyer A, Peng B, Wu J, Hannafon BN, Ding W-Q. Chloroquine is a zinc ionophore. *PLoS ONE.* (2014) 9:e109180. doi: 10.1371/journal.pone.0109180
189. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) *in vitro*. *Cell Res.* (2020) 30:269–71. doi: 10.1038/s41422-020-0282-0
190. Finzi E. Treatment of SARS-CoV-2 with high dose oral zinc salts: a report on four patients. *Int J Infect Dis.* (2020) 99:307–9. doi: 10.1016/j.ijid.2020.06.006
191. Hemilä H, Chalker E. Zinc for preventing and treating the common cold. *Cochrane Database Syst Rev.* (2017) 2017:CD012808. doi: 10.1002/14651858.CD012808
192. Read SA, Obeid S, Ahlenstiel C, Ahlenstiel G. The role of zinc in antiviral immunity. *Adv Nutr.* (2019) 10:696–710. doi: 10.1093/advances/nmz013
193. Dagdag RP, Burkhead JL, Bortz E. Influenza versus host: the role of novel copper-related host factors in antiviral immunity. *FASEB J.* (2020) 34:1. doi: 10.1096/fasebj.2020.34.s1.06545
194. Minoshima M, Lu Y, Kimura T, Nakano R, Ishiguro H, Kubota Y, et al. Comparison of the antiviral effect of solid-state copper and silver compounds. *J Hazard Mater.* (2016) 312:1–7. doi: 10.1016/j.jhazmat.2016.03.023
195. Ishida T. Antiviral activities of Cu2+ ions in viral prevention, replication, RNA degradation, and for antiviral efficacies of lytic virus, ROS-mediated virus, copper chelation. *World Sci News.* (2018) 99:148–68. Available online at: www.worldscientificnews.com
196. Han M, Lin Z, Zhang Y. The alteration of copper homeostasis in inflammation induced by lipopolysaccharides. *Biol Trace Elem Res.* (2013) 154:268–74. doi: 10.1007/s12011-013-9725-5
197. Percival SS. Copper and immunity. *Am J Clin Nutr.* (1998) 67:1064–8S. doi: 10.1093/ajcn/67.5.1064S
198. Li C, Li Y, Ding C. The role of copper homeostasis at the host-pathogen axis: from bacteria to fungi. *Int J Mol Sci.* (2019) 20:175. doi: 10.3390/ijms20010175
199. Liu L, Geng X, McDermott J, Shen J, Corbin C, Xuan S, et al. Copper deficiency in the lungs of TNF-α transgenic mice. *Front Physiol.* (2016) 7:234. doi: 10.3389/fphys.2016.00234
200. Bonham M, O'Connor JM, Hannigan BM, Strain JJ. The immune system as a physiological indicator of marginal copper status? *Br J Nutr.* (2002) 87:393–403. doi: 10.1079/BJN2002558
201. Raha S, Mallick R, Basak S, Duttaroy AK. Is copper beneficial for COVID-19 patients? *Med Hypotheses.* (2020) 142:109814. doi: 10.1016/j.mehy.2020.109814
202. Sagripanti JL, Routson LB, Lytle CD. Virus inactivation by copper or iron ions alone and in the presence of peroxide. *Appl Environ Microbiol.* (1993) 59:4374–6. doi: 10.1128/AEM.59.12.4374-4376.1993
203. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and its inactivation with biocidal agents. *J Hosp Infect.* (2020) 104:246–51. doi: 10.1016/j.jhin.2020.01.022
204. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med.* (2020) 382:1564–7. doi: 10.1056/NEJMc2004973
205. González-Gallego J, García-Mediavilla MV, Sánchez-Campos S, Tuñón MJ. Chapter 32 - anti-inflammatory and immunomodulatory properties of dietary flavonoids. In: Watson RR, Preedy VR, Zibadi S, editors. *Polyphenols in Human Health and Disease*. San Diego, CA: Academic Press (2014). p. 435–52.
206. Kawser Hossain M, Abdal Dayem A, Han J, Yin Y, Kim K, Kumar Saha S, et al. Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids. *Int J Mol Sci.* (2016) 17:569. doi: 10.3390/ijms17040569
207. Pan MH, Lai CS, Ho CT. Anti-inflammatory activity of natural dietary flavonoids. *Food Funct.* (2010) 1:15–31. doi: 10.1039/c0fo00103a
208. Vernarelli JA, Lambert JD. Flavonoid intake is inversely associated with obesity and C-reactive protein, a marker for inflammation, in US adults. *Nutr Diabetes.* (2017) 7:e276. doi: 10.1038/nutd.2017.22
209. Geraets L, Haegens A, Brauers K, Haydock JA, Vernooy JHJ, Wouters EFM, et al. Inhibition of LPS-induced pulmonary inflammation by specific flavonoids. *Biochem Biophys Res Commun.* (2009) 382:598–603. doi: 10.1016/j.bbrc.2009.03.071
210. Lago JHG, Toledo-Arruda AC, Mernak M, Barrosa KH, Martins MA, Tibério IFLC, et al. Structure-activity association of flavonoids in

lung diseases. *Molecules.* (2014) 19:3570–95. doi: 10.3390/molecules19033570
211. Vazhappilly CG, Ansari SA, Al-Jaleeli R, Al-Azawi AM, Ramadan WS, Menon V, et al. Role of flavonoids in thrombotic, cardiovascular, and inflammatory diseases. *Inflammopharmacology.* (2019) 27:863–9. doi: 10.1007/s10787-019-00612-6
212. Zakaryan H, Arabyan E, Oo A, Zandi K. Flavonoids: promising natural compounds against viral infections. *Arch Virol.* (2017) 162:2539–51. doi: 10.1007/s00705-017-3417-y
213. Somerville VS, Braakhuis AJ, Hopkins WG. Effect of flavonoids on upper respiratory tract infections and immune function: a systematic review and meta-analysis. *Adv Nutr.* (2016) 7:488–97. doi: 10.3945/an.115.010538
214. Rane J, Chatterjee A, Ray S. Targeting SARS-CoV-2 spike protein of COVID-19 with naturally occurring phytochemicals: an *in silico* study for drug development. *ChemRxiv*. (2020). doi: 10.26434/chemrxiv.12094203.v1
215. Adem S, Eyupoglu V, Sarfraz I, Rasul A, Ali M. Identification of potent COVID-19 main protease (Mpro) inhibitors from natural polyphenols: An *in silico* strategy unveils a hope against CORONA. *Preprints*. (2020). doi: 10.20944/preprints202003.0333.v1
216. Nguyen TTH, Woo H-J, Kang H-K, Nguyen VD, Kim Y-M, Kim D-W, et al. Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in Pichia pastoris. *Biotechnol Lett.* (2012) 34:831–8. doi: 10.1007/s10529-011-0845-8
217. Jena A, Kanungo N, Nayak V, Chainy GBN, Dandapat J. Catechin and Curcumin interact with corona (2019-nCoV/SARS-CoV2) viral S protein and ACE2 of human cell membrane: insights from Computational study and implication for intervention. *Res Square*. (2020). doi: 10.21203/rs.3.rs-22057/v1
218. Gennadi G. Genomics-guided tracing of SARS-CoV-2 targets in human cells identifies Vitamin D and Quercetin as candidate medicinal agents for mitigation of the severity of pandemic COVID-19. *ChemRxiv*. (2020). doi: 10.26434/chemrxiv.12052512.v4
219. Suravajhala R, Parashar A, Malik B, Nagaraj AV, Padmanaban G, Kishor PK, et al. Comparative Docking Studies on Curcumin with COVID-19 Proteins. *Preprints*. (2020). doi: 10.20944/preprints202005.0439.v2
220. Rocha FAC, de Assis MR. Curcumin as a potential treatment for COVID-19. *Phytother Res*. (2020) 34:2085–7. doi: 10.1002/ptr.6745
221. Marinella MA. Indomethacin and resveratrol as potential treatment adjuncts for SARS-CoV-2/COVID-19. *Int J Clin Pract.* (2020) 74:e13535. doi: 10.1111/ijcp.13535
222. Legrand D. Lactoferrin, a key molecule in immune and inflammatory processes. *Biochem Cell Biol.* (2012) 90:252–68. doi: 10.1139/o11-056
223. Lang J, Yang N, Deng J, Liu K, Yang P, Zhang G, et al. Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. *PLoS ONE.* (2011) 6:e23710. doi: 10.1371/journal.pone.0023710
224. Puddu P, Borghi P, Gessani S, Valenti P, Belardelli F, Seganti L. Antiviral effect of bovine lactoferrin saturated with metal ions on early steps of human immunodeficiency virus type 1 infection. *Int J Biochem Cell Biol.* (1998) 30:1055–62. doi: 10.1016/S1357-2725(98)00066-1
225. Superti F, Siciliano R, Rega B, Giansanti F, Valenti P, Antonini G. Involvement of bovine lactoferrin metal saturation, sialic acid and protein fragments in the inhibition of rotavirus infection. *Biochim et Biophys Acta.* (2001) 1528:107–15. doi: 10.1016/S0304-4165(01)00178-7
226. Pietrantoni A, Dofrelli E, Tinari A, Ammendolia MG, Puzelli S, Fabiani C, et al. Bovine lactoferrin inhibits Influenza A virus induced programmed cell death *in vitro*. *BioMetals.* (2010) 23:465–75. doi: 10.1007/s10534-010-9323-3
227. Kell DB, Heyden EL, Pretorius E. The biology of lactoferrin, an iron-binding protein that can help defend against viruses and bacteria. *Front Immunol.* (2020) 11:1221. doi: 10.3389/fimmu.2020.01221
228. Serrano G, Kochergina I, Albors A, Diaz E, Oroval M, Hueso G, et al. Liposomal lactoferrin as potential preventative and cure for COVID-19. *Int J Res Health Sci.* (2020) 8:8–15. doi: 10.5530/ijrhs.8.1.3
229. Sun J, Ren F, Xiong L, Zhao L, Guo H. Bovine lactoferrin suppresses high-fat diet induced obesity and modulates gut microbiota in C57BL/6J mice. *J Funct Foods.* (2016) 22:189–200. doi: 10.1016/j.jff.2016.01.022
230. García OP, Ronquillo D, Caamaño MdC, Camacho M, Long KZ, Rosado JL. Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: results from a cross-sectional study. *Nutr Metab.* (2012) 9:59. doi: 10.1186/1743-7075-9-59
231. Kumar NG, Contaifer D, Madurantakam P, Carbone S, Price ET, Van Tassell B, et al. Dietary bioactive fatty acids as modulators of immune function: implications on human health. *Nutrients.* (2019) 11:2974. doi: 10.3390/nu11122974
232. Itariu BK, Zeyda M, Hochbrugger EE, Neuhofer A, Prager G, Schindler K, et al. Long-chain n−3 PUFAs reduce adipose tissue and systemic inflammation in severely obese nondiabetic patients: a randomized controlled trial. *Am J Clin Nutr.* (2012) 96:1137–49. doi: 10.3945/ajcn.112.037432
233. Flock MR, Rogers CJ, Prabhu KS, Kris-Etherton PM. Immunometabolic role of long-chain omega-3 fatty acids in obesity-induced inflammation. *Diabetes Metab Res Rev.* (2013) 29:431–45. doi: 10.1002/dmrr.2414
234. Martinez-Fernandez L, Laiglesia LM, Huerta AE, Martinez JA, Moreno-Aliaga MJ. Omega-3 fatty acids and adipose tissue function in obesity and metabolic syndrome. *Prostaglandins Other Lipid Mediat.* (2015) 121(Pt. A):24–41. doi: 10.1016/j.prostaglandins.2015.07.003
235. Torrinhas R, Calder P, Lemos G, Waitzberg D. Parenteral fish oil, an adjuvant pharmacotherapy for COVID-19? *Nutrition*. (2020) 81:110900. doi: 10.1016/j.nut.2020.110900
236. Adili R, Hawley M, Holinstat M. Regulation of platelet function and thrombosis by omega-3 and omega-6 polyunsaturated fatty acids. *Prostaglandins Other Lipid Mediat.* (2018) 139:10–8. doi: 10.1016/j.prostaglandins.2018.09.005
237. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* (2020) 191:145–7. doi: 10.1016/j.thromres.2020.04.013

Conflict of Interest: FU was employed by Reven Pharmaceutcal (Golden, CO).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Sahin, Orhan, Uckun and Sahin. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

MINI REVIEW
published: 28 October 2020
doi: 10.3389/fimmu.2020.590716

Tea Bioactive Modulate Innate Immunity: In Perception to COVID-19 Pandemic

Pritom Chowdhury[1*] *and Anoop Kumar Barooah*[2]

[1] *Department of Biotechnology, Tocklai Tea Research Institute, Tea Research Association, Jorhat, India,* [2] *Directorate, Tocklai Tea Research Institute, Tea Research Association, Jorhat, India*

OPEN ACCESS

Edited by:
Alexandros Tsoupras, University of Limerick, Ireland

Reviewed by:
Tanmoy Karak, Tea Research Association, India
Yue-Rong Liang, Zhejiang University, China

***Correspondence:**
Pritom Chowdhury
pritomc@gmail.com

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: *02 August 2020*
Accepted: *25 September 2020*
Published: *28 October 2020*

Citation:
Chowdhury P and Barooah AK (2020) Tea Bioactive Modulate Innate Immunity: In Perception to COVID-19 Pandemic. Front. Immunol. 11:590716. doi: 10.3389/fimmu.2020.590716

Innate immunity impairment led to disruption in cascade of signaling pathways upregulating pro-inflammatory cytokines, diminish interferons, depleted natural killer cells and activate reactive oxygen species production. These conditions severely affected body's ability to fight against infectious diseases and also plays a pivotal role in disease progression. Here, in emphasis is on nutritional immunity for regulating effective innate immune response for combating against infectious diseases like novel coronavirus disease (COVID 19). Drawing from discoveries on in-vitro experiments, animal models and human trials, tea polyphenols, micronutrients, and vitamins has the potential to modulate and enhance innate immune response. This article provides a comprehensive review on tea (*Camellia sinensis* L*)* infusion (a hot water extract of dried processed tea leaves prepared from young shoots of tea plant) as an innate immunity modulator. Tea infusion is rich in polyphenols; epigallocatechin gallate (EGCG) and theaflavin (TF), major green and black tea polyphenols, respectively. Studies showed their immunomodulatory competence. Tea infusions are also rich in alkaloids; caffeine and its intermediates, theophylline and theobromine, which have anti-inflammatory properties. Tea plant being an acidophilic perennial crop can accumulate different micronutrients, *viz.*, copper (Cu), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn) from growing medium, i.e., from soil, which led to their considerable presence in tea infusion. Micronutrients are integral part of innate immune response. Overall, this review presents tea infusion as an important source of nutritional immunity which can enhance innate immune response in order to mitigate the unprecedented COVID-19 pandemic.

Keywords: coronavirus, cytokines, innate immunity, micronutrients, nutritional immunity, tea polyphenols, vitamins

INTRODUCTION

Coronaviruses belongs to a group of enveloped, non-segmented positive-sense RNA viruses belongs to the β genus, *Nidovirales* order of the *Coronaviridae* family (1). A novel coronavirus, since named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in 2019, when it was first reported from Wuhan city of China causing respiratory illness (2). Soon it spread worldwide with cases reported from 182 countries and 33 territories and the World Health Organization has named it as coronavirus disease (COVID-19). Total reported cases surge past 26 millions among which more than 0.88 million succumbed to infection, which accounts for almost 3.26% mortality as on 07 September 2020 (3). On 11 March 2020, WHO declared COVID-19 as pandemic after almost 3 months from its first report (4). Information on COVID-19 pathophysiology is still emerging, but lots have been known till date, particularly on the role of immune response in disease progression (5, 6). Understanding immune regulation triggered by SARS-CoV-2 holds the key for development of vaccines and therapeutics regimen, which are in developing stages. Low neutralizing antibodies reported in a study, which is around 30% of COVID-19 recovered patients is a matter of concern (7). Variation in neutralizing antibodies response and antibody dependent enhancement should be a matter of investigation for effective vaccine candidates (7, 8). Earlier studies on SARS-CoV patients and present evidence on COVID-19 suggested that exacerbate host immune response rather than virulence of the infected virus is responsible for pulmonary injury and multiple organ dysfunctions (9, 10). Therefore, an effective therapeutic needs to address both anti-viral action as well as specific cytokine targeted immunomodulation. This complexity of COVID-19 warrants "multiple approaches" to combat disease progression. In addition to vaccines and effective drugs, a pivotal approach would be of nutritional immunity. This will be important not only to patients with underlying conditions like diabetes, hypertension, etc., but also for other infected and healthy population. Natural compounds in plants are our regular source of nutritious supplements. Among many sources, the tea plant (*Camellia sinensis*) being rich in antioxidant compound polyphenols, micronutrients, and vitamins in tea infusion makes it as an unique beverage. Tea being a popular and most consumed manufactured drink in the world can play an important role in nutritional immunity (11). The tea consumption worldwide stands at around 4.84 million tons (11). Furthermore, consumption of tea is popular thought out the world due to its easy availability as well as its economic viability. In this review, we have highlighted the importance of tea constituents on regulating an effective innate immune response in special perspective to present unprecedented pandemic. The Innate immunity is the first line of body's defense against intruded pathogen and will be most important to constraint the pandemic spread in terms of role of nutritional immunity.

Abbreviations: IFN, Interferon; NK, Natural killer cells; IL, Interleukin; EGCG, Epigallocatechin-3-gallate; TF, Theaflavin; LPS, Lipopolysaccharide; NF-Kb, Nuclear factor kappa B; MAPK, Mitogen-activated protein kinase; Mpro, Main protease; RdRp, RNA-dependent RNA polymerase; RIG-I, Retinoic acid-inducible gene I; TLR, Toll like receptor; ICAM-1, Intercellular adhesion molecule; VCAM, Vascular cell adhesion molecule.

TEA POLYPHENOLS AND ALKALOIDS ROLE IN INNATE IMMUNITY

Immunological responses reported till now suggested a "cytokine storm" mediated disease severity (12, 13). Earlier studies on SARS-CoV and MERS-CoV, showed down regulation of type I Interferon (IFN), as a strategy to evade immune response (9). Present virus SARS-CoV-2 share close genomic proximity with SARS-CoV and MERS-CoV and it is most likely that SARS-CoV-2 has similar mechanism to evade innate immune response (14). Recent findings confirmed that critical COVID-19 patients have an impaired type I IFN production and a lower viral clearance (10). Concurrently, down regulation of interferon stimulated genes and also plasma levels of IFN- α2 protein in critical patient was observed whereas IFN-ß was undetectable in the mild, moderate, and critical categories of patients studied (10). It is noteworthy that patients with underlying disease conditions were excluded from the study. Studies showed that green tea polyphenol (−)epigallocatechin-3-gallate (EGCG) can induced IFN-λ 1, antiviral interferon stimulated genes expression in both Hepatitis C Virus (HCV), Japanese fulminant hepatitis (JFH) -1-infected and uninfected human hepatoma (Huh7) cells (15). The HCV is an RNA virus similar to SARS-CoV-2 and share same mode of action in reference to impairing interferons through down regulation of receptors like toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I) (16). Earlier studies on SARS-CoV reported the role of TLR3 adapter protein, Toll/interleukin-1 receptor domain-containing adapter-inducing interferon-β (TRIF) and TLR4 related TRIF-related adaptor molecule (TRAM) signaling in controlling viral loads in mice alveolar tissues (17). This protective signaling *via* TLR3/TRIF and TLR4/TRAM is postulated to be unique in the pathophysiology of coronaviruses unlike in influenza where their depletion does not change the pathogenesis (17). The same should be looked into SARS-CoV-2 interrogating their role both in clinical and experimental aspect. These may lead to importance of TLR agonist in controlling disease progression. Experimental studies with murine derived macrophage and dendritic cells showed that EGCG intervene TLR4 and TLR2 expression through down streaming mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-Kb) signaling leading to inhibition of pro-inflammatory cytokines (18, 19). Highly expressed TLR2 in alveolar epithelial lung tissue is another target which should be investigated (20). EGCG and theaflavin 3,3′ digallate (TFDG) were found to be a potent RIG-I inhibitors (21). Individuals at high risk to SARS-CoV-2 are those with underlying diseases, including diabetes, hypertension, and cardiovascular disease (14). Children are at lower risk which may be due to their adequate innate immune response. These facts strongly indicate that innate immune response is a critical factor for disease outcome. Mounting evidence suggested that polyphenols and micronutrients improve defense function, i.e.,

resistance to infection, by modulating immune regulation; this may have a strong implication in controlling COVID-19 particularly for cytokine storms (22) EGCG has inhibitory effect on neutrophil transmigration through monolayers of endothelial cells which in turn can reduce vascular permeability (23). It can also reduce neutrophil elastase, a proteolytic molecule implicated with increase permeability in alveolar epithelium (24). Loss of endothelial cells barrier integrity and production of reactive oxygen species by neutrophils leads to homeostatic imbalance which may explain the reason for acute respiratory distress syndrome as observed in COVID-19 patients. This can be characterized by alveolar tissue injury by inflammatory cytokines due to vascular leakage. The inhibitory action of EGCG and theaflavin (TF) against ROS and neutrophil elastase at a concentration available in blood (**Table 1**) is quite encouraging (23, 31, 32). EGCG and TF, a major black tea polyphenol suppresses the lipopolysaccharide (LPS)-induced intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 expression through blockage of nuclear factor-kappa B (NF-κB) and c-Jun N-terminal kinase (JNK) activation pathway (33, 34). These findings probably explain the inhibitory mechanism of neutrophils by tea polyphenols as ICAM-1 and VCAM-1 are expressed on endothelial cells. Evidence of the stimulatory activity of tea polyphenols of different origins on natural killer (NK) cells with a biphasic effect was reported (35, 36). Treatment with polyphenols in mice model with upper airway inflammation showed increase in NK cells degranulation (37). Although further investigation will be required for elucidating the role of tea polyphenols on NK cells, it may be assumed that a similar action will allow us to highlight the pivotal implication it may have on COVID-19 patients. The NK cells were reported to deplete in COVID-19 patients marking the weakening immune response at the early stage of SARS-CoV-2 infection (37, 38). The NK cells depletion was inversely correlated with increase in expression of Natural Killer Cell Receptors (NKG2A) (39). The same has been observed in COVID-19 patients where NKG2A expression was up-regulated (40). Therefore, the available evidences suggest that the tea polyphenols down regulating NKG2A to induce NK cells can play an important role in early stages of virus infection. Another important aspect is "gut-lung" crosstalk, in recent times this phenomenon has gained lot of attention and it is widely recognized that an ideal gut microenvironment is essential for a balanced immune response not only for gastrointestinal track but also for maintaining respiratory homeostasis (41). Experimental and clinical evidences have indicated that host stress response activates macrophages and neutrophils in alveolar cells, leading to increase in pro-inflammatory cytokines and catecholamines (42). This signal is strongly correlated with growth and virulence of bacterial community like streptococcus pneumonia, klebsiella pneumonia perpetuating alveolar inflammation (42). This change in the inflamed lungs also leads to "gut dysbiosis" altering gut microbiota. This may explain severity of gastrointestinal problem, e.g., diarrhea in COVID 19 patients (41). The "gut-lung" axis is a bidirectional phenomenon, which can explain that an altered bacterial diversity in gut also can trigger a cascade of inflammatory signals in the lungs through butyrate, propionate and secondary bile acids secreted by bacteroides which can bind to receptors of dendritic cells and macrophages (41, 42). Murine and clinical intervention studies indicated that tea catechins have prebiotic activities which help in improving gut barrier integrity, decrease LPS containing gram negative bacterial community in

TABLE 1 | Immune activities, content and recovery from human plasma of major green tea polyphenol, epigallocatechin gallate (EGCG), and black tea polyphenol, theaflavin (TF).

Tea Bioactives	Content in tea infusion (mg g^{-1})	Immune activities	Concentration in plasma (peak time)	References
Polyphenols				
EGCG	113.6 ± 5.85[a]	Enhances interferon secretion; enhances NK cell activity; regulates NF-κB, RIG-I dependent signal transduction; Inhibits ROS activity; regulates apoptosis of neutrophils; regulates Th1/Th2 polarization	55 nM (2 h)[b] 37 nM♂–42 nM♀ (2 h)[c]	(25) (26) (27)
TF	0.70 ± 0.08[a]	Down regulate NF-κB,ICAM-1,VCAM-1; inhibits pro-inflammatory cytokines, e.g., IL-6, regulates neutrophils; inhibits ROS activity	8 nM♂–16 nM♀ (45 min)[d]	(28)
Alkaloids				
CAF	Green tea (GT):34.92 ± 0.93[a]; Black tea (BT):35.13 ± 3.02[a]	Regulates neutrophils; suppress monocyte chemotaxis; inhibits TNF-α; neuroprotective effect	30.89 μM (60 min)[e]	(29, 30)
TBR	GT:0.16 ± 0.03[a] BT:0.38 ± 0.10[a]	Vasodilator; muscle relaxant	23.17 μM (120 min)[e]	
TPI	GT:1.54 ± 0.02[a] BT:1.61 ± 0.05[a]	Vasodilator; anti-inflammatory in bronchial airways	23.17 μM (120 min)[e]	

[a]*Tea infusion was prepared with 1 gram of green or black tea in 100 ml of boiled distilled water.*
[b]*Subject followed overnight fasting before taking 500 ml of green tea. Blood samples were collected at 0, 0.5, 1, 2, 4, 6, 8, and 24-h time interval in reference to drinking tea.*
[c]*Subjects were given 300 mg of EGCG (Teavigo® capsules, 94% EGCG) with 100 ml of water. Blood sample was collected before the intervention and at 30, 60, 90, 120, 180, 240, and 360 min after the ingestion. The European Food Safety Agency (EFSA) based on clinical trial has recommended intake of EGCG/day to less than 800 mg.*
[d]*Volunteers consumed 700 mg TF mixed in 150 ml hot water. Symbol designation: ♂, male; ♀, women volunteers.*
[e]*Subjects followed 4 h fasting before taking 270 mg of caffeine (CAF) and 250 mg of each theobromine (TBR) and theophylline (TPI). Blood sample was collected before the intervention and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, till 10 h after methylxanthine dose.*

the gut, and regulate intestinal tight junction protein expressions (42). Tea also contain alkaloids methylxanthine, namely, caffeine (CAF) and its intermediates, theophylline (TPI), and theobromine (TBR) (43). They have structural with purine nucleosides, which enable them to act as competitive inhibitor of adenosine receptor (43). This G-protein coupled receptor is widely present in immune cells in the form of different subtypes and plays a pivotal role in regulating innate immune response (43). These interactions of methylxanthines with adenosine receptor may be attributed to functional role of CAF in suppression of neutrophil, monocyte chemotaxis and inhibition of TNF-alpha in human blood (44). TPI is a proven vasodilator and used in the treatment of breathing condition (Apnea of prematurity) of neonates (43). Due to its anti-inflammatory action on bronchial airways, it may prove beneficial to treat ARDS patients when treated for shorter duration. TBR too has similar activity but is a weaker diuretic and muscle relaxant in comparison to TPI (45). Methylxanthine has also the capability to cross the blood brain barrier and regulate gamma-aminobutyric acid (GABA), a principle neurotransmitter in CNS (43). Studies in mice model showed GABA can control inflammatory cytokines in peripheral macrophages (46). This may have a positive implication in neuroprotection of COVID-19 patients with CNS dysfunction. Studies are warranted to explore this dimension with methylxanthines.

MICRONUTRIENTS AND VITAMINS IN INNATE IMMUNITY

Micronutrients and vitamins are important component in the diet for an effective innate immune response (47). Although their status in COVID 19 patients is not yet known but earlier studies documented that selenium (Se), zinc (Zn), and vitamin B6 are associated with adverse clinical outcome in patients of infectious diseases (48). While the micronutrients and vitamin deficiency will lead to overall weakening of the innate immune response, the individual elements does play an important role in balancing immunity (47). Tea is also a source of these micronutrients and vitamins, but their contents; particularly micronutrients may vary with its geographical origin (48, 49). Here, we consider seven essential micronutrients like copper (Cu), iron (Fe), manganese (Mn), selenium (Se), zinc (Zn), and vitamins (B_2, B_{12}) which are present in tea infusion (**Table 2**). This will reflect the uptake of elements in the gastrointestinal tract constituting epithelial cells to predict the effect of habitual tea drinking on innate immunity. Se supplementation has been reported to increase the lymphocyte proliferation, NK cell activity and IFN production in human subjects (54). Se deficiency has been related with severe lung pathology in mice infected with influenza virus (55). It was postulated that Se deficiency has a role in increased viral mutation resulting in more virulent phenotype (56). Recent study from China documented association between higher death rates observed in COVID-19 patients from regions known to be Se deficient in population to that of other regions (57). This observation strongly supported earlier studies findings, where Se deficiency leads to higher pathogenicity in RNA viruses (55, 56). But this also highlights the need for further research on Se status in COVID-19 patients taking into account various confounding factors. Zn is essential for maintaining homeostasis in respiratory alveolar epithelium cells due to its antioxidant and anti-inflammatory activity (58). In vitro studies showed the inhibitory activity of Zn ionophore pyrithione against replication of SARS-coronavirus by effecting

TABLE 2 | Immune activities and content of micronutrients and vitamins in tea infusion.

Micronutrients and vitamins	Immune activities	Content in tea infusion	Actual body requirement	Reference
Selenium	Acts as immunostimulatory; helps in regulating oxidative stress; regulates phagocytic activity of macrophages; enhances T-cell proliferation, NK-cell mediated cytotoxicity; increases NK cell, lymphocytes, and leukocyte counts; modulates antioxidant capacity of lungs	Se-rich green tea (GT) - 1.44 µg Se/g DW; Regular GT - 0.13 µg Se/g DW; Se-rich black tea (BT) - 1.42 µg Se/g DW; Regular-BT - 0.08 µg Se/g DW	55 ug/day	(50)
Iron	Promotes activation of NF-kB cells; enhances host resistance to intracellular pathogens; regulates anti-microbial activity of macrophages	GT: 0.142–0.099 mg/L BT: 0.100–0.084 mg/L	12–15 mg/day	(51)
Zinc	Enhances phagocyte activities and cytokine secretion of macrophages; acts as antioxidant against reactive oxygen and nitrogen species; important for development and function of neutrophils and NK cells; Zn deficiency leads to decreased lymphocyte counts	GT: 0.130–0.588 mg/L BT: 0.113–0.057 mg/L	10–15 mg/day	(51)
Copper	Target invading pathogens in the phagosome with activated macrophages. Neutrophil and monocytes activities impaired with deficiency in Cu. Enhances NK cells	GT: 0.033–0.191 mg/L; BT: 0.072–0.441 mg/L	900 ug/day	(51)
Manganese	Stimulation of interferon production	GT: 0.78–3.94 mg/L; BT: 0.56–7.49 mg/L	1.8–2.3 mg/day	(51)
Vitamin B_2	Essential for proper functioning of macrophages; B2 deficiency leads to decreases pathogen recognition and impaired immune response	GT: 3.26–2.80 µg/gm BT: 3.34–0.58 µg/g;	1.8–2.5 mg/day	(52)
Vitamin B_{12}	Acts as immunomodulator; enhances NK cell activity; increases lymphocyte count; enhances T-cell proliferation	GT: 0.046–0.263 µg/g DW BT: 0.104–0.859 µg/g DW;	2.4 ug/day	(53)

Selenium enriched tea plantation is being practiced particularly in regions with low soil Se content and human Se deficient areas, as part of Se-biofortification. Infusions were prepared with 1 g of each tea incubated for 5 min in 50–100 ml of Milli-Q (MQ), or boiled distilled water.

enzyme RNA polymerase (RNA dependent RNA polymerase, RdRp) (59). Zn deficiency in lungs, in relation to zinc-binding protein metallothionein (MT), mediates zinc partitioning from lung to liver during hyperoxia, which resulted in lung injury (60). In sepsis condition, Zn deficiency resulted in increased NF-κB p65 mRNA expression resulting in upregulation of target genes interleukin (IL)-1β, TNFα, and ICAM-1 and increase in proinflammatory cytokines IL-6, IL-8, and TNF (61). These cytokines are reported to induce cytokine storm in COVID patients. Zn supplementation reduced neutrophil infiltration and myeloperoxidase mediated oxidative damage which mediates protection in airway inflammation (61). Extensive macrophage infiltration was also reported from post-mortem lungs and kidneys of COVID-19 patients (62, 63). It is established in nutritional immunology that Zn homeostasis is crucial for proper functioning of macrophages (64). Macrophages also play an essential role in iron homeostasis, by recycling iron through phagocytosis of senescent red blood cells for hemoglobin production (65). Vice versa Fe has an effect on regulating macrophages in reducing NF-κB p65 translocation into the nucleus which leads to inhibition of pro-inflammatory cytokine expression (66). Cu too is essential micronutrient acting as co-factor for enzymatic reaction and signal transduction (66). It found itself in a razor edge between essentiality and toxicity in regards to its accumulation in cells beyond its requirement. However, concept in innate immunity is emerging that immune cells uses this toxic level of Cu to attack invading pathogens (67). Activated macrophages during infection transport Cu to phagosome through high affinity copper uptake protein 1 and ATPase Copper Transporting Alpha (67). Cu deficiency has been related with impaired neutrophil and macrophage function (67). Recent literature supported a potential treatment using Cu as ant-viral and inflammatory along with N-acetylcysteine (NAC), colchicine, Nitric oxide and an effective anti-viral drug (68). Mn supplementation was found to increase TYPE I IFN production in murine models and in THP-1, a human macrophage cell line (69). Studies in mice model observed relation between Mn deficiency and impaired anti-viral response. Mn^{2+} enhances activity of PAMP molecule for binding to cyclic-GMP-AMP synthase (cGAS). Overall it helps in mediating stimulation of cGAS- STING (stimulator of interferon genes) signaling pathway for interferon release (69). Vitamins also has role in inhibition of inflammatory cytokines (70). Tea infusion is also an important source of vitamin B_{12} (53, 71). Studies showed that drinking tea can improve considerably B_{12} status in B_{12}-deficient rats (72). It is an essential for innate immunity and its deficiency resulted in reduced white blood cells resulting in increased susceptibility to infections. Tea infusion is also source of another important vitamin, riboflavin (vitamin B_2) (52). Its deficiency is a matter of concern for both developing and developed nations. Elderly, pregnant women and alcohol abusers are usually at high risk of immune suppression associated with its deficiency. It affects balanced macrophage functioning which results in reduction of cell viability and excess release of TNF-α (73). It is also known to down-regulate the NF-κB activation initiated by ROS (73), which are the potent activators of a plethora of general pro-inflammatory cytokines such as IL-6 and TNF-α; the two most prominent in "cytokine storm" in COVID-19 patients (5).

DISCUSSION

Innate immunity is pivotal as the first line of defense against viral infections. Ability of an infectious agent to evade innate immune response is determinant for its pathogenecity, augmenting severe symptoms or even fatality. In critical patients infected with SARS-CoV-2; respiratory failure, systemic shock and multi organ failure are common characteristics (74). Almost 30% of COVID 19 patients require intensive care unit for ventilation support (74). Acute respiratory distress syndrome is a common complication for viral pneumonia (74), which is same for SARS-CoV-2, SERS-CoV, and MERS augmented pneumonia cases. Pulmonary and alveolar tissue specific inflammatory responses are result of innate immune response leading to cascade of inflammatory host signaling pathways (38). The cross talk between innate and adaptive immunity is hallmark for immune strategies to combat disease progression. NK cells are one such example of crosstalk, which can recognize antigen and generate antigen dependent memory (75). Although understanding the immune response in COVID-19 patients is in growing stage, observations reported till now showed apparent involvement of hyper-inflammation and vascular permeability contributing towards the disease severity (12, 13, 38). Mounting evidence suggested that micronutrients improve defense function, i.e., resistance to infection, by modulating immune regulation (48). This include inhibition of pro-inflammatory cytokines, promotion of anti-inflammatory functions, modulation of cell-mediated immunity, alteration of antigen-presenting cell functions, and communication between the innate and adaptive immune systems. Evidence presented here suggested that tea constituting free radical scavengers polyphenols, micronutrients and vitamins has the functional ability to regulate all these functions. Numerous research work in recent times on TFs and EGCG, showed their importance as immunomodulator (**Figure 1**). Several medicinal plants highlighted for their medicinal properties in connection with human health in recent past. However, tea itself as a non-alcoholic beverage did not get much attention as a source of nutritional supplements. Being the most widely consumed drink globally, it is of paramount importance to highlight and harness the benefits of tea drinking. There are numerous different types of tea like black tea, green tea, oolong tea, white tea, yellow tea, etc., which differ due to difference in processing methods but have originated from the same plant *Camellia sinensis* L. Different tea has different major constituents like TFs in black tea and EGCG for green tea. Sometimes number of constituents in the same type of tea varies as per the geographical origin. Assam variety cultivars have been found to possess more polyphenol content than to China or Cambodia variety (76). Likewise, Darjeeling tea is rich in aroma which can be attributed to rich volatile compounds (77). These compounds may

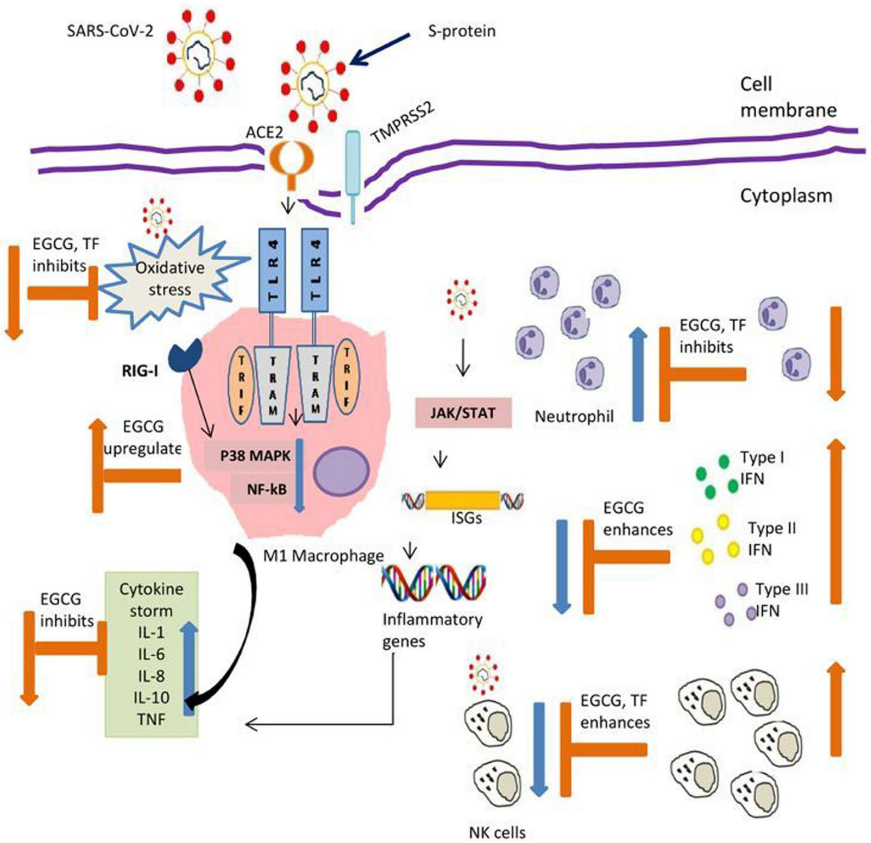


FIGURE 1 | Possible action by tea polyphenols contributing to balance innate immunity in COVID-19. EGCG, Epigallocatechin gallate; TF, Theaflavins; ACE2-Angiotensin, converting enzyme 2; TMPRSS2 Transmembrane protease, serine 2; S-protein, Spike protein; TLR-4, Toll like receptor 4; TRIF, Toll/interleukin-1 receptor (TIR)-domain-containing adapter-inducing interferon-β; TRAM, TRIF-related adaptor molecule; RIG-1, Retinoic acid-inducible gene I; MAPK, Mitogen-activated protein kinases; NF-Kb, Nuclear factor kappa B; JAK, Janus kinase; STAT, Signal transducer and activator of transcription; ISGs, Interferon stimulating genes; IL- Interleukin; TNF, Tumor necrosis factor; IFN, Interferon; NK, Natural killer cells. Symbol denotes: Blue arrow reflects effect of virus; Orange arrow reflect action of polyphenols.

havebeneficial effect on our respiratory system. Similarly, teas of other region have some unique characteristics, may be in terms of micronutrient contents. These all make tea an important component for nutritional research. Interestingly, computational modeling studies showed the potency of theaflavin-3,3'-digallate, a major TFs, and EGCG in inhibiting RNA-dependent RNA polymerase (RdRp) and the main protease (M^{pro}), respectively. Both are key components of coronavirus replications and can be major drug target for SARS-CoV-2 (78–80). Earlier studies on HCV showed ability of TFs acting directly against virions particle, changing its morphology, making it unable to enter cells (81). The absorption of polyphenols involves small intestine, liver, and epithelial colon cells of large intestine through process of methylation, glucuronidation, and sulfation (82). They are too hydrophilic to penetrate the gut wall by passive diffusion and mainly active transport mechanism is involved in its absorption (82). Studies in mice model and human volunteers showed their presence in numerous tissues (83, 84). They are also found to be associated with better pulmonary functions in epidemiological studies (85). These findings are encouraging to consider polyphenols as an integral part of nutritional immunity particularly innate immune response, and tea being widely consumed drink is an important and unique source of essential micronutrients and vitamins as well.

CONCLUSION

It is evident that innate immunity plays an important role in COVID-19 disease progression. This review showed the importance of nutritional immunity on innate immune response and tea as an important source of it. Recent advancements on increasing the bioavailability of tea polyphenols and its popularity as a beverage advocates for random control clinical trials to translate the basic research findings into clinical use. Nevertheless, this article will further boost tea as a health drink and give an add-on to nutritional supplements augmenting innate immunity in

controlling the present pandemic. In present scenario, it will be interesting to study the effect of tea polyphenols on the respiratory and alveolar human cell lines regulating the inflammatory markers by mitigating the cytokine storm of SARS-CoV-2 infection. This will further justify the need for in-vivo studies and clinical trials for developing it as a nutraceuticals and popular health drink at large.

AUTHOR CONTRIBUTIONS

PC conceptualized, performed literature review, wrote, and presented (table and figures) the MS. AB conceptualized and helped in critically reviewing the MS. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors are thankful to Trishna Bora, Scientist, Medical Rural Research Health Unit, ICMR-Regional Medical Research Centre, Northeast Region, Dibrugarh, India for discussing the content of the article. Finally, we would like to express our sincere gratitude to the two reviewers for their valuable suggestions.

REFERENCES

1. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol* (2015) 1282:1–23. doi: 10.1007/978-1-4939-2438-7_1
2. Du Z, Wang L, Cauchemez S, Xu X, Wang X, Cowling BJ, et al. Risk for transportation of coronavirus disease from Wuhan to other cities in China. *Emer Infect Dis* (2020) 26:1049–52. doi: 10.3201/eid2605.200146
3. World Health Organization. *WHO Coronavirus disease (COVID-19) dashboard* (2020). Available at: https://covid19.who.int (Accessed on Sept, 7th 2020).
4. World Health Organization. *Rolling updates on coronavirus disease (COVID-19)* (2020). Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen (Accessed on July, 24th 2020).
5. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol* (2020) 20:363–74. doi: 10.1038/s41577-020-0311-8
6. Ong EZ, Chan YFZ, Leong WY, Lee NMY, Kalimuddin S, Mohideen SMH, et al. A Dynamic Immune Response Shapes COVID-19 Progression. *Cell Host Microbe* (2020) 27:879–82. doi: 10.1016/j.chom.2020.03.021
7. Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications(2020). (Accessed April15, 2020).
8. Iwasaki A, Yang Y. The potential danger of suboptimal antibody responses in COVID-19. *Nat Rev Immunol* (2020) 20:339–41. doi: 10.1038/s41577-020-0321-6
9. Channappanavar R, Zhao J. Perlman S. T cell-mediated immune response to respiratory coronaviruses. *Immunol Res* (2014) 59:118–28. doi: 10.1007/s12026-014-8534-z
10. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Péré H, et al. Impaired type I interferon activity and exacerbated inflammatory responses in severe Covid-19 patients. *Science* (2020) 369:718–24. doi: 10.1126/science.abc6027
11. Food and Agricultural organization. *FAO intergovernmental group on tea. world tea production and trade: current and future development* (2015). Available at: http://www.fao.org/3/a-i4480e.pdf (Accessed on Sept, 15th 2020).
12. Pedersen SF. Ho Ya-Chi. SARS-CoV-2: A storm is raging. *J Clin Invest* (2020) 130:2202–05. doi: 10.1172/JCI137647
13. Schett G, Sticherling M, Neurath MF. COVID-19: risk for cytokine targeting in chronic inflammatory diseases? *Nat Rev Immunol* (2020) 20:271–72. doi: 10.1038/s41577-020-0312-7
14. Gua YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. *Mil Med Res* (2020) 7:11. doi: 10.1186/s40779-020-00240-0
15. Wang Y, Li J, Wang X, Peña JC, Li K, Zhang T, et al. (–)-Epigallocatechin-3-gallate enhances hepatitis c virus double-stranded RNA intermediates-triggered innate immune responses in hepatocytes. *Sci Rep* (2016) 6:21595. doi: 10.1038/srep21595
16. Katze MG, Fornek JL, Palermo RE, Walters KA, Korth MJ. Innate immune modulation by RNA viruses: emerging insights from functional genomics. *Nat Rev Immunol* (2008) 8:644–54. doi: 10.1038/nri2377
17. Totura AL, Whitmore A, Agnihothram S, Schäfer A, Katze MG, Heise MT, et al. Toll-like receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. *mBIO* (2015) 6:e00638–15. doi: 10.1128/mBio.00638-15
18. Byun EH, Omura T, Yamada K, Tachibana H. Green tea polyphenol epigallocatechin-3-gallate Inhibits TLR2 signaling induced by peptidoglycan through the polyphenol sensing molecule 67-kda laminin receptor. *FEBS Lett* (2011) 585:814–20. doi: 10.1016/j.febslet.2011.02.010
19. Byun EB, Choi HG, Sung NY, Byun EH. Green tea polyphenol epigallocatechin-3-gallate inhibits TLR4 signaling through the 67-kDa laminin receptor on lipopolysaccharide-stimulated dendritic cells. *Biochem Biophys Res Commun* (2012) 426:480–5. doi: 10.1016/j.bbrc.2012.08.096
20. Heounjeong G, Jaemoon K, Hye Sung K, Yoon Kyung J, Doo Hyun C. Expression of toll-like receptor 2 and 4 is increased in the respiratory epithelial cells of chronic idiopathic interstitial pneumonia patients. *Respir Med* (2014) 108:783–92. doi: 10.1016/j.rmed.2013.12.007
21. Kumar CTR, Lai Y, Sarisky RT, Kao CC. Green tea catechin, epigallocatechin gallate, suppresses signaling by the dsRNA innate immune receptor RIG-I. *PloS One* (2010) 5:e12878. doi: 10.1371/journal.pone.0012878
22. Cena H, Chieppa M. Coronavirus disease (COVID-19–SARS-CoV-2) and nutrition: is infection in Italy suggesting a connection? *Front Immunol* (2020) 11:944. doi: 10.3389/fimmu.2020.00944
23. Dona M, Dell'Aica I, Calabrese F, Benelli R, Morini M, Albini A. Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. *J Immunol* (2003) 170:4335–41. doi: 10.4049/jimmunol.170.8.4335
24. Xiaokaiti Y, Wu H, Chen Y, Yang H, Duan J, Li X. EGCG reverses human neutrophil elastase-induced migration in A549 cells by directly binding to HNE and by regulating α1-AT. *Sci Rep* (2015) 5:11494. doi: 10.1038/srep11494
25. Koch W, Kukula-Koch W, Komsta L, Marzec Z, Szwerc W, Glowniak K. Green tea quality evaluation based on its catechins and metal composition in combination with chemometric analysis. *Molecules* (2018) 23:16989. doi: 10.3390/molecules23071689
26. Fernández VA, Toledano LA, Lozano NP, Tapia EN, Roig MDG, Fornell RDLT, et al. Bioavailability of epigallocatechin gallate administered with different nutritional strategies in healthy volunteers. *Antioxidants* (2020) 9:440. doi: 10.3390/antiox9050440
27. Stalmach A, Troufflard S, Serafini M, Crozier A. Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. *Mol Nutr Food Res* (2009) 53:44–53. doi: 10.1002/mnfr.200800169
28. Mulder TPJ, Platerink van CJ, Schuyl W, Amelsvoort van JMM. Analysis of theaflavins in biological fluids using liquid chromatography–electrospray mass spectrometry. *J Chromatogr B* (2001) 760:271–79. doi: 10.1016/S0378-4347(01)00285-7
29. Konieczynski P, Viapiana A, Wesolowski M. Comparison of infusions from black and green teas (*Camellia sinensis* L. Kuntze) and *Erva-mate* (*Ilex paraguariensis* A. St.-Hil.) based on the content of essential elements, secondary metabolites, and antioxidant activity. *Food Anal Methods* (2017) 10:3063–70. doi: 10.1007/s12161-017-0872-8
30. Lelo A, Birkett DJ, Robson RA, Miners JO. Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine

and theophylline in man. *Br J Clin Pharmacol* (1986) 22:177–82. doi: 10.1111/j.1365-2125.1986.tb05246.x
31. Wu H, Li Q, Zhou X, Kolosov VP, Perelman JM. Theaflavins extracted from black tea inhibit airway mucous hypersecretion induced by cigarette smoke in rats. *Inflammation* (2012) 35:271–9. doi: 10.1007/s10753-011-9314-8
32. Sartor L, Pezzato E, Garbisa S. (-)Epigallocatechin-3-gallate inhibits leukocyte elastase: Potential of the phyto-factor in hindering inflammation, emphysema, and invasion. *J Leukoc Biol* (2002) 71:73–9. doi: 10.1189/jlb.71.1.73
33. Song YA, Park YL, Yoon SH, Kim KY, Cho SB, Lee WS, et al. Black tea polyphenol theaflavin suppresses LPS-induced ICAM-1 and VCAM-1 expression via blockage of NF-κB and JNK activation in intestinal epithelial cells. *Inflammation Res* (2011) 60:493–500. doi: 10.1007/s00011-010-0296-z
34. Li J, Ye L, Wang X, Liu J, Wang Y, Zhou Y. (–)-Epigallocatechin gallate inhibits endotoxin-induced expression of inflammatory cytokines in human cerebral microvascular endothelial cells. *J Neuroinflamm* (2012) 9:161. doi: 10.1186/1742-2094-9-161
35. Grudzien M, Rapak A. Effect of natural compounds on NK cell activation. *J Immunol Res* (2018) 2018:4868417. doi: 10.1155/2018/4868417
36. Li Q, Huyan T, Ye LJ, Li J, Shi JL, Huang QS. Concentration-dependent biphasic effects of resveratrol on human natural killer cells in vitro. *J Agric Food Chem* (2014) 62:10928–935. doi: 10.1021/jf502950u
37. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol* (2020) 17:533–35. doi: 10.1038/s41423-020-0402-2
38. McKechnie JL, Blish CA. The innate immune system: fighting on the front lines or fanning the flames of COVID-19? *Cell Host Microbe* (2020) 27:863–69. doi: 10.1016/j.chom.2020.05.009
39. Zhang C, Wang XM, Li SR, Twelkmeyer T, Wang WH, Zhang SY, et al. NKG2A is a NK cell exhaustion checkpoint for HCV persistence. *Nat Commun* (2019) 10:1507. doi: 10.1038/s41467-019-09212-y
40. Antonioli L, Fornai M, Pellegrini C, Blandizzi C. NKG2A and COVID-19: another brick in the wall. *Cell Mol Immunol* (2020) 17:672–74. doi: 10.1038/s41423-020-0450-7
41. Dhar D, Mohanty A. Gut microbiota and Covid-19- possible link and implications. *Virus Res* (2020) 285:198018. doi: 10.1016/j.virusres.2020.198018
42. Hodges JK, Zhu J, Yu Z, Vodovotz Y, Borck G, Sasaki GY. Intestinal-level anti-inflammatory bioactivities of catechin-rich green tea: Rationale, design, and methods of a double-blind, randomized, placebo-controlled crossover trial in metabolic syndrome and healthy adults. *Contemp Clin Trials Commun* (2020) 17:100495. doi: 10.1016/j.conctc.2019.100495
43. Onatibia-Astibia A, Martinez-Pinilla E, Franco R. The potential of methylxanthine-based therapies in pediatric respiratory tract diseases. *Res Med* (2016) 112:1–9. doi: 10.1016/j.rmed.2016.01.022
44. Horrigan LA, Kelly JP, Connor TJ. Immunomodulatory effects of caffeine: friend or foe? *Pharmacol Ther* (2006) 111:877–92. doi: 10.1016/j.pharmthera.2006.02.002
45. Yoneda M, Sugimoto N, Katakura M, Matsuzaki K, Tanigami H, Yachie A, et al. Theobromine up-regulates cerebral brain-derived neurotrophic factor and facilitates motor learning in mice. *J Nut Biochem* (2017) 39:110–16. doi: 10.1016/j.jnutbio.2016.10.002
46. Bhat R, Axtell R, Mitra A, Miranda M, Lock C, Tsien RW, et al. Inhibitory role for GABA in autoimmune inflammation. *PNAS* (2010) 107:2580–85. doi: 10.1073/pnas.0915139107
47. Erickson KL, Medina EA, Hubbard NE. Micronutrients and innate immunity. *J Infect Dis* (2000) 182:5–10. doi: 10.1086/315922
48. Dizdar OS, Baspınar O, Kocer D, Dursun ZB, Avci D, Karakükcü C, et al. Nutritional risk, micronutrient status and clinical outcomes: A prospective observational study in an infectious disease clinic. *Nutrients* (2016) 29:124. doi: 10.3390/nu8030124
49. Karak T, Kutu FR, Nath JR, Sonar I, Paul RK, Boruah RK, et al. Micronutrients (B, Co, Cu, Fe, Mn, Mo, and Zn) content in made tea (*Camellia sinensis* L.) and tea infusion with health prospect: A critical review. *Crit Rev Food Sci Nutr* (2017) 57:2996–34. doi: 10.1080/10408398.2015.1083534
50. Nordin NA. *The impact of selenium-rich green and black tea water extracts on bone health in vitro, and in an animal model of osteoporosis [Ph.D. thesis]*. Palmerston North, New Zealand: Massey University (2017).
51. Street R, Szakova J, Drabek O, Mladkova L. The Status of Micronutrients (Cu, Fe, Mn, Zn) in tea and tea infusions in selected samples imported to the Czech Republic. *Czech J Food Sci* (2006) 24:62–71. doi: 10.17221/3301-CJFS
52. Tezcan F, Erim FB. Determination of Vitamin B_2 Content in black, green, sage, and rosemary tea infusions by capillary electrophoresis with laser-induced fluorescence detection. *Beverages* (2018) 4:86. doi: 10.3390/beverages4040086
53. Katsurai Kittaka H, Watanebe F, Nakan Y. Occurrence of vitamin B_{12} in green, blue, red and black tea leaves. *J Nutr Sci Vitaminol* (2004) 50:438–40. doi: 10.3177/jnsv.50.438
54. Broome CS, McArdle F, Kyle JA, Andrews F, Lowe NM, Hart CA, et al. An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. *Am J Clin Nutr* (2004) 80:154–62. doi: 10.1093/ajcn/80.1.154
55. Beck MA, Shi Q, Van Dael P, Schiffrin EJ, Blum S, Barclay D, et al. Selenium deficiency increases the pathology of an influenza virus infection. *FASEB J* (2001) 15:1481–83. doi: 10.1096/fj.00-0721fje
56. Nelson HK, Shi Q, Van Dael P, Schiffrin EJ, Blum S, Barclay D, et al. Host nutritional selenium status as a driving force for influenza virus mutations. *FASEB J* (2001) 15:1486–88. doi: 10.1096/fj.01-0108com
57. Zhang J, Taylor EW, Bennett K, Saad R, Rayman MP. Association between regional selenium status and reported outcome of COVID-19 cases in China. *Am J Clin Nutr* (2020) 111:1297–99. doi: 10.1093/ajcn/nqaa095
58. Bao S, Knoell DL. Zinc modulates cytokine-induced lung epithelial cell barrier permeability. *Am J Physiol Lung Cell Mol Physiol* (2006) 291:1132–41. doi: 10.1152/ajplung.00207.2006
59. Skalny AV, Rink L, Ajsuvakova OP, Aschner M, Gritsenko VA, Alekseenko SI. Zinc and respiratory tract infections: Perspective for COVID-19. *Int J Mol Med* (2020) 46:17–26. doi: 10.3892/ijmm.2020.4575
60. Lee SM, McLaughlin JN, Frederick DR, Zhu L, Thambiayya K, Wasserloos KJ, et al. Metallothionein-induced zinc partitioning exacerbates hyperoxic acute lung injury. *Am J Physiol Lung Cell Mol Physiol* (2013) 304:350–60. doi: 10.1152/ajplung.00243.2012
61. Bao S, Liu MJ, Lee B, Besecker B, Lai JP, Guttridge DC. Zinc modulates the innate immune response in vivo to polymicrobial sepsis through regulation of NF-κb. *Am J Physiol Lung Cell Mol Physiol* (2010) 298:744–54. doi: 10.1152/ajplung.00368.2009
62. Carsana L, Sonzogni A, Nasr A, Rossi RS, Pellegrinelli A, Zerbi P, et al. Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. *Lancet Infect Dis* (2020) S1473:30434–5. doi: 10.1016/S1473-3099(20)30434-5
63. Diao B, Wang C, Wang R, Feng Z, Tan Y, Wang H, et al. Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Infection(2020). (Accessed May 15, 2020).
64. Hamon R, Homan CC, Tran HB, Mukaro VR, Lester SE, Roscioli E, et al. Zinc and zinc transporters in macrophages and their roles in efferocytosis in COPD. *PloS One* (2014) 9:e110056. doi: 10.1371/journal.pone.0110056
65. Agoro R, Taleb M, Quesniaux Valerie FJ, Mura C. Cell iron status influences macrophage polarization. *PloS One* (2018) 13:e0196921. doi: 10.1371/journal.pone.0196921
66. Kardos J, Héja L, Simon A, Jablonkai I, Kovács R, Jemnitz K. Copper signalling: causes and consequences. *Cell Comm Sign* (2018) 16:71. doi: 10.1186/s12964-018-0277-3
67. Festa RA, Thiele DJ. Copper at the front line of the host- pathogen battle. *PloS Pathog* (2012) 8:e1002887. doi: 10.1371/journal.ppat.1002887
68. Andreou A, Trantza S, Filippou D, Sipsas N, Tsiodras S. COVID-19: The potential role of copper and N-acetylcysteine (NAC) in a combination of candidate antiviral treatments against SARS-CoV-2. *Vivo* (2020) 34:1567–88. doi: 10.21873/invivo.11946
69. Haase H. Innate Immune Cells Speak Manganese. *Immunity* (2018) 48:616–18. doi: 10.1016/j.immuni.2018.03.031
70. Gombart AF, Pierre A. Maggini S. A review of micronutrients and the immune system–working in harmony to reduce the risk of infection. *Nutrients* (2020) 12:236. doi: 10.3390/nu12010236
71. Teng F, Bito T, Takenaka S, Yabuta Y, Watanabe F. Determination of Vitamin B_{12} in chinese black tea leaves. *Food Nutr Sci* (2014) 5:1326–32. doi: 10.4236/fns.2014.514144

72. Katsurai Kittaka H, Ebara S, Watanabe F, Nakano Y. Characterization of corrinoid compounds from a Japanese black tea (batabata-cha) fermented by bacteria. *J Agric Food Chem* (2004) 52:909–11. doi: 10.1021/jf030585r
73. Suwannasom N, Kao I, Pruß A, Georgieva R, Bäumler H. Riboflavin: The health benefits of a forgotten natural vitamin. *Int J Mol Sci* (2020) 21:950. doi: 10.3390/ijms21030950
74. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* (2020) 395:497–06. doi: 10.1016/S0140-6736(20)30183-5
75. O'Sullivan TE, Sun JC, Lanier LL. Natural killer cell memory. *Immunity* (2015) 43:634–45. doi: 10.1016/j.immuni.2015.09.013
76. Sabhapondit S, Karak T, Bhuyan LP, Goswami BC, Hazarika M. Diversity of catechin in northeast indian tea cultivars. *Sci World J* (2012) 2012:485193. doi: 10.1100/2012/485193
77. Schuh C, Schieberle P. Characterization of the key aroma compounds in the beverage prepared from darjeeling black tea: Quantitative differences between tea leaves and infusion. *J Agric Food Chem* (2006) 54:916–24. doi: 10.1021/jf052495n
78. Lung J, Lin YS, Yang YH, Chou YL, Shu LH, Cheng YC, et al. The potential chemical structure of anti-SARS-CoV-2 RNA-dependent RNA polymerase. *J Med Virol* (2020) 92:693–97. doi: 10.1002/jmv.25761
79. Bhardwaj VK, Singh R, Sharma J, Rajendran V, Purohit R, Kumar S. Identification of bioactive molecules from tea plant as SARS-CoV-2 main protease inhibitors. *J Biomol Struct Dyn* (2020), 1–10. doi: 10.1080/07391102.2020.1766572
80. Ghosh R, Chakraborty A, Biswas A, Chowdhuri A. Evaluation of green tea polyphenols as novel corona virus (SARS CoV-2) main protease (Mpro) inhibitors – an *in silico* docking and molecular dynamics simulation study. *J Biomol Struct Dyn* (2020), 1–13. doi: 10.1080/07391102.2020.1779818
81. Chowdhury P, Sahuc ME, Rouille Y, Rivière C, Bonneau N, Vandeputte A, et al. Theaflavins, polyphenols of black tea, inhibit entry of hepatitis C virus in cell culture. *PloS One* (2018) 13:1–16. doi: 10.1371/journal.pone.0198226
82. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr* (2004) 79:727–47. doi: 10.1093/ajcn/79.5.727
83. Kim SB, Lee MJ, Li C, Smith TJ, Yang GY, et al. Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. *Nutr Cancer* (2000) 37:41–8. doi: 10.1207/s15327914nc3701_5
84. Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol Biomarkers Prev* (1998) 7:351–54.
85. Pounis G, Arcari A, Costanzo S, Castelnuovo Di A, Bonaccio M, Persichillo M. Favorable association of polyphenol-rich diets with lung function: Cross sectional findings from the Moli-sani study. *Respir Med* (2018) 136:48–56. doi: 10.1016/j.rmed.2017.12.007

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Chowdhury and Barooah. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

REVIEW
published: 28 October 2020
doi: 10.3389/fimmu.2020.574029

The Long History of Vitamin C: From Prevention of the Common Cold to Potential Aid in the Treatment of COVID-19

Giuseppe Cerullo[1†], Massimo Negro[2†], Mauro Parimbelli[2], Michela Pecoraro[3], Simone Perna[4], Giorgio Liguori[1], Mariangela Rondanelli[5,6], Hellas Cena[6,7] and Giuseppe D'Antona[2,6]*

[1] Department of Movement Sciences and Wellbeing, University of Naples Parthenope, Naples, Italy, [2] Centro di Ricerca Interdipartimentale nelle Attività Motorie e Sportive (CRIAMS)—Sport Medicine Centre, University of Pavia, Voghera, Italy, [3] Department of Pharmacy, University of Salerno, Fisciano, Italy, [4] Department of Biology, College of Science, University of Bahrain, Sakhir, Bahrain, [5] IRCCS Mondino Foundation, Pavia, Italy, [6] Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy, [7] Clinical Nutrition and Dietetics Service, Unit of Internal Medicine and Endocrinology, ICS Maugeri IRCCS, University of Pavia, Pavia, Italy

OPEN ACCESS

Edited by:
Ioannis Zabetakis,
University of Limerick, Ireland

Reviewed by:
Ran Wei,
The State University of New Jersey, United States
Anitra C. Carr,
University of Otago, New Zealand

***Correspondence:**
Massimo Negro
massimo.negro@unipv.it

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: 18 June 2020
Accepted: 21 September 2020
Published: 28 October 2020

Citation:
Cerullo G, Negro M, Parimbelli M, Pecoraro M, Perna S, Liguori G, Rondanelli M, Cena H and D'Antona G (2020) The Long History of Vitamin C: From Prevention of the Common Cold to Potential Aid in the Treatment of COVID-19. Front. Immunol. 11:574029. doi: 10.3389/fimmu.2020.574029

From Pauling's theories to the present, considerable understanding has been acquired of both the physiological role of vitamin C and of the impact of vitamin C supplementation on the health. Although it is well known that a balanced diet which satisfies the daily intake of vitamin C positively affects the immune system and reduces susceptibility to infections, available data do not support the theory that oral vitamin C supplements boost immunity. No current clinical recommendations support the possibility of significantly decreasing the risk of respiratory infections by using high-dose supplements of vitamin C in a well-nourished general population. Only in restricted subgroups (e.g., athletes or the military) and in subjects with a low plasma vitamin C concentration a supplementation may be justified. Furthermore, in categories at high risk of infection (i.e., the obese, diabetics, the elderly, etc.), a vitamin C supplementation can modulate inflammation, with potential positive effects on immune response to infections. The impact of an extra oral intake of vitamin C on the duration of a cold and the prevention or treatment of pneumonia is still questioned, while, based on critical illness studies, vitamin C infusion has recently been hypothesized as a treatment for COVID-19 hospitalized patients. In this review, we focused on the effects of vitamin C on immune function, summarizing the most relevant studies from the prevention and treatment of common respiratory diseases to the use of vitamin C in critical illness conditions, with the aim of clarifying its potential application during an acute SARS-CoV2 infection.

Keywords: vitamin C supplementation, viral infections, COVID-19, pneumonia, immune function, athletes, non-communicable diseases, frail elderly subjects

INTRODUCTION

Vitamin C (ascorbic acid) plays an important role in the normal functioning of the immune system (1–4) and its use in preventing and/or treating infections has strongly attracted the interest of physicians and investigators for almost a century. A plethora of papers have been published on this topic, but, although it is well known that a deficiency of vitamin C due to a low nutritional intake leads to a greater susceptibility to infections (5), the possibility of reducing the incidence of viral diseases in a well-nourished population through the use of dietary supplements based on vitamin C is not adequately supported in literature. At present, very little evidence supports the benefit of high doses of vitamin C supplementation on immune function in healthy individuals (4, 6) and several authors have underlined that this practice is ineffective in preventing the common cold and viral infections in most subjects (7–12). Despite this, the popular belief that an extra intake of vitamin C can boost the immune system is still widespread and every year the market claims that the use of supplements is a winter remedy to prevent infectious disease.

While scientists' current position is not to recommend the use of vitamin C supplementation to prevent viral invasions in healthy subjects, more promising—though questioned—data seem instead to emerge from the intravenous administration (IA) of this vitamin in acute respiratory conditions or critical illnesses. Furthermore a potential pharmacological role during early phases of the new coronavirus (SARS-CoV2) infection and its related disease (COVID-19) was recently put forward (13–18). The rapid worldwide spread of SARS-CoV2 and the consequent pandemic emergency recognized by World Health Organization urgently requires a global effort to identify anything that can treat symptoms and reduce deaths. At the beginning of June, more than 6,600,000 cases of infection and 391,000 deaths related to COVID-19 had been reported globally and the number of cases is constantly increasing worldwide (19). Currently, no specific antiviral therapy has been approved for the cure of COVID-19 (20), and this has led researchers to speculate on a possible adjuvant treatment based on indirect evidence from severely ill patients and patients with sepsis conditions (21). Sepsis is a life-threatening organ dysfunction caused by an impaired host response to infection, characterized by a dramatic failure of the circulatory, metabolic, and immune systems, and recognized as the primary cause of death from infection: patients who develop septic shock can have hospital mortality rates of up to 50% (22). On these clinical conditions literature shows that high doses of vitamin C by intravenous infusion may reduce the inflammatory cytokine-related production and potentially improve important outcomes such as the duration of mechanical ventilation time and mortality rates (13–18). This is of particular importance since acute respiratory distress syndrome (ARDS) is one of the most frequent severe conditions registered in COVID-19 patients (23). ARDS is a serious and, in some cases fatal, syndrome characterized by a strong inflammatory response with massive alveolar damage and multiple organ failure, requiring intensive care unit (ICU) treatment (24). Authors reported a percentage of ARDS cases of around 15% among hospitalized patients with SARS-CoV2 infection (25).

Data on the use of intravenous-administered vitamin C in COVID-19 patients are still unavailable, but clinical trials to explore the efficacy of this treatment are currently in progress in several countries (26) and important results will be available soon.

Based on the above, the aim of this review is firstly to summarize the immunological role of vitamin C with a description of its potential effects as a dietary supplement, on the mechanisms involved during respiratory viral infections, and in relation to the inflammatory response considering different subject categories and clinical conditions; secondly, the manuscript describes the updated literature on the IA of vitamin C in the treatment of severe sepsis and ARDS conditions, with the aim of establishing whether the current clinical background on this topic offers strong enough perspectives to propose vitamin C for a pharmacological application to reduce the dramatic cytokine production and regulate other recognized COVID-19-related immune responses.

PHYSIOLOGY OF VITAMIN C

Bioavailability

Vitamin C is an essential nutrient that must be taken through the diet as humans are unable to synthesize it (27). Thus, our body has developed an effective adaptation system which maintains the organic reserves of vitamin C and prevents its deficiency due to a low dietary intake. These adaptations include a higher absorption and recycling capacity of vitamin C compared to other animal species (e.g., goat and reptiles), which can normally produce it (28, 29). Animal studies have shown that vitamin C is preferentially stored in the brain, adrenal gland, liver and lungs (30–33) but its levels in these organs are rapidly depleted after about one week of dietary insufficiency (31). In humans, skeletal muscle represents the major pool of vitamin C (34). Muscle fibers also lose vitamin C content very rapidly under inadequate dietary intake. However, a consumption of half a kiwifruit per day seems to be enough to saturate the muscle tissue's vitamin C concentrations in non-smoking men (35). Vitamin C homeostasis is finely regulated by at least four mechanisms: intestinal absorption, transport to tissue, renal reuptake, and urine excretion, mainly regulated by a family of proteins named Sodium-Dependent Vitamin C Transporters (SVCT) (36). Considering the individual variability in healthy subjects, studies suggest that a daily intake of vitamin C from 100 to 400 mg ensures 100% of the bioavailability and blood saturation with a steady state of plasma concentration that reaches a maximum level of approximately 70–80 μmol/L (37, 38). Generally, if the intake of vitamin C exceeds 500 mg/day, a further increase in plasma concentration is inhibited and the bioavailability can decrease close to 30% when more than 1,000 mg of vitamin C is administered in a single bout (39). This occurs because when 500–1,000 mg of vitamin C is administered orally, the intestinal transporter (SVCT1) rapidly achieves its maximal

saturation, while the urine excretion of the vitamin is progressively increased (38, 39). The measure of plasma vitamin C concentration may be considered, even though the circulant values cannot be used as a reliable marker of the body stores (about 5 g) (40). A plasma concentration value of vitamin C lower than 23 μmol/L reflects a depletion of the vitamin C pool (state of hypovitaminosis), while clinical symptoms of scurvy occur when plasma values are lower than 11 μmol/L (41).

Recommended Dietary Allowances

To maintain adequate body stores, recommended dietary allowance (RDA) for vitamin C has been proposed over the years. The RDAs vary among countries: e.g., current recommendations in the United States and Canada is 90 mg/day for adult men and 75 mg/day for adult women (42), while in Italy the suggested intakes are 105 mg/day and 85 mg/day for adult men and women, respectively (43). This variation in RDAs can be explained by the different criteria used by various authorities to define the estimated average requirement (EAR) for vitamin C, including prevention of scurvy, immune cell support, maintenance of an adequate plasma vitamin C level, and optimizing health (44). Furthermore, RDAs vary among subjects as several factors can modify vitamin C requirements, including gender, age, smoking, pregnancy, and lactation (44).

Several authors and guidelines indicated that males need more vitamin C than females (45–51) probably due to the higher body weight and fat-free mass of men compared to women (52).

In children and adolescents the RDAs for vitamin C are generally derived from adult needs and adjusted in relation to their lower body mass (46, 47, 53) as reported by Carr and Lykkesfeldt (44). In Italy, for example, SINU recommends an intake of 45 mg for children from 4 to 6 years of age (45). Epidemiological studies indicate that a lower vitamin C status can be found in the elderly, suggesting that they require a higher intake compared to adults (54–56). However, currently only France has developed specific guidelines for subjects from 75 years of age, indicating a daily intake of 120 mg (57).

Vitamin C requirements are higher in women during pregnancy (44): the hemodilution due to the increase of blood volume and the active absorption of vitamin C by the fetus during its development lead to a reduction of the vitamin status (58). Even lactation increases the vitamin C requirement of women, to satisfy the vitamin needs of the infant normal growth. Most countries recommend an extra daily intake of 10–20 mg for pregnant women and an extra daily intake of 20–60 mg/day for women during lactation (44).

Smokers usually have lower plasma values than non-smokers, probably due to increased oxidative stress and higher turnover of vitamin C. In addition to this, a reduced vitamin C status in smokers is also due to the dietary intake of vitamin C, which is usually lower compared to non-smokers (59). Therefore, in order to compensate these conditions, authorities have recommended an additional intake of 20 to 80 mg/day of vitamin C for these subjects, setting the RDAs for smokers at 120–155 mg/day (44).

Some other factors have been recognized as reducing vitamin C status (60), even though they were not considered in general guidelines and the daily additional values of vitamin C potentially required are not currently available. These factors include: 1) passive exposure to tobacco smoking and environmental pollutants, which can enhance oxidative stress; 2) geographic influence, socioeconomic and cultural status, which may have an impact on production, selection and consumption of foods typically rich in vitamin C; 3) food preparation and cooking methods, which can reduce the content of vitamin C in foods since this vitamin is water-soluble and heat-labile.

The potential variability of the metabolism of vitamin C among various ethnic groups is practically unknown and this topic should certainly be further explored. Only one study reported that lower vitamin C concentrations were significantly associated with a higher leukocyte count in African Americans but not in Caucasians, suggesting hypothetical metabolic or pharmacokinetic differences among races (61).

VITAMIN C AND IMMUNE SYSTEM REGULATION

Besides an extensive range of biochemical pathways in which vitamin C is involved, it also participates in the response of the innate and adaptive immune system (1). The intracellular content of vitamin C in immune cells depends on the plasma availability. In healthy adults the content of vitamin C in leukocytes can be saturated with an intake of at least 100 mg of vitamin C per day, through foods, obtaining a concentration of about 3.5, 3, and 1.5 mmol/L, respectively, in lymphocytes, monocytes and neutrophils (39, 62–65). Leukocytes' absorption of vitamin C from the blood is very efficient, through SVCT proteins (66), resulting in an intracellular content which is 50 to 100 times greater than the plasma concentration (67, 68). As an effective antioxidant, vitamin C contributes to protecting neutrophils from oxidative stress during the early stages of an immune response, when neutrophils activate phagocytosis and produce reactive oxygen species (ROS) to destroy antigens (69, 70). Once the phagocytic capacity is exhausted and neutrophils start to die, vitamin C seems to regulate the process in favor of apoptosis, through the activation of a caspase-dependent cascade, inhibiting the transition to necrosis, and resulting in a more efficient resolution of inflammation (71).

Vitamin C is also involved in the migration of phagocytes (neutrophils and macrophages) toward the infection sites in response to chemoattractants (72, 73). This is particularly important since an impaired neutrophilic chemotaxis has been observed in patients with severe infection (74–76). Furthermore in subjects with low blood concentrations of vitamin C (<50 μmol/L), a daily intake of 250 mg of vitamin C can result in a 20% increase of neutrophils' migration capacity (6). Conversely, in individuals with a physiological blood concentration of vitamin C, neutrophils' mobility cannot be enhanced, as demonstrated by Bozonet et al. (4), in which neutrophils isolated from healthy volunteers and incubated with a vitamin C solution (200 μmol/L) to artificially increase their content of ascorbate did not show a major chemotactic ability.

Similarly to neutrophils, vitamin C protects lymphocytes from oxidative damage (77) and has a pivotal role in the development and function of these cells, even though the exact mechanisms have not yet been clarified (3). In T lymphocytes, vitamin C stimulates differentiation and proliferation from precursors to mature T cells, in a dose-dependent way (78). Studies on the influence of vitamin C on subtypes of T cells are mainly related to the Th1/Th2 balance. Reports underline that vitamin C can induce a shift of immune responses from Th2 to Th1 (3), while only one study suggests that vitamin C can induce the Th17 polarization of naïve CD4+ cells in murine model, affecting epigenetic mechanism (79). At present, no studies exploring the effects of vitamin C on cytotoxic T cells are available (3). In B lymphocytes, vitamin C seems to affect the production of antibodies, despite conflicting evidence (80–87). Physiological levels of Vitamin C are also necessary for normal natural killer (NK) cell development and function (3). In vitamin C-deficient mice, NK cytotoxic activity (NKCA) was lower than that in mice with normal levels of vitamin C (88), while supraphysiological levels of ascorbate do not further increase NKCA (89).

Vitamin C also regulates inflammatory response. In animal studies, vitamin C deficiency has been linked with higher circulating histamine levels, which can be rebalanced once vitamin C blood level has been normalized (90–92). Furthermore, vitamin C can reduce the production of pro-inflammatory leukocyte-derived cytokines (e.g., TNFα and IL-6), through the modulation of nuclear transcription factor kappa B (NFkB) (2, 93, 94) in at least two ways: 1) through its reduced form (ascorbate), by scavenging cellular ROS and inhibiting ROS-related signaling for the transcription of NFkB (95–98); 2) through its oxidized form (dehydroascorbate), produced as a consequence of quenching ROS, by directly inhibiting the activity of several kinases involved in the TNFα-mediated activation of NFkB (p38 mitogen-activated protein kinase, IkB kinase α and β) (99–101).

However, the effect of vitamin C on the balance of cytokine responses (pro- and anti-inflammatory) is very complex and seems to be dependent on cell type and/or inflammatory condition (1). Moreover, authors (102, 103) have recently suggested that vitamin C may interact with molecular pathways related to inflammatory stress and immune dysfunction during sepsis, involving particular mediators: Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase-1 (MAPK1), Proto-Oncogene c (JUN), C–C chemokine Receptor type 5 (CCR5), Mitogen Activated Protein Kinase 3 (MAPK3), Angiotensin II Receptor type 2 (AGTR2), and Signal Transducer and Activator of Transcription-3 (STAT3).

The effects of vitamin C on immune function may also be expressed through epigenetic regulations, although this topic is still poorly understood (104, 105). The epigenetic remodeling associated with immune cell activation and differentiation includes DNA and histone modification (106). Vitamin C plays an important role by increasing the activity of epigenetic enzymes, including ten-eleven translocation (TET) proteins and Jumonij-C domain-containing histone demethylases (JHDMs) (107). In fact, since TET and JHDMs belong to the Fe^{2+}/α-ketoglutarate-dependent dioxygenases superfamily (105), vitamin C, as ascorbate, being able to donate electrons, acts as a cofactor for these enzymes, reducing Fe^{3+} to its catalytically active form (Fe^{2+}) (63). TET proteins are involved in the demethylation of cytosine residues in DNA, while JHDMs regulate the methylation of lysine and arginine residues in histones, resulting in modifications of gene transcription (63, 108, 109) that are involved in the response of both the innate and the adaptive immune system (106, 110). The utility of these recently-discovered gene-regulatory functions of vitamin C for the assessment of dietary recommendations has not yet been elucidated and further research is needed to indicate the minimum dose at which vitamin C may have an effective impact on functional or clinical outcomes through epigenetic changes (44).

ORAL SUPPLEMENTATION OF VITAMIN C FOR THE PREVENTION AND TREATMENT OF THE COMMON COLD AND UPPER RESPIRATORY TRACT INFECTIONS

The common cold is one of the most widespread viral upper respiratory tract infections (URTI), characterized by coughing, tiredness, fever, sore throat, and muscle pain, which persist for a period ranging from a few days to not more than 3 weeks (111, 112). The term "common cold" refers to an unspecific syndrome caused by several viruses, although the rhinovirus is the most frequent pathogen involved, being found in 30% to 50% of sufferers (113). Despite symptomatology usually being very mild, the common cold is a major cause of absenteeism from work and school (114). The popular myth that a very high intake of vitamin C may lead to a lower susceptibility to respiratory tract infections originates from Linus Pauling's theories published in the seventies. According to Pauling, a daily vitamin C intake of 1,000 mg can reduce the incidence of colds by about 45% and the optimal daily intake of vitamin C to live healthily and prevent disease should be at least 2.3 g (115, 116). The response of the US market to this pioneering point of view was immediate and the sales of vitamin C dietary supplements almost doubled over a couple of years (117). However, other clinical studies with similar aims failed to demonstrate its efficacy (118–121) and, in general, contemporary authors completely refuted Pauling's ideas, mainly based on non-randomized controlled trials or incorrect application of animal background to humans (122, 123). Although a high daily dose of vitamin C does not seem to prevent viral infections in the general population, some categories of subjects with potentially higher risk of viral infection may require particular consideration. These subjects include individuals undergoing a daily heavy physical workload such as soldiers and athletes, who may develop an immune stress condition.

General Population

If we exclude some results that reported only small or inconsistent effects attributable to vitamin C (124), after decades of investigations, the scientific community established that a high intake of vitamin C is useless in preventing the common cold (7–11), and therefore, a regular daily supplementation is not justified in the general population (12). Recent meta-analysis has reached similar conclusions regarding the incidence of infection, underlining, however, the possibility of reducing the duration of a cold. Gómez et al. (125), demonstrated that 8,472 subjects from eight randomized clinical trials (RCTs), showed very strong evidence that vitamin C intake above 80 mg/day does not prevent the common cold in healthy adults and children. Vorilhon et al. (126), analyzed eight RCTs and confirmed that vitamin C supplementation (dosage from 0.5 g to 2 g/day) is not effective, compared to placebo, in reducing the incidence of upper respiratory tract infections (URTI) in 3,135 children (from 3 months to 18 years of age), although the administration can reduce the duration of URTI by 14%, as previously highlighted by Rondanelli et al. (127). Positive results on the duration of colds was also suggested by the meta-analysis of Ran et al. (128) in which the combination of a small, long-term daily dose of vitamin C (no more than 1 g/day) to sustain immunity and a larger dose of vitamin C during the onset of the common cold (usually 3–4 g/day) was associated with the ability to relieve chest pain, fever, and chills, reducing the staying indoors duration, as well as the mean duration of disease.

People Under Heavy Physical Stress

Some authors have reported a high incidence of respiratory infections in military training centers, probably also due to an overcrowding of individuals often coming from different geographical areas (129–131). More data are available on athletes, for whom daily high-intensity training and competitions have been associated with transient immune perturbations, inflammation conditions, and increased susceptibility to infections (132–134). Furthermore, compared to the general population, athletes have a higher exposure to pathogens, due to frequent travel and sports events (135, 136), which may potentially increase the risk of developing viral infections.

Data in literature referring to the effect of vitamin C supplementation on the prevention of the common cold in these subjects are interesting, despite being limited at present. As was well described by Hemilä and Chalker, (12) vitamin C supplementation may decrease the incidence of colds by about 50% in people under extreme physical stress. More recently, Kim et al. (137) carried out a large randomized, double-blind, placebo-controlled trial in 1,444 Korean soldiers, 695 of whom received vitamin C (6 g/day) for 30 days. They showed that the vitamin C group had a 0.80-fold lower risk of getting the common cold compared to the placebo group (n = 749).

The theoretical basis for the use of vitamin C in physically stressed subjects resides in the significant increase of ROS production due to intense exercise (138), with remarkable tissue damage/inflammatory response that may have harmful consequences on preventing URTI (139), possibly requiring a higher antioxidant intake compared to the general population (140). Despite this, it has recently been established that the administration of a high dose of antioxidants can negatively interfere with exercise-induced redox signaling and muscle adaptations (141–147) and the use of high doses of vitamin C to abolish ROS, especially over a long period, should be avoided (148, 149).

Even though the effects of isolated vitamin C on oxidative stress, inflammatory markers, muscle damage and immune response following exercise remain to be clarified in depth (146), a recent scientific society position stand (150), a recent review (140), and a meta-analysis (146) agree on recommending vitamin C supplementation (0.25–1.0 g/day) as an option to prevent URTI symptoms in athletes under heavy exertion and/or during periods of increased risk of infection (e.g., travel abroad) (140); athletes with low initial blood concentrations of vitamin C are the major candidates for supplementation (146, 149, 151, 152).

ORAL SUPPLEMENTATION OF VITAMIN C FOR THE PREVENTION AND TREATMENT OF PNEUMONIA

Pneumonia is a lower respiratory tract infection characterized by a cough, difficulty in breathing, chest pain, fever, and lung inflammation (153). Pneumonia is the first cause of death by infection in the United States and the fifth most common cause of death overall (154, 155). *Streptococcus pneumoniae* and *Haemophilus influenzae* are recognized as the most common agents responsible for pneumonia (156) but other pathogens are also able to induce pneumonia, including viruses and fungi (153, 154).

Results obtained in rats and mice suggested that orally supplemented vitamin C may be useful in reducing susceptibility to viral pneumonia and potentially in reducing the development of ARDS (157, 158), which is recognized as the most severe form of acute respiratory infection (159). However, human findings on vitamin C supplementation and pneumonia remain scarce, with few dated observations mainly based on particular subjects and conditions (e.g., military people, developing countries) and not generalizable (5, 160). On this topic, the most recent meta-analysis including 2,774 participants from seven clinical studies, underlined that current evidence is insufficient to sustain the efficacy of vitamin C supplementation in preventing or treating pneumonia, due to the small number of trials and very low quality of the existing results (161). However, the meta-analysis of Padhani et al. (161) considered studies from different populations, including three studies on children, without subgroup analysis. Since the pharmacokinetics of vitamin C varies between subgroups and is not yet known in children, an analysis of data should have been done independently and, therefore, conclusions of this study may be questionable.

ORAL SUPPLEMENTATION OF VITAMIN C FOR THE PREVENTION OF COVID-19

COVID-19 is a new, worldwide recognized form of viral pneumonia, caused by SARS-CoV2 infection (162, 163). The symptomatology often begins within 2 weeks from contagion and mainly includes fever, fatigue, cough, and shortness of breath (164). Current knowledge suggests that while the majority of infected subjects (80%–90%) exhibit mild symptoms or can be asymptomatic, about 5% may develop pneumonia, ARDS and multi-organ dysfunction leading to death (165).

It is unquestionable that an optimal nutritional status effectively reduces inflammation and oxidative stress, improving the immune system regulation (166). However, no data are currently available on the regular use of high doses of oral vitamin C to reduce the risk of infection by SARS-CoV2 in a healthy general population (167–169) and further studies are needed to explore the role of vitamin C in prevention of COVID-19 (169). For heavily stressed subjects (athletes in particular), specific data are not currently reported regarding the incidence, prevalence, or natural history of disease related to COVID-19 (134), despite these subjects' potentially high risk of exposure to this virus (136, 170). Furthermore, scientific opinions have not been expressed regarding the use of oral vitamin C to prevent SARS-CoV2 infection in extreme exercisers. However, a vitamin C supplementation may be effective for improving the health status of patients considered at high risk of viral infections (171).

ORAL SUPPLEMENTATION OF VITAMIN C FOR PATIENTS WITH METABOLIC DISORDERS, CARDIOVASCULAR DISEASE, AND FRAIL ELDERLY SUBJECTS: POTENTIAL RELATIONSHIP WITH COVID-19

There are notably some factors that increase the risk of developing SARS-CoV2 infection and affect the severity of COVID-19 (172). People with pre-existing non-communicable diseases (NCDs) appear to be more susceptible to developing COVID-19 (173, 174). NCDs include obesity, diabetes mellitus, chronic lung diseases, cardiovascular diseases (CVD) and various other conditions which are characterized by systemic inflammation which impairs immune response and may exacerbate the cytokine storm related to COVID-19 (173, 174).

Obese Subjects

Some studies have shown that obesity is associated to a more severe form of COVID-19 (175, 176), even in younger patients (age < 50) (177), and a BMI > 40 kg/m^2 was identified as a one of the strongest risks of hospitalization due to SARS-CoV2 infection (178). These findings are worrying considering that obesity is a global phenomenon and in countries such as the U.S about 36% of population is obese (179). This association could be linked to inflammatory mechanisms, since authors suggest that, compared to individuals with a normal weight, obese subjects have a higher plasma concentration of C-reactive Protein (CRP), an inflammatory biomarker used to predict cardiovascular disease (180). Based on this, a vitamin C supplementation in these subjects may be useful in reducing inflammation, considering data that showed how a treatment of oral vitamin C (1,000 mg/day) for two months can significantly reduce plasma CRP in healthy, overweight, non-smokers with baseline CRP ≥ 1.0 mg/L (181). This finding is very interesting, taking into account that participants had an adequate dietary intake of vitamin C, with a baseline mean plasma level of 57.8 μmol/L, and it suggests that the RDAs for vitamin C in obese individuals may be underestimated, as was recently underlined by Rychter et al. (182, 183). Research is needed to understand whether by reducing the CRP with vitamin C it could be possible to influence the incidence and/or the progression of inflammation-mediated diseases associated with obesity, including infections and potentially COVID-19 (171).

Diabetic Subjects

A recent meta-analysis including 33 studies (16,003 patients) confirmed that diabetics have a two-fold higher increase in mortality, as well as severity of COVID-19, compared to non-diabetic COVID-19 patients (184). Type 2 diabetes mellitus (T2DM) is the most common form of diabetes (185), characterized by chronic hyperglycemia, inflammation and oxidative stress (186). The inflammatory condition observable in diabetes may possibly be a mechanism that increases the susceptibility to COVID-19. Low plasma concentrations of vitamin C in people with T2DM was observed (187, 188), despite adequate vitamin C intake (189, 190). Two mechanisms could particularly explain lower vitamin C levels in these patients: 1) increased urinary excretion, especially in those with microalbuminuria (191); 2) higher depletion of vitamin C caused by an increase of oxidative stress (190, 192). An interesting study on the use of oral vitamin C in diabetic subjects was reported by Mason et al. (193). It showed an approximately two-fold enhanced SVCT2 expression in skeletal muscle after vitamin C supplementation (1,000 mg for 4 months) in people with T2DM, with an increase of muscle concentration of vitamin C and a decrease of muscle oxidative stress. However, given the small number of subjects investigated in this study (seven participants, six males and one female), larger studies are needed to confirm similar results. Findings from an RCT showed that vitamin C supplementation (1,000 mg/day for 8 weeks) significantly reduced CRP, IL-6, fasting blood glucose (FBG), and triglycerides (TG) in 64 obese, hypertensive and/or diabetic patients, with high levels of CRP ≥ 6 mg/L (194). In addition, meta-analytic data indicated that vitamin C supplementation for more than 30 days with a dosage ranging from 200 to 1,000 mg significantly reduces FBG in patients with T2DM (195). Based on the above, vitamin C supplementation may represent a promising option to modulate inflammation and blood glucose in patients with hyperglycemia and elevated CRP, it could potentially be able to improve the health of these individuals and reduce the susceptibility to infections. Investigations are

strongly encouraged to establish a possible correlation between an extra intake of vitamin C and a possible decrease of incidence, severity and mortality for COVID-19.

Subjects With CVDs

CVDs (as well as hypertension) are the most common comorbidities among COVID-19 patients (174, 196, 197). Individuals with a CVD are five-fold more at risk of developing the critical stage of the disease, as indicated in a meta-analysis involving over 3,000 patients with COVID-19 (198). In this case, the main reason for a higher risk of SARS-Cov2 infection is related to the high angiotensin-converting enzyme 2 (ACE2) expression observed in these patients (199–201), since this enzyme is used by the virus to invade cells, promoting viral colonization (202). It is known that low plasma concentrations of vitamin C are predictive of heightened CVD risk (203–205), but the current literature provides little support for a widespread use of vitamin C supplements to reduce CVD risk or mortality (206), and available data are also controversial. Many cohort studies and RCTs have shown no relationship between vitamin C intake and CVD risk. However, in most RCTs the participants were not prescreened for a depleted status and this seriously limits the possibility of concluding on the results, as the potential impact of the vitamin C supplementation on the outcomes considered may vary from highly significant to negligible in relation to their vitamin C status at study start (207).

A few other studies have suggested moderate benefits, and some references have registered a slight increase in risk (206). A significant barrier to the comprehension of the relationship between vitamin C and CVDs is the lack of mechanistic studies in humans (206). At present, there are no recommendations for an additional daily dose of vitamin C in CVDs to potentially prevent diseases, including pulmonary infections and COVID-19.

Frail Elderly Subjects

It is particularly important to consider elderly communities when trying to prevent COVID-19. The elderly are more vulnerable compared to the general population due to an increased risk of malnourishment and infections and a high prevalence of NCDs (208). Age itself is a risk factor for developing COVID-19 (209), due to a functional decline of the immune system (210, 211). Furthermore, malnourishment in these subjects is very frequent for several reasons (e.g., poor socioeconomic conditions, mental status, social status) (212) and nutritional deficiencies (including vitamin C) have been reported (213). Malnourishment can worsen an impaired immune system in the elderly, making them more susceptible to infections (214). In elderly hospitalized subjects (mean age 81 years) suffering from acute bronchitis or pneumonia, a mean plasma vitamin C level at baseline of 23 μmol/L was reported and a concentration of 11 μmol/L was found in one third of the patients (215). This is particularly important since a low vitamin C concentration (<17 μmol/L) in older people (aged 75–82 years) is considered a strong predictor of all-cause mortality (216). Notably, in Hunt's study (215) administration of vitamin C (0.2 g/day) reduced the respiratory symptom score in the more severe patients. However, at present, it is not known whether a regular supplementation with vitamin C can protect these subjects from chronic inflammation NCDs-related and/or can prevent the onset of viral infections including COVID-19.

ORAL SUPPLEMENTATION AND SIDE EFFECTS

Vitamin C has an excellent safety profile, primarily due to its high water solubility and rapid clearance of excess levels by the kidneys (44, 217). Although it is not possible to establish a UL for vitamin C, values of 1,000–2,000 mg/day have been suggested as prudent limits by some countries, based on a potential risk of osmotic diarrhea and related gastrointestinal distress in some individuals at higher doses (44, 53).

Since vitamin C is partially converted to oxalate and excreted in the urine, high doses of vitamin C could be associated with calcium oxalate stone formation (218, 219). Ferraro et al. (220) studied 156,735 women and 40,536 men, who reported episodes of kidney stones during an average follow-up of 11.3–11.7 years. The authors significantly correlated the total vitamin C intake with a higher risk of incident kidney stones in men, but not in women. However, it is important to outline that this study had limitations to be considered. The presence of confounding factors (e.g., comorbidities, dehydration, dietary intakes of oxalate-forming foods) were not taken into account during the follow-up, and the authors assessed vitamin C intake only through a questionnaire (without measuring blood levels) and with very long time intervals (every 4 years).

INTRAVENOUS ADMINISTRATION OF VITAMIN C: A POTENTIAL ROLE IN THE TREATMENT OF COVID-19?

While an extra dietary intake of vitamin C to counter pneumonia does not seem promising, several interesting data have emerged from the use of vitamin C through IA, providing an encouraging, but questioned, hypothesis on its potential pharmacological use for the treatment of pneumonia caused by SARS-CoV2 infection. In fact, as opposed to oral supplementation, in which the maximum peak plasma concentration that was achieved with a high-dose (3 g every 4 h) was 220 μmol/L (221), the IA of vitamin C, bypassing the limitations induced by intestinal transporters (SVCT1), may lead to a higher plasma level (e.g., up to 3,000 μmol/L at day 4 with 200 mg/kg/day, administered in 50 mg/kg/dose every 6 h).

Although the potential antisepsis therapeutic mechanism exerted by vitamin C has not yet been understood (103), besides the effects described in the previous paragraphs, the use of vitamin C in an infectious emergency may be justified for some reasons: 1) significant clinical evidence from pneumonia, critical illnesses and other acute infections suggests that plasma levels of vitamin C can rapidly drop off (e.g., <30 μmol/L) during the inflammatory response (2, 93, 94, 222–228) probably due to an increased

consumption of vitamin C by leukocytes. Considering that intracellular ascorbate concentrations in mononuclear cells and in granulocytes are respectively 80 and 25 times greater than in plasma, an increased replacement and turnover of these cells during these medical conditions can contribute to a decrease of vitamin C in the blood (229); 2) a negative regulation of SVCT2 transporters induced by inflammatory cytokines, in particular IL-1β and TNFα (230); 3) the antioxidant defense system of the pulmonary epithelium involves enzymes and vitamin C (231) and according to Banerjee and Kaul, a sustained high dose of vitamin C available in respiratory secretion could exhibit an effective anti-viral activity (232). This last point, however, is still a hypothesis at present, since the level of vitamin C in the bronchoalveolar fluid is normally too low to achieve anti-viral activity and furthermore very little is known about the potential increase of vitamin C concentration in bronchial tissue and fluid secretion following a high-dose IA (36).

Therefore, considering the aspects mentioned above, the infusion of vitamin C has recently been suggested to treat COVID-19 in ICU hospitalized patients (13–18). Below, we summarize the most substantial evidence obtained in critical illness studies based on IA of vitamin C regarding the most relevant outcomes (inflammation, ventilation time, and mortality) which may relate to SARS-CoV2-induced ARDS.

Effects of IA of Vitamin C on Inflammation Markers

In COVID-19 patients the inflammatory response is very dramatic and has been defined as a "cytokine storm", associated with increased plasma concentration of IL-1β, IL-2, IL-6, IL-10, IFNγ, and TNF-α (233, 234), able to induce an acute lung injury (ALI) which results in ARDS and requires urgent ICU interventions (162). Physiopathology of ARDS involves alteration of pulmonary permeability, rapid lung leukocyte infiltration with a large increase of tissue oxidative stress, leading to respiratory failure and death, which in most cases is due to massive alveolar damage (24, 235). A promising background on the use of vitamin C in an experimental model of ALI was found (236–241), with positive evidence on rebalancing cytokine production and specific physiopathological mechanisms involving neutrophils (i.e., neutrophil extracellular traps) (4) which may contribute to organ damage and mortality in COVID-19 (233) (**Figure 1**). Two studies by Fowler et al. are currently available on IA of vitamin C in ICU hospitalized patients and inflammatory response, with mixed results (227, 242). In the first preliminary study (227), vitamin C showed a significant reduction in proinflammatory biomarkers (CRP and procalcitonin) over the first 96 h, without adverse effects registered during the infusion, but the number of ARDS-affected patients treated with vitamin C was too small (50 mg/kg/24 h, n = 8; 200 mg/kg/24 h, n = 8) to allow safe conclusions. In the second larger study (242), 167 patients with sepsis and ARDS were randomized to receive vitamin C (50 mg/kg every 6 h for 96 h) or placebo; no changes in CRP and thrombomodulin were detected, although the study was criticized for the choice of the inflammatory markers assessed (243).

Effects of IA of Vitamin C on Intensive Care Ventilation Time

The above-mentioned study (242) also reported a lower duration time of mechanical ventilation support in the supplemented group, with a higher number of ventilator-free days in the vitamin C group than in the placebo group (mean values: 10.6 vs. 13.1 days, respectively). Ventilator-free days were defined as the number of extubated days, considering the time between ICU hospitalization and day 28. Another previous randomized controlled trial (RCT), including burn patients with severe respiratory dysfunction receiving a very high dose of vitamin C

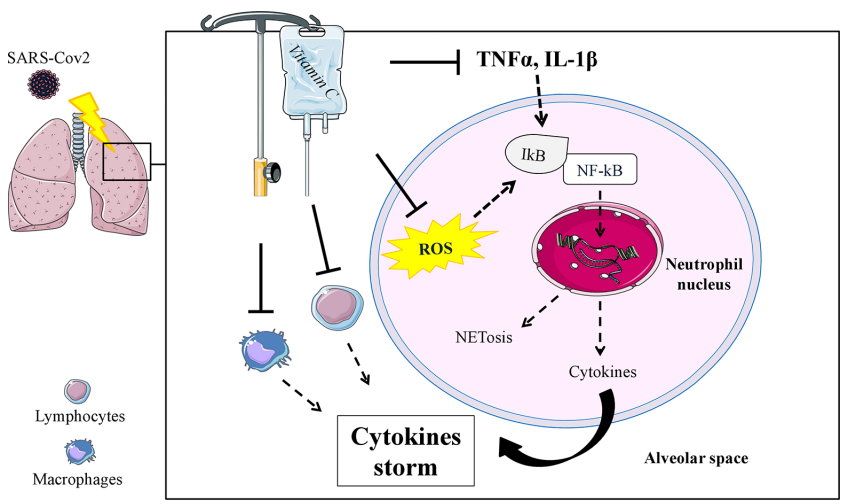


FIGURE 1 | Schematic mechanism in which an IA of vitamin C could modulate specific functions of neutrophils (ROS and TNFα, IL-1β mediated), inhibiting pathways involved in the Neutrophil Extracellular Trap formation (NETosis) and reducing the uncontrollable inflammatory cytokine production in the alveolar space. Potential effects on reducing cytokine production have also been speculated in lymphocytes and macrophages. ROS, reactive oxygen species; NFkB, nuclear transcription factor kappa B; ⊥, inhibition stimulus; dashed arrow, reduced effect or production.

(66 mg/kg/h for 24 h), showed a significant decrease of the time of ventilation (mean values: 12.1 vs. 21.3 days, respectively) in those who received vitamin C infusion compared to the control group (85). The pulmonary benefit reported by these authors is probably due to the antioxidant, anti-inflammatory and microvascular action of the vitamin C (244).

Different positions on the topic derive from systematic evaluation of the literature, suffering because of the gross limitations of the available primary data. For example, the meta-analysis of Langlois et al. (245), failed to find any improvement on ventilation time. This work, however, included studies with vitamin C administrated through different routes (enteral or parenteral), most of which (9 out of a total of 11 studies) used antioxidant mixtures instead of vitamin C alone; Zhang and Jativa (244) analyzed the efficacy of IA of vitamin C on vasopressor sparing effects and the lower need for mechanical ventilation in critical illness, underlining several weaknesses of the available studies considered (four RCTs and one retrospective review), such as the paucity of the sample size, the heterogeneity of subjects enrolled, hospitalization setting, dosages and follow-up; recently, the meta-analysis from Hemilä and Chalker (246), including eight trials and 685 patients, with promising results on ventilation time, pointed out that the great variation in the reported effects of vitamin C may be linked to the non-homogeneous severity of the illness which mostly impacts the ventilation time required. From this point of view, vitamin C shortened ventilation time on average by 25% when the analysis was restricted to patients requiring mechanical support for more than 10 h.

Effects of IA of Vitamin C on Mortality

Of the critical outcomes considered the potential effect of vitamin C on mortality rates appears to be the most controversial one, with RCTs studies that underline promising results which are not supported by recent meta-analysis. A significant reduction of 28-day mortality during ICU hospitalization was observed in a small group of patients with sepsis treated with IA of vitamin C (25 mg/kg every 6 h, for 72 h) compared to the control group (14.28% Vs. 64.28%, respectively) (247). More recently, findings from the CITRIS-ALI study (242) showed a reduced mortality at day 28 in the vitamin C group (29.8%) compared to the placebo group (46.3%). Conversely, according to the meta-analysis of Zhang and Jativa, although vitamin C IA seems to be linked to positive vasopressor effects, temporally reducing the need for mechanical ventilation, no positive effect in favor of overall mortality emerged (244), leading the authors to conclude that it does seem improbable that vitamin C, considered as a single agent, could be so dramatically decisive on the physiopathology of a critical illness as to influence the incidence of mortality (244). Similar conclusions were drawn by Wei et al. (248), who, by including recently published retrospective studies in their meta-analysis, suggest the lack of benefit on 28-day mortality, both in ICU and in-hospital patients with sepsis.

It is important to consider that the effect of vitamin C infusion seems to exert different results on mortality in relation to the type of critical patients involved, especially when administration is in association with other drugs. From this point of view, two retrospective studies showed that vitamin C (1.5 g every 6 h), in combination with hydrocortisone (50 mg every 6 h), and thiamine (200 mg every 12 h) may dramatically reduce mortality by 56% in ICU patients with severe pneumonia (249) and by 79% in severe sepsis (250), compared with patients who did not receive vitamin C and thiamine. Unlike these data, a recently published RCT (VITAMINS) showed no benefit from the combination of IA of vitamin C, hydrocortisone and thiamine in comparison to hydrocortisone alone among patients with septic shock (251). However, as underlined by Carr (252), since the VITAMINS trial did not include a monotherapy vitamin C subgroup, this trial does not provide any information as to whether IA of vitamin C offers some benefit to septic patients in the absence of corticosteroid administration, and further trials are needed in this direction.

Another critical issue that should be highlighted is the timing of treatment administration. On this topic, important results

TABLE 1 | Summary of research findings on the use of vitamin C in humans.

General population		There are no recommendations that vitamin C supplementation impacts the incidence and duration of the common cold or pneumonia, and regular supplementation is not currently justified in the general population. Data on pneumonia are less clear in children, because the pharmacokinetics of vitamin C in this category is unknown at present. A supplementation of 0.2 g/day of vitamin C may be reasonable in subjects with low plasma vitamin C concentration.
Subjects with NCDs		Subjects with noncommunicable diseases (NCDs) and frail, elderly subjects are recognized as being at high risk of viral infections. Vitamin C supplementation (0.2–1 g/day) can reduce inflammation and, although direct evidence is still lacking, it may decrease the susceptibility to infections and/or the severity of the disease. Vitamin C supplementation (0.2 g/day) may reduce the respiratory symptoms in elderly patients.
Heavy stressed subjects		Vitamin C supplementation may decrease the incidence of colds in people under extreme physical stress. Vitamin C supplementation does not seem to improve physical performance in well-nourished athletes, and regular high-dose vitamin C supplementation may interfere with the exercise-induced redox signaling adaptation and should be avoided. Athletes should consider a vitamin C supplementation (0.25–1 g/day) to prevent URTI symptoms during limited periods of enhanced heavy stress (e.g., sports competitions).
Hospitalized patients		In critically ill patients, vitamin C deficiency is common, and IA of high doses has been used to normalize plasma vitamin C levels. To date, no recommendations are available as few data on inflammatory markers, mechanical ventilation time and mortality rates have been reported in these patients. Further controlled studies are encouraged, especially for COVID-19.

come from a retrospective cohort study of 208 patients in septic shock (253), which suggested that the ICU mortality ratio [based on the APACHE (Acute Physiology and Chronic Health Evaluation)–predicted ICU mortality] of patients who received vitamin C with thiamine and hydrocortisone, increased linearly with the delay in treatment from initial sepsis presentation. Indeed, the APACHE-adjusted ICU mortality was significantly reduced only in patients who received vitamin C, thiamine, and steroids within 6 h from sepsis presentation (253).

CONCLUSION

Apart from some specific individuals and conditions (**Table 1**), the evidence described is insufficient to support the efficacy of a regular supplementation with vitamin C for the prevention or treatment of the common cold or pneumonia in the general population. Interesting data on the possible use of vitamin C to prevent infections regard special conditions (e.g., soldiers and athletes) and subjects with metabolic disorders, CVDs or frailty, in which the potential control of inflammation by a vitamin C supplementation could represent an effective aid in reducing the risk of infection, even for COVID-19. However, this last statement needs to be properly supported by future RCTs. Even though the IA of vitamin C could be an adjuvant therapy to quickly restore plasma levels of vitamin C during severe sepsis and ARDS in ICU hospitalized patients (254), its effects on inflammation response, ventilation time and mortality rates still remain uncertain and results from further RCTs, especially in COVID-19 patients, are urgently needed.

Although a significant increase in vitamin C sales was registered immediately after the global pandemic state of emergency was declared, at present, there is no evidence that vitamin C supplementation can protect people from the SARS-CoV2 infection (255). At the current state of knowledge, health care professionals have the responsibility to guarantee that patients have correct information regarding the lack of data supporting the efficacy of this supplement for the prevention and/or treatment of COVID-19 (167, 168).

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version. GC and MN conceived the original idea of the manuscript and contributed equally to this work as main authors. GD'A revised the manuscript before submission.

ACKNOWLEDGMENTS

The authors thank Simona Aldrovandi for generating table illustrations and Emily Riley for the English revision of the manuscript.

REFERENCES

1. Carr AC, Maggini S, Vitamin C. and immune function. *Nutrients* (2017) 9:1211. doi: 10.3390/nu9111211
2. Carr AC, Rosengrave PC, Bayer S, Chambers S, Mehrtens J, Shaw GM. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. *Crit Care* (2017) 21:200. doi: 10.1186/s13054-017-1891-y
3. Van Gorkom GNY, Klein Wolterink RGJ, Van Elssen CHMJ, Wieten L, Germeraad WTV, Bos GMJ. Influence of Vitamin C on lymphocytes: An overview. *Antioxidants* (2018) 7:41. doi: 10.3390/ANTIOX7030041
4. Bozonet SM, Carr AC. The role of physiological vitamin c concentrations on key functions of neutrophils isolated from healthy individuals. *Nutrients* (2019) 11:1363. doi: 10.3390/nu11061363
5. Hemilä H. Vitamin C and infections. *Nutrients* (2017) 52:222–3. doi: 10.3390/nu9040339
6. Bozonet SM, Carr AC, Pullar JM, Vissers MCM. Enhanced human neutrophil vitamin C status, chemotaxis and oxidant generation following dietary supplementation with vitamin C-rich SunGold kiwifruit. *Nutrients* (2015) 7:2574–88. doi: 10.3390/nu7042574
7. Allan GM, Arroll B. Prevention and treatment of the common cold: Making sense of the evidence. *CMAJ* (2014) 186:190–99. doi: 10.1503/cmaj.121442
8. Douglas RM, Hemilä H. Vitamin C for preventing and treating the common cold. *PloS Med* (2005), CD000980. doi: 10.1371/journal.pmed.0020168
9. Douglas RM, Hemilä H, Chalker E, Treacy B. Vitamin C for preventing and treating the common cold. *Cochrane Database Syst Rev* (2007) CD000980. doi: 10.1002/14651858.CD000980.pub3
10. Heimer KA, Hart AM, Martin LG, Rubio-Wallace S. Examining the evidence for the use of vitamin C in the prophylaxis and treatment of the common cold. *J Am Acad Nurse Pract* (2009) 21:295–300. doi: 10.1111/j.1745-7599.2009.00409.x
11. Hemilä H. Vitamin C supplementation and the common cold - Was Linus Pauling right or wrong? *Int J Vitam Nutr Res* (1997) 67:329–35.
12. Hemilä H, Chalker E. Vitamin C for preventing and treating the common cold. *Cochrane Database Syst Rev* (2013) CD000980. doi: 10.1002/14651858.CD000980.pub4
13. Boretti A, Banik BK. Intravenous vitamin C for reduction of cytokines storm in acute respiratory distress syndrome. *PharmaNutrition* (2020) 12:100190. doi: 10.1016/j.phanu.2020.100190
14. Cheng RZ. Can early and high intravenous dose of vitamin C prevent and treat coronavirus disease 2019 (COVID-19)? *Med Drug Discovery* (2020) 5:100028. doi: 10.1016/j.medidd.2020.100028
15. Erol A. High-dose intravenous vitamin C treatment for COVID-19. *OSF Prepr* (2020). doi: 10.31219/osf.io/p7ex8
16. Hernández A, Papadakos PJ, Torres A, González DA, Vives M, Ferrando C, et al. Two known therapies could be useful as adjuvant therapy in critical patients infected by COVID-19. *Rev Esp Anestesiol Reanim* (2020) 67:245–52. doi: 10.1016/j.redar.2020.03.004
17. Matthay MA, Aldrich JM, Gotts JE. Treatment for severe acute respiratory distress syndrome from COVID-19. *Lancet Respir Med* (2020) 8:433–34. doi: 10.1016/S2213-2600(20)30127-2
18. Zhang L, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. *J Med Virol* (2020) 92:479–90. doi: 10.1002/jmv.25707
19. Worldometer. *COVID-19 Coronavirus Pandemic* (2020). Available at: https://www.worldometers.info/coronavirus/.
20. Yu F, Du L, Ojcius DM, Pan C, Jiang S. Measures for diagnosing and treating infections by a novel coronavirus responsible for a pneumonia outbreak originating in Wuhan, China. *Microbes Infect* (2020) 22:74–9. doi: 10.1016/j.micinf.2020.01.003
21. Alhazzani W, Hylander Møller M, Arabi YM, Loeb M, Ng Gong M, Fan E, et al. Surviving Sepsis Campaign: Guidelines on the Management of Critically Ill Adults with Coronavirus Disease 2019 (COVID-19). *Crit Care Med J* (2020) 48:e440–69. doi: 10.1097/CCM.0000000000004363
22. Singer M, Deutschman CS, Seymour C, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis

and septic shock (sepsis-3). *JAMA* (2016) 315:801–10. doi: 10.1001/jama.2016.0287
23. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
24. The ARDS Definition Task Force*. Acute Respiratory Distress Syndrome. The Berlin Definition of ARDS. *JAMA* (2012) 307:2526–33. doi: 10.1001/jama.2012.5669
25. Sun P, Qie S, Liu Z, Ren J, Li K, Xi J. Clinical characteristics of 50 466 hospitalized patients with 2019-nCoV infection. *J Med Virol* (2020) 92:612–17. doi: 10.1002/jmv.25735
26. Available at: https://clinicaltrials.gov/.
27. Linster CL, Van Schaftingen E. Vitamin C: Biosynthesis, recycling and degradation in mammals. *FEBS J* (2007) 274:1–22. doi: 10.1111/j.1742-4658.2006.05607.x
28. Frikke-Schmidt H, Tveden-Nyborg P, Lykkesfeldt J. L-dehydroascorbic acid can substitute l-ascorbic acid as dietary vitamin C source in guinea pigs. *Redox Biol* (2016) 7:8–13. doi: 10.1016/j.redox.2015.11.003
29. Michels AJ, Hagen TM, Frei B. Human Genetic Variation Influences Vitamin C Homeostasis by Altering Vitamin C Transport and Antioxidant Enzyme Function. *Annu Rev Nutr* (2013) 33:45–70. doi: 10.1146/annurev-nutr-071812-161246
30. Hasselholt S, Tveden-Nyborg P, Lykkesfeldt J. Distribution of vitamin C is tissue specific with early saturation of the brain and adrenal glands following differential oral dose regimens in guinea pigs. *Br J Nutr* (2015) 113:1539–49. doi: 10.1017/S0007114515000690
31. Kim H, Bae S, Yu Y, Kim Y, Kim H-R, Hwang Y, et al. The Analysis of Vitamin C Concentration in Organs of Gulo -/- Mice Upon Vitamin C Withdrawal. *Immune Netw* (2012) 12:18–26. doi: 10.4110/in.2012.12.1.18
32. Harrison FE, Green RJ, Dawes SM, May JM. Vitamin C distribution and retention in the mouse brain. *Brain Res* (2010) 1348:181–86. doi: 10.1016/j.brainres.2010.05.090
33. Toutain PL, Béchu D, Hidiroglou M. Ascorbic acid disposition kinetics in the plasma and tissues of calves. *Am J Physiol - Regul Integr Comp Physiol* (1997) 273:R1587–97. doi: 10.1152/ajpregu.1997.273.5.r1585
34. Omaye St, Schaus Ee, Kutnink Ma, Hawkes Wc.. Measurement of Vitamin C in Blood Components by High-Performance Liquid Chromatography: Implication in Assessing Vitamin C Status. *Ann N Y Acad Sci* (1987) 498:389–401. doi: 10.1111/j.1749-6632.1987.tb23776.x
35. Carr AC, Bozonet SM, Pullar JM, Simcock JW, Vissers MCM. Human skeletal muscle ascorbate is highly responsive to changes in vitamin C intake and plasma concentrations. *Am J Clin Nutr* (2013) 97:800–7. doi: 10.3945/ajcn.112.053207
36. Lykkesfeldt J, Tveden-Nyborg P. The pharmacokinetics of vitamin C. *Nutrients* (2019) 11:2412. doi: 10.3390/nu11102412
37. Frei B, Birlouez-Aragon I, Lykkesfeldt J. Authors' perspective: What is the optimum intake of vitamin C in humans? *Crit Rev Food Sci Nutr* (2012) 52:815–29. doi: 10.1080/10408398.2011.649149
38. Levine M, Padayatty SJ, Espey MG. Vitamin C: A Concentration-Function Approach Yields Pharmacology and Therapeutic Discoveries. *Adv Nutr* (2011) 2:78–88. doi: 10.3945/an.110.000109
39. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, et al. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proc Natl Acad Sci U S A* (1996) 93:3704–9. doi: 10.1073/pnas.93.8.3704
40. Nygaard G. On a Novel, Simplified Model Framework Describing Ascorbic Acid Concentration Dynamics. In: *41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS* (2019). p. 2880–6. doi: 10.1109/EMBC.2019.8857675
41. Lykkesfeldt J, Poulsen HE. Is vitamin C supplementation beneficial? Lessons learned from randomised controlled trials. *Br J Nutr* (2010) 103:1251–9. doi: 10.1017/S0007114509993229
42. Bechthold A. New reference values for Vitamin C intake. *Ann Nutr Metab* (2015) 67:13–20. doi: 10.1159/000434757
43. VITAMINE. *Assunzione raccomandata per la popolazione (PRI) e assunzione adeguata (AI). sinu.it*. Available at: https://sinu.it/2019/07/09/assunzione-raccomandata-per-la-popolazione-pri-e-assunzione-adeguata-ai/ (Accessed June 11, 2020).
44. Carr AC, Lykkesfeldt J. Discrepancies in global vitamin C recommendations: a review of RDA criteria and underlying health perspectives. *Crit Rev Food Sci Nutr* (2020) 1–14. doi: 10.1080/10408398.2020.1744513
45. Italian Society of Human Nutrition (SINU). *LARN Levels of reference intake for nutrients and energy for the Italian population*. (2014). Available at: https://sinu.it/tabelle-larn-2014/.
46. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on Dietary Reference Values for vitamin C. *EFSA J* (2013) 11:3418. doi: 10.2903/j.efsa.2013.3418
47. German Nutrition Society. New Reference Values for Vitamin C Intake. *Ann Nutr Metab* (2015) 67:13–20. doi: 10.1159/000434757
48. Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington (DC): National Academies Press (US) (2000). doi: 10.17226/9810
49. Galan P, Viteri FE, Bertrais S, Czernichow S, Faure H, Arnaud J, et al. Serum concentrations of β-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur J Clin Nutr* (2005) 59:1181–90. doi: 10.1038/sj.ejcn.1602230
50. Canoy D, Wareham N, Welch A, Bingham S, Luben R, Day N, et al. Plasma ascorbic acid concentrations and fat distribution in 19 068 British men and women in the European Prospective Investigation into Cancer and Nutrition Norfolk cohort study. *Am J Clin Nutr* (2005) 82:1203–09. doi: 10.1093/ajcn/82.6.1203
51. Hampl JS, Taylor CA, Johnston CS. Vitamin C Deficiency and Depletion in the United States: The Third National Health and Nutrition Examination Survey, 1988 to 1994. *Am J Public Health* (2004) 94:870–5. doi: 10.2105/AJPH.94.5.870
52. Jungert A, Neuhäuser-Berthold M. The lower vitamin C plasma concentrations in elderly men compared with elderly women can partly be attributed to a volumetric dilution effect due to differences in fat-free mass. *Br J Nutr* (2015) 113:859–64. doi: 10.1017/S0007114515000240
53. National Health and Medical Research Council. *Nutrient Reference Values for Australia and New Zealand: Executive Summary*. (2006).
54. Faure H, Preziosi P, Roussel AM, Bertrais S, Galan P, Hercberg S, et al. Factors influencing blood concentration of retinol, α-tocopherol, vitamin C, and β-carotene in the French participants of the SU.VI.MAX trial. *Eur J Clin Nutr* (2006) 60:706–17. doi: 10.1038/sj.ejcn.1602372
55. Birlouez-Aragon I, Delcourt C, Tessier F, Papoz L. Associations of age, smoking habits and diabetes with plasma vitamin C of elderly of the POLA study. *Int J Vitam Nutr Res* (2001) 71:53–9. doi: 10.1024/0300-9831.71.1.53
56. Ravindran RD, Vashist P, Gupta SK, Young IS, Maraini G, Camparini M, et al. Prevalence and risk factors for vitamin C deficiency in North and South India: A two centre population based study in people aged 60 years and over. *PloS One* (2011) 6:e28588. doi: 10.1371/journal.pone.0028588
57. Martin A. The "apports nutritionnels conseillés (ANC)" for the French population. *Reprod Nutr Dev* (2001) 41:119–28. doi: 10.1051/rnd:2001100
58. Juhl B, Lauszus FF, Lykkesfeldt J. Is diabetes associated with lower vitamin C status in pregnant women? A prospective study. *Int J Vitam Nutr Res* (2016) 86:184–89. doi: 10.1024/0300-9831/a000407
59. Lykkesfeldt J, Priemé H, Loft S, Poulsen HE. Effect of smoking cessation on plasma ascorbic acid concentration. *Br Med J* (1996) 313:91. doi: 10.1136/bmj.313.7049.91
60. Carr AC, Rowe S. Factors affecting vitamin c status and prevalence of deficiency: A global health perspective. *Nutrients* (2020) 12:1963. doi: 10.3390/nu12071963
61. Suarez EC, Schramm-Sapyta NL. Race differences in the relation of vitamins A, C, E, and β-carotene to metabolic and inflammatory biomarkers. *Nutr Res* (2014) 34:1–10. doi: 10.1016/j.nutres.2013.10.001
62. Levine M, Wang Y, Padayatty SJ, Morrow J. A new recommended dietary allowance of vitamin C for healthy young women. *Proc Natl Acad Sci U S A* (2001) 98:9842–6. doi: 10.1073/pnas.171318198
63. Camarena V, Wang G. The epigenetic role of vitamin C in health and disease. *Cell Mol Life Sci* (2016) 73:1645–58. doi: 10.1007/s00018-016-2145-x
64. Mangge H. Antioxidants, inflammation and cardiovascular disease. *World J Cardiol* (2014) 6:462–77. doi: 10.4330/wjc.v6.i6.462

65. Ginter E, Simko V, Panakova V. Antioxidants in health and disease. *Bratislava Med J* (2014) 115:603–6. doi: 10.4149/BLL_2014_116
66. Hong JM, Kim JH, Kang JS, Lee WJ, Hwang Y. Vitamin C is taken up by human T cells via sodium-dependent vitamin C transporter 2 (SVCT2) and exerts inhibitory effects on the activation of these cells in vitro. *Anat Cell Biol* (2016) 49:88–98. doi: 10.5115/acb.2016.49.2.88
67. Bergsten P, Amitai G, Kehrl J, Dhariwal KR, Klein HG, Levine M. Millimolar concentrations of ascorbic acid in purified human mononuclear leukocytes. Depletion and reaccumulation. *J Biol Chem* (1990) 265:2584–87.
68. Evans RM, Currie L, Campbell A. The distribution of ascorbic acid between various cellular components of blood, in normal individuals, and its relation to the plasma concentration. *Br J Nutr* (1982). doi: 10.1079/bjn19820059
69. Winterbourn CC, Vissers MCM. Changes in ascorbate levels on stimulation of human neutrophils. *BBA - Mol Cell Res* (1983) 763:175–9. doi: 10.1016/0167-4889(83)90041-1
70. Oberritter H, Glatthaar B, Moser U, Schmidt KH. Effect of functional stimulation on ascorbate content in phagocytes under physiological and pathological conditions. *Int Arch Allergy Appl Immunol* (1986) 81:46–50. doi: 10.1159/000234106
71. Vissers MCM, Wilkie RP. Ascorbate deficiency results in impaired neutrophil apoptosis and clearance and is associated with up-regulation of hypoxia-inducible factor 1α. *J Leukoc Biol* (2007) 8:1236–44. doi: 10.1189/jlb.0806541
72. Goldschmidt MC. Reduced bactericidal activity in neutrophils from scorbutic animals and the effect of ascorbic acid on these target bacteria in vivo and in vitro. *Am J Clin Nutr* (1991) 54:1214S–20S. doi: 10.1093/ajcn/54.6.1214s
73. Goldschmidt MC, Masin WJ, Brown LR, Wyde PR. The effect of ascorbic acid deficiency on leukocyte phagocytosis and killing of Actinomyces viscosus. *Int J Vitam Nutr Res* (1988) 58:326–34.
74. Demaret J, Venet F, Friggeri A, Cazalis M-A, Plassais J, Jallades L, et al. Marked alterations of neutrophil functions during sepsis-induced immunosuppression. *J Leukoc Biol* (2015) 98:1081–90. doi: 10.1189/jlb.4a0415-168rr
75. Arraes SMA, Freitas MS, Da Silva SV, De Paula Neto HA, Alves-Filho JC, Martins MA, et al. Impaired neutrophil chemotaxis in sepsis associates with GRK expression and inhibition of actin assembly and tyrosine phosphorylation. *Blood* (2006) 108:2906–13. doi: 10.1182/blood-2006-05-024638
76. Chishti AD, Shenton BK, Kirby JA, Baudouin SV. Neutrophil chemotaxis and receptor expression in clinical septic shock. *Intensive Care Med* (2004) 30:605–11. doi: 10.1007/s00134-004-2175-y
77. Lenton KJ, Therriault H, Fülöp T, Payette H, Wagner JR. Glutathione and ascorbate are negatively correlated with oxidative DNA damage in human lymphocytes. *Carcinogenesis* (1999) 20:607–13. doi: 10.1093/carcin/20.4.607
78. Huijskens MJAJ, Walczak M, Koller N, Briedé JJ, Senden-Gijsbers BLMG, Schnijderberg MC, et al. Technical Advance: Ascorbic acid induces development of double-positive T cells from human hematopoietic stem cells in the absence of stromal cells. *J Leukoc Biol* (2014) 96:1165–75. doi: 10.1189/jlb.1ta0214-121rr
79. Song MH, Nair VS, Oh KI. Vitamin C enhances the expression of IL17 in a Jmjd2-dependent manner. *BMB Rep* (2017) 50:49–54. doi: 10.5483/BMBRep.2017.50.1.193
80. Vallance S. Relationships between ascorbic acid and serum proteins of the immune system. *Br Med J* (1977) 2:437–8. doi: 10.1136/bmj.2.6084.437
81. Anderson R, Oosthuizen R, Maritz R, Theron A, Van Rensburg AJ. The effects of increasing weekly doses of ascorbate on certain cellular and humoral immune functions in normal volunteers. *Am J Clin Nutr* (1980) 33:71–6. doi: 10.1093/ajcn/33.1.71
82. Prinz W, Bloch J, Gilich G, Mitchell G. A systematic study of the effect of vitamin C supplementation on the humoral immune response in ascorbate-dependent mammals. I. The antibody response to sheep red blood cells (a T-dependent antigen) in guinea pigs. *Int J Vitam Nutr Res* (1980) 50:294–300.
83. Feigen GA, Smith BH, Dix CE, Flynn CJ, Peterson NS, Rosenberg LT, et al. Enhancement of antibody production and protection against systemic anaphylaxis by large doses of vitamin C. *Res Commun Chem Pathol Pharmacol* (1982) 38:313–33. doi: 10.1016/s0022-5347(17)52586-0
84. Kennes B, Dumont I, Brohee D, Hubert C, Neve P. Effect of vitamin C supplements on cell-mediated immunity in old people. *Gerontology* (1983) 29:305–10. doi: 10.1159/000213131
85. Tanaka H, Matsuda T, Miyagantani Y, Yukioka T, Matsuda H, Shimazaki S. Reduction of resuscitation fluid volumes in severely burned patients using ascorbic acid administration: A randomized, prospective study. *Arch Surg* (2000) 135:326–31. doi: 10.1001/archsurg.135.3.326
86. Albers R, Bol M, Bleumink R, Willems AA, Pieters RHH. Effects of supplementation with vitamins A, C, and E, selenium, and zinc on immune function in a murine sensitization model. *Nutrition* (2003) 19:940–6. doi: 10.1016/S0899-9007(03)00178-3
87. Hesta M, Ottermans C, Krammer-Lukas S, Zentek J, Hellweg P, Buyse J, et al. The effect of vitamin C supplementation in healthy dogs on antioxidative capacity and immune parameters. *J Anim Physiol Anim Nutr (Berl)* (2009) 93:26–34. doi: 10.1111/j.1439-0396.2007.00774.x
88. Kim JE, Cho HS, Yang HS, Jung DJ, Hong SW, Hung CF, et al. Depletion of ascorbic acid impairs NK cell activity against ovarian cancer in a mouse model. *Immunobiology* (2012) 217:873–81. doi: 10.1016/j.imbio.2011.12.010
89. Heuser G, Vojdani A. Enhancement of natural killer cell activity and T and B cell function by buffered vitamin C in patients exposed to toxic chemicals: The role of protein kinase - C. *Immunopharmacol Immunotoxicol* (1997) 19:291–312. doi: 10.3109/08923979709046977
90. Chatterjee IB, Gupta SD, Majumder AK, Nandi BK, Subramanian N. Effect of ascorbic acid on histamine metabolism in scorbutic guinea-pigs. *J Physiol* (1975) 25:271–9. doi: 10.1113/jphysiol.1975.sp011091
91. Nandi BK, Subramanian N, Majumder AK, Chatterjee IB. Effect of ascorbic acid on detoxification of histamine under stress conditions. *Biochem Pharmacol* (1974) 23:643–7. doi: 10.1016/0006-2952(74)90629-7
92. Johnston CS, Solomon RE, Corte C. Vitamin c depletion is associated with alterations in blood histamine and plasma free carnitine in adults. *J Am Coll Nutr* (1996) 15:586–91. doi: 10.1080/07315724.1996.10718634
93. Bonham MJD, Abu-Zidan FM, Simovic MO, Sluis KB, Wilkinson A, Winterbourn CC, et al. Early ascorbic acid depletion is related to the severity of acute pancreatitis. *Br J Surg* (1999) 86:1296–301. doi: 10.1046/j.1365-2168.1999.01182.x
94. Bakaev VV, Duntau AP. Ascorbic acid in blood serum of patients with pulmonary tuberculosis and pneumonia. *Int J Tuberc Lung Dis* (2004) 8:263–6.
95. Peng Y, Kwok KHH, Yang PH, Ng SSM, Liu J, Wong OG, et al. Ascorbic acid inhibits ROS production, NF-κB activation and prevents ethanol-induced growth retardation and microencephaly. *Neuropharmacology* (2005) 48:426–34. doi: 10.1016/j.neuropharm.2004.10.018
96. Maiuolo J, Maretta A, Gliozzi M, Musolino V, Carresi C, Bosco F, et al. Ethanol-induced cardiomyocyte toxicity implicit autophagy and NFkB transcription factor. *Pharmacol Res* (2018) 133:141–50. doi: 10.1016/j.phrs.2018.04.004
97. Moniruzzaman M, Ghosal I, Das D, Chakraborty SB. Melatonin ameliorates H2O2-induced oxidative stress through modulation of Erk/Akt/ NFkB pathway. *Biol Res* (2018) 51:17. doi: 10.1186/s40659-018-0168-5
98. Thoma A, Lightfoot AP. Nf-kb and inflammatory cytokine signalling: Role in skeletal muscle atrophy. In: J Xiao, editor. *Advances in Experimental Medicine and Biology*, vol. 1088. Singapore: Springer (2018). p. 267–79. doi: 10.1007/978-981-13-1435-3_12
99. Bowie AG, O'Neill LAJ. Vitamin C Inhibits NF-κB Activation by TNF Via the Activation of p38 Mitogen-Activated Protein Kinase. *J Immunol* (2000) 165:7180–8. doi: 10.4049/jimmunol.165.12.7180
100. Cárcamo JM, Pedraza A, Bórquez-Ojeda O, Golde DW. Vitamin C suppresses TNFα-induced NFκB activation by inhibiting IκBα phosphorylation. *Biochemistry* (2002) 41:12995–3002. doi: 10.1021/bi0263210
101. Cárcamo JM, Pedraza A, Bórquez-Ojeda O, Zhang B, Sanchez R, Golde DW. Vitamin C Is a Kinase Inhibitor: Dehydroascorbic Acid Inhibits IκBα Kinase β. *Mol Cell Biol* (2004) 24:6645–52. doi: 10.1128/mcb.24.15.6645-6652.2004
102. Li R, Guo C, Li Y, Liang X, Yang L, Huang W. Therapeutic target and molecular mechanism of vitamin C-treated pneumonia: a systematic study of network pharmacology. *Food Funct* (2020) 11:4765–72. doi: 10.1039/d0fo00421a

103. Li R, Guo C, Li Y, Qin Z, Huang W. Therapeutic targets and signaling mechanisms of vitamin C activity against sepsis: a bioinformatics study. *Brief Bioinform* (2020) bbaa079. doi: 10.1093/bib/bbaa079
104. Kuiper C, Vissers MCM. Ascorbate as a cofactor for Fe-and 2-oxoglutarate dependent dioxygenases: Physiological activity in tumour growth and progression. *Front Oncol* (2014) 4:359. doi: 10.3389/fonc.2014.00359
105. Young JI, Züchner S, Wang G. Regulation of the Epigenome by Vitamin C. *Annu Rev Nutr* (2015) 35:545–64. doi: 10.1146/annurev-nutr-071714-034228
106. Lio CWJ, Rao A. TET enzymes and 5hMC in adaptive and innate immune systems. *Front Immunol* (2019) 10:210. doi: 10.3389/fimmu.2019.00210
107. Lee Chong T, Ahearn EL, Cimmino L. Reprogramming the Epigenome With Vitamin C. *Front Cell Dev Biol* (2019) 7:128. doi: 10.3389/fcell.2019.00128
108. Blaschke K, Ebata KT, Karimi MM, Zepeda-Martínez JA, Goyal P, Mahapatra S, et al. Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. *Nature* (2013) 500:222–6. doi: 10.1038/nature12362
109. Minor EA, Court BL, Young JI, Wang G. Ascorbate induces ten-eleven translocation (Tet) methylcytosine dioxygenase-mediated generation of 5-hydroxymethylcytosine. *J Biol Chem* (2013) 288:13669–74. doi: 10.1074/jbc.C113.464800
110. Ang A, Pullar JM, Currie MJ, Vissers MCM, Vitamin C. and immune cell function in inflammation and cancer. *Biochem Soc Trans* (2018) 46:1147–59. doi: 10.1042/BST20180169
111. Eccles R. Understanding the symptoms of the common cold and influenza. *Lancet Infect Dis* (2005) 5:718–25. doi: 10.1016/S1473-3099(05)70270-X
112. Arroll B. Common cold. *BMJ Clin Evid* (2011) 2011:1510. doi: 10.5124/jkma.1998.41.11.1188
113. Heikkinen T, Järvinen A. The common cold. *Lancet* (2003) 361:51–9. doi: 10.1016/S0140-6736(03)12162-9
114. Dicpinigaitis PV, Eccles R, Blaiss MS, Wingertzahn MA. Impact of cough and common cold on productivity, absenteeism, and daily life in the United States: ACHOO Survey. *Curr Med Res Opin* (2015) 31:1519–25. doi: 10.1185/03007995.2015.1062355
115. Pauling L. Evolution and the need for ascorbic acid. *Proc Natl Acad Sci U S A* (1970) 67:1643–8. doi: 10.1073/pnas.67.4.1643
116. Pauling L. The significance of the evidence about ascorbic acid and the common cold. *Proc Natl Acad Sci U S A* (1971) 68:2678–81. doi: 10.1073/pnas.68.11.2678
117. Duerbeck NB, Dowling DD, Duerbeck JM. Vitamin C: Promises not kept. *Obstet Gynecol Surv* (2016) 71:187–93. doi: 10.1097/OGX.0000000000000289
118. Anderson TW, Reid DB, Beaton GH. Vitamin C and the common cold: a double-blind trial. *Can Med Assoc J* (1972) 107:503–8.
119. Karlowski TR, Chalmers TC, Frenkel LD, Kapikian AZ, Lewis TL, Lynch JM. Ascorbic Acid for the Common Cold: A Prophylactic and Therapeutic Trial. *JAMA J Am Med Assoc* (1975) 231:1038–42. doi: 10.1001/jama.1975.03240220018013
120. Chalmers TC. Effects of ascorbic acid on the common cold. An evaluation of the evidence. *Am J Med* (1975) 58:532–6. doi: 10.1016/0002-9343(75)90127-8
121. Dykes MHM, Meier P. Ascorbic Acid and the Common Cold: Evaluation of Its Efficacy and Toxicity. *JAMA J Am Med Assoc* (1975) 231:1073–9. doi: 10.1001/jama.1975.03240220051025
122. Bourne GH. Vitamin C and Immunity. *Br J Nutr* (1949) 2:341–7. doi: 10.1079/bjn19480063
123. Stone I. Hypoascorbemia, the genetic disease causing the human requirement for exogenous ascorbic acid. *Perspect Biol Med* (1966) 10:133–4. doi: 10.1353/pbm.1966.0037
124. Elwood PC, Hughes SJ, Leger St AS. A randomized controlled trial of the therapeutic effect of vitamin C in the common cold. *Practitioner* (1977) 218:133–7.
125. Gómez E, Quidel S, Bravo Soto G, Ortigoza Á.. Does vitamin C prevent the common cold? *Medwave* (2018) 18:e7235. doi: 10.5867/medwave.2018.04.7236
126. Vorilhon P, Arpajou B, Vaillant Roussel H, Merlin É, Pereira B, Cabaillot A. Efficacy of vitamin C for the prevention and treatment of upper respiratory tract infection. A meta-analysis in children. *Eur J Clin Pharmacol* (2019) 75:303–11. doi: 10.1007/s00228-018-2601-7
127. Rondanelli M, Miccono A, Lamburghini S, Avanzato I, Riva A, Allegrini P, et al. Self-Care for Common Colds: The Pivotal Role of Vitamin D, Vitamin C, Zinc, and Echinacea in Three Main Immune Interactive Clusters (Physical Barriers, Innate and Adaptive Immunity) Involved during an Episode of Common Colds - Practical Advice on Dosages. *Evidence-Based Complement Altern Med* (2018) 2018:5813095. doi: 10.1155/2018/5813095
128. Ran L, Zhao W, Wang J, Wang H, Zhao Y, Tseng Y, et al. Extra Dose of Vitamin C Based on a Daily Supplementation Shortens the Common Cold: A Meta-Analysis of 9 Randomized Controlled Trials. *BioMed Res Int* (2018) 2018:1837634. doi: 10.1155/2018/1837634
129. Hemilä H. Vitamin C Supplementation and Respiratory Infections: a Systematic Review. *Mil Med* (2004) 169:920–5. doi: 10.7205/milmed.169.11.920
130. Pazzaglia G, Pasternack M. Recent Trends of Pneumonia Morbidity in US Naval Personnel. *Mil Med* (1983) 148:647–51. doi: 10.1093/milmed/148.8.647
131. Kleemola M, Saikku P, Visakorpi R, Wang SP, Grayston JT, Kleemola M. Epidemics of pneumonia caused by twar, a new chlamydia organism, in military trainees in finland. *J Infect Dis* (1988) 157:230–6. doi: 10.1093/infdis/157.2.230
132. Scheffer D da L, Latini A. Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs. *Biochim Biophys Acta - Mol Basis Dis* (2020) 1866:165823. doi: 10.1016/j.bbadis.2020.165823
133. Simpson RJ, Campbell JP, Gleeson M, Krüger K, Nieman DC, Pyne DB, et al. Can exercise affect immune function to increase susceptibility to infection? *Exerc Immunol Rev* (2020) 26:8–22.
134. Hull JH, Loosemore M, Schwellnus M. Respiratory health in athletes: facing the COVID-19 challenge. *Lancet Respir Med* (2020) 8:557–8. doi: 10.1016/S2213-2600(20)30175-2
135. Schwellnus MP, Derman WE, Jordaan E, Page T, Lambert MI, Readhead C, et al. Elite athletes travelling to international destinations <5 time zone differences from their home country have a 2–3-fold increased risk of illness. *Br J Sports Med* (2012) 46:816–21. doi: 10.1136/bjsports-2012-091395
136. Sassano M, McKee M, Ricciardi W, Boccia S. Transmission of SARS-CoV-2 and Other Infections at Large Sports Gatherings: A Surprising Gap in Our Knowledge. *Front Med* (2020) 7:277. doi: 10.3389/fmed.2020.00277
137. Kim TK, Lim HR, Byun JS. Vitamin C supplementation reduces the odds of developing a common cold in Republic of Korea Army recruits: randomised controlled trial. *BMJ Mil Heal* (2020). doi: 10.1136/bmjmilitary-2019-001384. bmjmilitary-2019-001384.
138. Nieman DC. Exercise immunology: Nutritional countermeasures. *Can J Appl Physiol* (2001) 26 Suppl:S45–55. doi: 10.1139/h2001-041
139. Powers SK, Jackson MJ. Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiol Rev* (2008) 88:1243–76. doi: 10.1152/physrev.00031.2007
140. Walsh NP. Nutrition and Athlete Immune Health: New Perspectives on an Old Paradigm. *Sport Med* (2019) 49:153–68. doi: 10.1007/s40279-019-01160-3
141. Peternelj TT, Coombes JS. Antioxidant supplementation during exercise training: Beneficial or detrimental? *Sport Med* (2011) 41:1043–69. doi: 10.2165/11594400-000000000-00000
142. Cobley JN, McHardy H, Morton JP, Nikolaidis MG, Close GL. Influence of vitamin C and vitamin E on redox signaling: Implications for exercise adaptations. *Free Radic Biol Med* (2015) 84:65–76. doi: 10.1016/j.freeradbiomed.2015.03.018 0
143. Margaritelis NV, Cobley JN, Paschalis V, Veskoukis AS, Theodorou AA, Kyparos A, et al. Principles for integrating reactive species into in vivo biological processes: Examples from exercise physiology. *Cell Signal* (2016) 28:256–71. doi: 10.1016/j.cellsig.2015.12.011
144. Merry TL, Ristow M. Do antioxidant supplements interfere with skeletal muscle adaptation to exercise training? *J Physiol* (2016) 594:5135–47. doi: 10.1113/JP270654
145. Ismaeel A, Holmes M, Papoutsi E, Panton L, Koutakis P. Resistance training, antioxidant status, and antioxidant supplementation. *Int J Sport Nutr Exerc Metab* (2019) 29:539–47. doi: 10.1123/ijsnem.2018-0339

146. Righi NC, Schuch FB, De Nardi AT, Pippi CM, de Almeida Righi G, Puntel GO, et al. Effects of vitamin C on oxidative stress, inflammation, muscle soreness, and strength following acute exercise: meta-analyses of randomized clinical trials. *Eur J Nutr* (2020) 59:2827–39. doi: 10.1007/s00394-020-02215-2
147. Rothschild JA, Bishop DJ. Effects of Dietary Supplements on Adaptations to Endurance Training. *Sport Med* (2020) 50:25–53. doi: 10.1007/s40279-019-01185-8
148. Pastor R, Tur JA. Antioxidant Supplementation and Adaptive Response to Training: A Systematic Review. *Curr Pharm Des* (2019) 25:1889–912. doi: 10.2174/1381612825666190701164923
149. Mason SA, Trewin AJ, Parker L, Wadley GD. Antioxidant supplements and endurance exercise: Current evidence and mechanistic insights. *Redox Biol* (2020) 35:101471. doi: 10.1016/j.redox.2020.101471
150. Kerksick CM, Wilborn CD, Roberts MD, Smith-Ryan A, Kleiner SM, Jäger R, et al. ISSN exercise & sports nutrition review update: Research & recommendations. *J Int Soc Sports Nutr* (2018) 15:38. doi: 10.1186/s12970-018-0242-y
151. Paschalis V, Theodorou AA, Kyparos A, Dipla K, Zafeiridis A, Panayiotou G, et al. Low vitamin C values are linked with decreased physical performance and increased oxidative stress: reversal by vitamin C supplementation. *Eur J Nutr* (2016) 55:45–53. doi: 10.1007/s00394-014-0821-x
152. Margaritelis NV, Paschalis V, Theodorou AA, Kyparos A, Nikolaidis MG. Antioxidants in personalized nutrition and exercise. *Adv Nutr* (2018) 9:813–23. doi: 10.1093/ADVANCES/NMY052
153. Mandell LA. Epidemiology and etiology of community-acquired pneumonia. *Infect Dis Clin North Am* (2004) 18:761–76. doi: 10.1016/j.idc.2004.08.003
154. Ellison RT, Donowitz GR. *Acute Pneumonia. Mandell, Douglas, and Bennett"s Principles and Practice of Infectious Diseases*. (2014). pp. 823–46.e5. doi: 10.1016/B978-1-4557-4801-3.00069-2.
155. Troeger C, Blacker B, Khalil IA, Rao PC, Cao J, Zimsen SRM, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis* (2018) 18:1191–210. doi: 10.1016/S1473-3099(18)30310-4
156. Naghavi M, Wang H, Lozano R, Davis A, Liang X, Zhou M, et al. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet* (2015) 385:117–71. doi: 10.1016/S0140-6736(14)61682-2
157. Erol N, Saglam L, Saglam YS, Erol HS, Altun S, Aktas MS, et al. The Protection Potential of Antioxidant Vitamins Against Acute Respiratory Distress Syndrome: a Rat Trial. *Inflammation* (2019) 42:1585–94. doi: 10.1007/s10753-019-01020-2
158. Cai Y, Li YF, Tang LP, Tsoi B, Chen M, Chen H, et al. A new mechanism of vitamin C effects on A/FM/1/47(H1N1) virus-induced pneumonia in restraint-stressed mice. *BioMed Res Int* (2015) 2015:675149. doi: 10.1155/2015/675149
159. Kim WY, Hong SB. Sepsis and acute respiratory distress syndrome: Recent update. *Tuberc Respir Dis (Seoul)* (2016) 79:53–7. doi: 10.4046/trd.2016.79.2.53
160. Hemilä H, Louhiala P. Vitamin C for preventing and treating pneumonia. *Cochrane Database Syst Rev* (2013) 8:CD005532. doi: 10.1002/14651858.CD005532.pub3
161. Padhani Z, Moazzam Z, Ashraf A, Bilal H, Salam R, Das J, et al. Vitamin C supplementation for prevention and treatment of pneumonia. *Cochrane Database Syst Rev* (2020) 4:CD013134. doi: 10.1002/14651858.CD013134.pub2
162. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020) 395:507–13. doi: 10.1016/S0140-6736(20)30211-7
163. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* (2020) 382:727–33. doi: 10.1056/NEJMoa2001017
164. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* (2020) 8:420–2. doi: 10.1016/S2213-2600(20)30076-X
165. Pascarella G, Strumia A, Piliego C, Bruno F, Del Buono R, Costa F, et al. COVID-19 diagnosis and management: a comprehensive review. *J Intern Med* (2020) 288:192–206. doi: 10.1111/joim.13091
166. Iddir M, Brito A, Dingeo G, Fernandez Del Campo S, Samouda H, La Frano M, et al. Strengthening the Immune System and Reducing Inflammation and Oxidative Stress through Diet and Nutrition: Considerations during the COVID-19 Crisis. *Nutrients* (2020) 12:1562. doi: 10.3390/nu12061562
167. Adams KK, Baker WL, Sobieraj DM. Myth Busters: Dietary Supplements and COVID-19. *Ann Pharmacother* (2020) 54:820–6. doi: 10.1177/1060028020928052
168. Bauer SR, Kapoor A, Rath M, Thomas SA. What is the role of supplementation with ascorbic acid, zinc, vitamin D, or N -acetylcysteine for prevention or treatment of COVID-19? *Cleve Clin J Med* (2020). doi: 10.3949/ccjm.87a.ccc046
169. BourBour F, Mirzaei Dahka S, Gholamalizadeh M, Akbari ME, Shadnoush M, Haghighi M, et al. Nutrients in prevention, treatment, and management of viral infections; special focus on Coronavirus. *Arch Physiol Biochem* (2020) 1–10. doi: 10.1080/13813455.2020.1791188
170. Toresdahl BG, Asif IM. Coronavirus Disease 2019 (COVID-19): Considerations for the Competitive Athlete. *Sport Heal A Multidiscip Approach* (2020) 12:221–4. doi: 10.1177/1941738120918876
171. Zabetakis I, Lordan R, Norton C, Tsoupras A. COVID-19: The Inflammation Link and the Role of Nutrition in Potential Mitigation. *Nutrients* (2020) 12:1466. doi: 10.3390/nu12051466
172. Costa FF, Rosário WR, Ribeiro Farias AC, de Souza RG, Duarte Gondim RS, Barroso WA. Metabolic syndrome and COVID-19: An update on the associated comorbidities and proposed therapies. *Diabetes Metab Syndr Clin Res Rev* (2020) 14:809–14. doi: 10.1016/j.dsx.2020.06.016
173. World Health Organization. *Information note on COVID-19 and NCDs. (2020)Information note on COVID-19 and NCDs* . Available at: https://www.who.int/publications/m/item/covid-19-and-ncds (Accessed July 4, 2020).
174. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities and its effects in coronavirus disease 2019 patients: A systematic review and meta-analysis. *Int J Infect Dis* (2020) 94:91–5. doi: 10.1016/j.ijid.2020.03.017
175. Cai Q, Chen F, Wang T, Luo F, Liu X, Wu Q, et al. Obesity and COVID-19 Severity in a Designated Hospital in Shenzhen, China. *Diabetes Care* (2020) 43:1392–8. doi: 10.2337/dc20-0576
176. Lighter J, Phillips M, Hochman S, Sterling S, Johnson D, Francois F, et al. Obesity in patients younger than 60 years is a risk factor for Covid-19 hospital admission. *Clin Infect Dis* (2020) 71:896–7. doi: 10.1093/cid/ciaa415
177. Bhasin A, Nam H, Yeh C, Lee J, Liebovitz D, Achenbach C. Is BMI higher in younger patients with COVID-19? Association between BMI and COVID-19 hospitalization by age. *Obesity* (2020) 28:1811–4. doi: 10.1002/oby.22947
178. Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell L, Chernyak Y, et al. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: Prospective cohort study. *BMJ* (2020) 369:m1966. doi: 10.1136/bmj.m1966
179. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA - J Am Med Assoc* (2014) (219):1–8. doi: 10.1001/jama.2014.732
180. Mah E, Matos MD, Kawiecki D, Ballard K, Guo Y, Volek JS, et al. Vitamin C Status Is Related to Proinflammatory Responses and Impaired Vascular Endothelial Function in Healthy, College-Aged Lean and Obese Men. *J Am Diet Assoc* (2011) 111:737–43. doi: 10.1016/j.jada.2011.02.003
181. Block G, Jensen CD, Dalvi TB, Norkus EP, Hudes M, Crawford PB, et al. Vitamin C treatment reduces elevated C-reactive protein. *Free Radic Biol Med* (2009) 46:70–7. doi: 10.1016/j.freeradbiomed.2008.09.030
182. Rychter AM, Ratajczak AE, Zawada A, Dobrowolska A, Krela-Kaźmierczak I. Non-systematic review of diet and nutritional risk factors of cardiovascular disease in obesity. *Nutrients* (2020) 12:814. doi: 10.3390/nu12030814
183. Rychter AM, Zawada A, Ratajczak AE, Dobrowolska A, Krela-Kaźmierczak I. Should patients with obesity be more afraid of COVID-19? *Obes Rev* (2020) 21:e13083. doi: 10.1111/obr.13083
184. Kumar A, Arora A, Sharma P, Anikhindi SA, Bansal N, Singla V, et al. Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis. *Diabetes Metab Syndr Clin Res Rev* (2020) 14:535–45. doi: 10.1016/j.dsx.2020.04.044

185. International Diabetes Federation. About Diabetes. (2020).. Available at: https://idf.org/aboutdiabetes/type-2-diabetes.html (Accessed July 6, 2020).
186. McArdle MA, Finucane OM, Connaughton RM, McMorrow AM, Roche HM. Mechanisms of obesity-induced inflammation and insulin resistance: Insights into the emerging role of nutritional strategies. *Front Endocrinol (Lausanne)* (2013) 4:52. doi: 10.3389/fendo.2013.00052
187. Will JC, Byers T. Does Diabetes Mellitus Increase the Requirement for Vitamin C? *Nutr Rev* (2009) 54:193–202. doi: 10.1111/j.1753-4887.1996.tb03932.x
188. Sargeant LA, Wareham NJ, Bingham S, Day NE, Luben RN, Oakes S, et al. Vitamin C and hyperglycemia in the European Prospective Investigation Into Cancer - Norfolk (EPIC-Norfolk) study. *Diabetes Care* (2000) 23:726–32. doi: 10.2337/diacare.23.6.726
189. Sinclair AJ, Taylor PB, Lunec J, Girling AJ, Barnett AH. Low Plasma Ascorbate Levels in Patients with Type 2 Diabetes Mellitus Consuming Adequate Dietary Vitamin C. *Diabetes Med* (1994) 11:893–8. doi: 10.1111/j.1464-5491.1994.tb00375.x
190. Wilson R, Willis J, Gearry R, Skidmore P, Fleming E, Frampton C, et al. Inadequate vitamin C status in prediabetes and type 2 diabetes mellitus: Associations with glycaemic control, obesity, and smoking. *Nutrients* (2017) 9:997. doi: 10.3390/nu9090997
191. Seghieri G, Martinoli L, Miceli M, Ciuti M, D'Alessandri G, Gironi A, et al. Renal excretion of ascorbic acid in insulin dependent diabetes mellitus. *Int J Vitam Nutr Res* (1994) 64:119–24.
192. Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. *J Physiol Pharmacol* (2019) 70(6). doi: 10.26402/jpp.2019.6.01
193. Mason SA, Della Gatta PA, Snow RJ, Russell AP, Wadley GD. Ascorbic acid supplementation improves skeletal muscle oxidative stress and insulin sensitivity in people with type 2 diabetes: Findings of a randomized controlled study. *Free Radic Biol Med* (2016) 93:227–38. doi: 10.1016/j.freeradbiomed.2016.01.006
194. Ellulu MS, Rahmat A, Ismail P, Khaza'ai H, Abed Y. Effect of vitamin C on inflammation and metabolic markers in hypertensive and/or diabetic obese adults: a randomized controlled trial. *Drug Des Devel Ther* (2015) 9:3405–12. doi: 10.2147/DDDT.S83144
195. Ashor AW, Werner AD, Lara J, Willis ND, Mathers JC, Siervo M. Effects of Vitamin C supplementation on glycaemic control: A systematic review and meta-analysis of randomised controlled trials. *Eur J Clin Nutr* (2017) 71:1371–80. doi: 10.1038/ejcn.2017.24
196. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med* (2020) 8:e21. doi: 10.1016/S2213-2600(20)30116-8
197. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
198. Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J, et al. Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. *J Infect* (2020) 81:e16–25. doi: 10.1016/j.jinf.2020.04.021
199. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong J-C, Turner AJ, et al. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System. *Circ Res* (2020) 126:1456–74. doi: 10.1161/circresaha.120.317015
200. Chen L, Hao G. The role of angiotensin-converting enzyme 2 in coronaviruses/influenza viruses and cardiovascular disease. *Cardiovasc Res* (2020) 116:1932–6. doi: 10.1093/cvr/cvaa093
201. Touyz RM, Li H, Delles C. ACE2 the Janus-faced protein – from cardiovascular protection to severe acute respiratory syndrome-coronavirus and COVID-19. *Clin Sci* (2020) 134:747–50. doi: 10.1042/CS20200363
202. Tan HW, Xu Y, Lau ATY. Angiotensin-converting enzyme 2: The old door for new severe acute respiratory syndrome coronavirus 2 infection. *Rev Med Virol* (2020) 30:e2122. doi: 10.1002/rmv.2122
203. Khaw KT, Bingham S, Welch A, Luben R, Wareham N, Oakes S, et al. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: A prospective population study. *Lancet* (2001) 357:657–63. doi: 10.1016/S0140-6736(00)04128-3
204. Pfister R, Sharp SJ, Luben R, Wareham NJ, Khaw KT. Plasma vitamin C predicts incident heart failure in men and women in European Prospective Investigation into Cancer and Nutrition-Norfolk prospective study. *Am Heart J* (2011) 162:246–53. doi: 10.1016/j.ahj.2011.05.007
205. Ingles DP, Cruz Rodriguez JB, Garcia H. Supplemental Vitamins and Minerals for Cardiovascular Disease Prevention and Treatment. *Curr Cardiol Rep* (2020) 22:22. doi: 10.1007/s11886-020-1270-1
206. Moser MA, Chun OK, Vitamin C. and heart health: A review based on findings from epidemiologic studies. *Int J Mol Sci* (2016) 17:1328. doi: 10.3390/ijms17081328
207. Lykkesfeldt J. On the effect of vitamin C intake on human health: How to (mis)interprete the clinical evidence. *Redox Biol* (2020) 34:101532. doi: 10.1016/j.redox.2020.101532
208. Chow N, Fleming-Dutra K, Gierke R, Hall A, Hughes M, Pilishvili T, et al. Preliminary Estimates of the Prevalence of Selected Underlying Health Conditions Among Patients with Coronavirus Disease 2019 — United States, February 12–March 28, 2020. *MMWR Morb Mortal Wkly Rep* (2020) 69:382–6. doi: 10.15585/mmwr.mm6913e2
209. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated with Acute Respiratory Distress Syndrome and Death in Patients with Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* (2020) 180:934–43. doi: 10.1001/jamainternmed.2020.0994
210. Pae M, Meydani SN, Wu D. The role of nutrition in enhancing immunity in aging. *Aging Dis* (2012) 3:91–129.
211. Meyer KC. The role of immunity and inflammation in lung senescence and susceptibility to infection in the elderly. *Semin Respir Crit Care Med* (2010) 31:561–74. doi: 10.1055/s-0030-1265897
212. Volkert D, Visser M, Corish CA, Geisler C, de Groot L, Cruz-Jentoft AJ, et al. Joint action malnutrition in the elderly (MaNuEL) knowledge hub: summary of project findings. *Eur Geriatr Med* (2020) 11:169–77. doi: 10.1007/s41999-019-00264-3
213. Power SE, Jeffery IB, Ross RP, Stanton C, O'Toole PW, O'Connor EM, et al. Food and nutrient intake of irish community-dwelling elderly subjects: Who is at nutritional risk? *J Nutr Heal Aging* (2014) 18:561–72. doi: 10.1007/s12603-014-0449-9
214. Haase H, Rink L. The immune system and the impact of zinc during aging. *Immun Ageing* (2009) 6:9. doi: 10.1186/1742-4933-6-9
215. Hunt C, Chakravorty NK, Annan G, Habibzadeh N, Schorah CJ. The clinical effects of vitamin C supplementation in elderly hospitalised patients with acute respiratory infections. *Int J Vitam Nutr Res* (1994) 64:212–9.
216. Fletcher AE, Breeze E, Shetty PS. Antioxidant vitamins and mortality in older persons: Findings from the nutrition add-on study to the Medical Research Council Trial of Assessment and Management of Older People in the Community. *Am J Clin Nutr* (2003) 78:999–1010. doi: 10.1093/ajcn/78.5.999
217. Yanase F, Fujii T, Naorungroj T, Belletti A, Luethi N, Carr AC, et al. Harm of IV High-Dose Vitamin C Therapy in Adult Patients. *Crit Care Med* (2020) 48:e620–8. doi: 10.1097/CCM.0000000000004396
218. Traxer O, Huet B, Poindexter J, Pak CYC, Pearle MS. Effect of ascorbic acid consumption on urinary stone risk factors. *J Urol* (2003) 170:397–401. doi: 10.1097/01.ju.0000076001.21606.53
219. Massey LK, Liebman M, Kynast-Gales SA. Ascorbate Increases Human Oxaluria and Kidney Stone Risk. *J Nutr* (2005) 135:1673–7. doi: 10.1093/jn/135.7.1673
220. Ferraro PM, Curhan GC, Gambaro G, Taylor EN. Total, dietary, and supplemental Vitamin C intake and risk of incident kidney stones. *Am J Kidney Dis* (2016) 67:400–7. doi: 10.1053/j.ajkd.2015.09.005
221. Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, Katz A, et al. Vitamin C Pharmacokinetics: Implications for Oral and Intravenous Use. *Ann Intern Med* (2004) 140:533–7. doi: 10.7326/0003-4819-140-7-200404060-00010
222. Galley HF, Davies MJ, Webster NR. Ascorbyl radical formation in patients with sepsis: Effect of ascorbate loading. *Free Radic Biol Med* (1996) 20:139–43. doi: 10.1016/0891-5849(95)02022-5
223. Schorah CJ, Downing C, Piripitsi A, Gallivan L, Al-Hazaa AH, Sanderson MJ, et al. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. *Am J Clin Nutr* (1996) 63:760–5. doi: 10.1093/ajcn/63.5.760

224. Metnitz PGH, Bartens C, Fischer M, Fridrich P, Steltzer H, Druml W. Antioxidant status in patients with acute respiratory distress syndrome. *Intensive Care Med* (1999) 25:180–5. doi: 10.1007/s001340050813
225. Kieffer P, Thannberger P, Wilhelm JM, Kieffer C, Schneider F. Multiple organ dysfunction dramatically improving with the infusion of vitamin C: More support for the persistence of scurvy in our "welfare" society. *Intensive Care Med* (2001) 27:448. doi: 10.1007/s001340000830
226. Long CL, Maull KI, Krishnan RS, Laws HL, Geiger JW, Borghesi L, et al. Ascorbic acid dynamics in the seriously ill and injured. *J Surg Res* (2003) 109:144–8. doi: 10.1016/S0022-4804(02)00083-5
227. Fowler AA, Syed AA, Knowlson S, Sculthorpe R, Farthing D, DeWilde C, et al. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J Transl Med* (2014) 12:32. doi: 10.1186/1479-5876-12-32
228. Carr AC, Spencer E, Dixon L, Chambers ST. Patients with community acquired pneumonia exhibit depleted vitamin c status and elevated oxidative stress. *Nutrients* (2020) 12:1318. doi: 10.3390/nu12051318
229. Oudemans-van Straaten HM, Spoelstra-de Man AME, de Waard MC. Vitamin C revisited. *Crit Care* (2014) 18:460. doi: 10.1186/s13054-014-0460-x
230. Seno T, Inoue N, Matsui K, Ejiri J, Hirata KI, Kawashima S, et al. Functional expression of sodium-dependent vitamin C transporter 2 in human endothelial cells. *J Vasc Res* (2004) 41:345–51. doi: 10.1159/000080525
231. Schmidt R, Luboeinski T, Markart P, Ruppert C, Daum C, Grimminger F, et al. Alveolar antioxidant status in patients with acute respiratory distress syndrome. *Eur Respir J* (2004) 24:994–9. doi: 10.1183/09031936.04.00120703
232. Banerjee D, Kaul D. Combined inhalational and oral supplementation of ascorbic acid may prevent influenza pandemic emergency: A hypothesis. *Nutrition* (2010) 26:128–32. doi: 10.1016/j.nut.2009.09.015
233. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. *J Exp Med* (2020) 217:e20200652. doi: 10.1084/jem.20200652
234. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmun Rev* (2020) 19:102537. doi: 10.1016/j.autrev.2020.102537
235. Pierrakos C, Karanikolas M, Scolletta S, Karamouzos V, Velissaris D. Acute respiratory distress syndrome: pathophysiology and therapeutic options. *J Clin Med Res* (2012) 4:7–16. doi: 10.4021/jocmr761w
236. Chen Y, Luo G, Yuan J, Wang Y, Yang X, Wang X, et al. Vitamin C Mitigates Oxidative Stress and Tumor Necrosis Factor-Alpha in Severe Community-Acquired Pneumonia and LPS-Induced Macrophages. *Mediators Inflammation* (2014) 2014:426740. doi: 10.1155/2014/426740
237. Härtel C, Strunk T, Bucsky P, Schultz C. Effects of vitamin C on intracytoplasmic cytokine production in human whole blood monocytes and lymphocytes. *Cytokine* (2004) 27:101–6. doi: 10.1016/j.cyto.2004.02.004
238. Fisher BJ, Seropian IM, Kraskauskas D, Thakkar JN, Voelkel NF, Fowler AA, et al. Ascorbic acid attenuates lipopolysaccharide-induced acute lung injury. *Crit Care Med* (2011) 39:1454–60. doi: 10.1097/CCM.0b013e3182120cb8
239. Fisher BJ, Kraskauskas D, Martin EJ, Farkas D, Wegelin JA, Brophy D, et al. Mechanisms of attenuation of abdominal sepsis induced acute lung injury by ascorbic acid. *Am J Physiol - Lung Cell Mol Physiol* (2012) 303:L20–32. doi: 10.1152/ajplung.00300.2011
240. Molina N, Morandi AC, Bolin AP, Otton R. Comparative effect of fucoxanthin and vitamin C on oxidative and functional parameters of human lymphocytes. *Int Immunopharmacol* (2014) 22:41–50. doi: 10.1016/j.intimp.2014.06.026
241. Zhang XY, Xu ZP, Wang W, Cao JB, Fu Q, Zhao WX, et al. Vitamin C alleviates LPS-induced cognitive impairment in mice by suppressing neuroinflammation and oxidative stress. *Int Immunopharmacol* (2018) 65:438–47. doi: 10.1016/j.intimp.2018.10.020
242. Fowler AA, Truwit JD, Hite RD, Morris PE, Dewilde C, Priday A, et al. Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients with Sepsis and Severe Acute Respiratory Failure: The CITRIS-ALI Randomized Clinical Trial. *JAMA* (2019) 322:1261–70. doi: 10.1001/jama.2019.11825
243. Marik PE, Payen D. CITRIS-ALI: How statistics were used to obfuscate the true findings. *Anaesth Crit Care Pain Med* (2019) 38:575–7. doi: 10.1016/j.accpm.2019.10.004
244. Zhang M, Jativa DF. Vitamin C supplementation in the critically ill: A systematic review and meta-analysis. *SAGE Open Med* (2018) 6:2050312118807615. doi: 10.1177/2050312118807615
245. Langlois PL, Manzanares W, Adhikari NKJ, Lamontagne F, Stoppe C, Hill A, et al. Vitamin C Administration to the Critically Ill: A Systematic Review and Meta-Analysis. *JPEN J Parenter Enteral Nutr* (2019) 43:335–46. doi: 10.1002/jpen.1471
246. Hemilä H, Chalker E. Vitamin C may reduce the duration of mechanical ventilation in critically ill patients: A meta-regression analysis. *J Intensive Care* (2020) 8:15. doi: 10.1186/s40560-020-0432-y
247. Zabet M, Mohammadi M, Ramezani M, Khalili H. Effect of high-dose Ascorbic acid on vasopressor's requirement in septic shock. *J Res Pharm Pract* (2016) 5:94–100. doi: 10.4103/2279-042x.179569
248. Wei X, Wang Z, Liao X, Guo W, Wen JY, Qin T, et al. Efficacy of vitamin C in patients with sepsis: An updated meta-analysis. *Eur J Pharmacol* (2020) 868:172889. doi: 10.1016/j.ejphar.2019.172889
249. Kim WY, Jo EJ, Eom JS, Mok J, Kim MH, Kim KU, et al. hydrocortisone, and thiamine therapy for patients with severe pneumonia who were admitted to the intensive care unit: Propensity score-based analysis of a before-after cohort study. *J Crit Care* (2018) 47:211–8. doi: 10.1016/j.jcrc.2018.07.004
250. Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study. *Chest* (2017) 151:1229–38. doi: 10.1016/j.chest.2016.11.036
251. Fujii T, Luethi N, Young PJ, Frei DR, Eastwood GM, French CJ, et al. Effect of Vitamin C, Hydrocortisone, and Thiamine vs Hydrocortisone Alone on Time Alive and Free of Vasopressor Support among Patients with Septic Shock: The VITAMINS Randomized Clinical Trial. *JAMA* (2020) 323:423–31. doi: 10.1001/jama.2019.22176
252. Carr AC. Is the VITAMINS RCT indicating potential redundancy between corticosteroids and vitamin C? *Crit Care* (2020) 24:129. doi: 10.1186/s13054-020-02853-2
253. Frommelt MA, Kory P, Long MT. Letter on Update to the Vitamin C, Thiamine, and Steroids in Sepsis (VICTAS) Protocol. *Trials* (2020) 21:350. doi: 10.1186/s13063-020-04289-z
254. Carr AC. Vitamin C in Pneumonia and Sepsis. In: Q Chen and MCM Vissers, editors. *Vitamin C: New Biochemical and Functional Insights*. Boca Raton, FL: CRC Press (2020). p. 115–35. doi: 10.1201/9780429442025-7
255. Romano S, Galante H, Figueira D, Mendes Z, Rodrigues AT. Time-trend analysis of medicine sales and shortages during COVID-19 outbreak: Data from community pharmacies. *Res Soc Adm Pharm* (2020) 1551-7411 (20):30611–2. doi: 10.1016/j.sapharm.2020.05.024

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Cerullo, Negro, Parimbelli, Pecoraro, Perna, Liguori, Rondanelli, Cena and D'Antona. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

MINI REVIEW
published: 30 October 2020
doi: 10.3389/fimmu.2020.570251

NLRP3 Inflammasome: The Stormy Link Between Obesity and COVID-19

Alberto López-Reyes[1,2], Carlos Martinez-Armenta[3], Rocio Espinosa-Velázquez[2], Paola Vázquez-Cárdenas[4], Marlid Cruz-Ramos[5], Berenice Palacios-Gonzalez[6], Luis Enrique Gomez-Quiroz[7] and Gabriela Angélica Martínez-Nava[8]*

[1] Laboratorio de Gerociencias, Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra, Secretaría de Salud, Ciudad de México, México, [2] Facultad de Ciencias de la Salud, Universidad Anáhuac, Ciudad de México, México, [3] Postgrado en Biología Experimental, Dirección de Ciencias Biológicas y de la Salud (DCBS), Universidad Autónoma Metropolitana Iztapalapa, Ciudad de México, México, [4] Centro de Innovación Médica Aplicada, Hospital General Dr. Manuel Gea González, Ciudad de México, México, [5] Cátedras de Consejo Nacional de Ciencia y Tecnología (CONACYT), Instituto Nacional de Cancerología, Ciudad de México, México, [6] Unidad de Vinculación Científica de la Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Instituto Nacional de Medicina Genómica, Ciudad de México, México, [7] Laboratorio de Fisiología Celular, Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana Iztapalapa, Ciudad de México, México, [8] Laboratorio de Líquido Sinovial, Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra, Ciudad de México, México

OPEN ACCESS

Edited by:
Christophe Matthys,
KU Leuven, Belgium

Reviewed by:
Christopher Lupfer,
Missouri State University,
United States
Teneema Kuriakose,
St. Jude Children's Research Hospital,
United States

***Correspondence:**
Alberto López-Reyes
allorey@yahoo.com

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: 07 June 2020
Accepted: 13 October 2020
Published: 30 October 2020

Citation:
López-Reyes A, Martinez-Armenta C, Espinosa-Velázquez R, Vázquez-Cárdenas P, Cruz-Ramos M, Palacios-Gonzalez B, Gomez-Quiroz LE and Martínez-Nava GA (2020) NLRP3 Inflammasome: The Stormy Link Between Obesity and COVID-19. Front. Immunol. 11:570251. doi: 10.3389/fimmu.2020.570251

Several countries around the world have faced an important obesity challenge for the past four decades as the result of an obesogenic environment. This disease has a multifactorial origin and it is associated with multiple comorbidities including type 2 diabetes, hypertension, osteoarthritis, metabolic syndrome, cancer, and dyslipidemia. With regard to dyslipidemia, hypertriglyceridemia is a well-known activator of the NLRP3 inflammasome, triggering adipokines and cytokines secretion which in addition induce a systemic inflammatory state that provides an adequate scenario for infections, particularly those mediated by viruses such as HIV, H1N1 influenza, and SARS-CoV-2. The SARS-CoV-2 infection causes the coronavirus disease 2019 (COVID-19) and it is responsible for the pandemic that we are currently living. COVID-19 causes an aggressive immune response known as cytokine release syndrome or cytokine storm that causes multiorgan failure and in most cases leads to death. In the present work, we aimed to review the molecular mechanisms by which obesity-associated systemic inflammation could cause a more severe clinical presentation of COVID-19. The SARS-CoV-2 infection could potentiate or accelerate the pre-existing systemic inflammatory state of individuals with obesity, *via* the NLRP3 inflammasome activation and the release of pro-inflammatory cytokines from cells trough Gasdermin-pores commonly found in cell death by pyroptosis.

Keywords: severe acute respiratory syndrome coronavirus 2, coronavirus disease 2019, pyroptosis, obesity, inflammasome

INTRODUCTION

Obesity has reached epidemic proportions globally; thus, the World Health Organization (WHO) identifies it as a serious public health problem, particularly in west countries (1). The global increase of obesity in the last 50 years has doubled, and it has been estimated that a third of the world population is obese or overweight (2). The WHO defines obesity as a complex entity in which there is an excessive

accumulation of fat that affects practically all body functions and compromises the individual's health (1). Furthermore, obesity is considered the fifth risk factor for mortality, as it is the main risk factor of diabetes, cardiovascular disease, hypertension, dyslipidemia, musculoskeletal disorders such as osteoarthritis, and other diseases (3–5). It is well known that obesity leads to a low-grade chronic inflammation promoted by the release of adipokines and cytokines (6). This dysfunctional state contributes to a systemic lipotoxicity affecting liver, muscle and pancreas, activating NOD-, LRR-, and the pyrin domain-containing protein 3 (NLRP3) inflammasome (7–10). Beyond cellular damage, organ dysfunction and metabolic compromise, the low-grade chronic inflammation could condition to viral diseases, such as those instigated by HIV (11, 12), H1N1 influenza virus (13–15), and SARS-CoV-2 (16).

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the coronavirus disease (COVID-19), which is responsible for more than 758,942 deaths worldwide (17). The WHO has listed COVID-19 as a global health crisis (18). Similar to obesity, this virus induces a systemic inflammation; however, the SARS-CoV-2 produces an uncontrolled increase of cytokine secretion causing multiple organ failure, followed by death (19–21). It should be noted that during the cytokine storm caused by SARS-CoV-2, the inflammasome could be involved in the maintenance of inflammation, as it happens in obesity (22–24). Given that obesity might be associated with the development of aggressive clinical symptoms in COVID-19, we aimed to suggest the possible role of NLRP3 inflammasome as a link between obesity and the increased risk for a severe COVID-19 outcome.

INFLAMMASOME AND PYROPTOSIS

The NLRP3 inflammasome is a multiprotein complex present in macrophages, dendritic cells and other non-immune cells. The activation of NLRP3 as the pivotal component of the innate immune system, plays a critical role in the host defense against bacteria, fungi and viruses among others; however, the NLRP3 is also associated with metabolic and inflammatory conditions such as gout, diabetes mellitus, insulin resistance, and obesity (25–28).

The inflammasome is coordinated by the NLRP3 sensor [Nucleotide-Binding Oligomerization Domain (NOD), Leucin-rich repeat (LRR), Pyrin domain (PYD), adaptor protein ASC, as well as the effector protein caspase 1] (29, 30). In most cases, the activation of NLRP3 is regulated by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that are recognized by the Toll-like receptors (29, 31). The canonical activation of NLRP3 requires two independent signals; an initial priming signal and a second one to be fully activated (**Figure 1**).

The priming signal begins when PAMPs and other inflammatory mediators such as interleukin 1-beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) bind to their respective receptors (Pattern Recognition Receptors, IL-1βR, TNF-αR). These receptors induce the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which promotes the transcription of NF-κB-dependent genes such as NLRP3, pro-IL-1β, and pro-interleukin-18 (IL-18) (31–33). NF-κB also modulates the gene expression of interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-12 (IL-12), TNF-α, and interferon-gamma inducible protein 10 (IP-10) (31, 34). These cytokines play a critical role in acute inflammation, as well as in promoting the synthesis of acute phase proteins that modify permeability and endothelial functions leading to the recruitment of other immune system cells. In particular, TNF-α and IL-6 regulate transcription and transduction of IL-1β (34).

The second activation signal of NLRP3 could be initiated by several extracellular stimulus including those mediated by crystals of cholesterol, uric acid, asbestos and β amyloid, which induce the release of ROS (reactive oxygen species), as well as lysosomal enzyme cathepsin B and Ca^{2+} caused by a destabilization and rupture of lysosome or endosome (35–37). The increase of cathepsin B and Ca^{2+} as well as aberrant ionic flux may trigger mitochondrial damage. Mitochondrial dysfunction occurs *via* an increase of mitochondrial ROS, oxidized mitochondrial DNA, cardiolipins and proteins that respond to viruses such as mitofusin-1 and -2 or mitochondrial antiviral signaling protein, promoting the oligomerization of NLRP3 (25, 38). This assembly allows caspase-1 to start the cleavage of pro-IL-1β and pro-IL-18 (**Figure 1**).

The final step of the NLRP3 inflammasome activation is the cleavage of Gasdermin D (GSDMD) by caspase-1, resulting in the release of GSDMD N-terminal fragments that are essential for pore formation on cell membranes (39). These pores lead to the release of cytosolic content causing unrestrained dissemination of inflammatory mediators including IL-1β, IL-18, that induce cell death by pyroptosis contributing to host immune defense (40, 41).

OBESITY, INFLAMMASOME, AND PYROPTOSIS

As a functional organ, adipose tissue (AT) is the main endocrine and immunological tissue implicated in the pathophysiology of obesity and metabolic dysfunction (42, 43). AT has a complex and heterogeneous composition that includes endothelium, extracellular proteins matrix, stem cells, fibroblasts and immune cells, and adipocytes (43–46). There are three types of AT. White AT is the organ responsible for storing energy mainly in the form of triglycerides for energy demand periods. In contrast, brown AT is composed of rich mitochondria and multivacuolar smaller adipocytes that are positive for the expression of uncoupling protein-1, which is responsible for thermogenesis and generation of heat rather than ATP from the oxidation of fatty acids (47). Finally, the most recently AT identified, beige adipocyte tissue, resembles BAT morphology and function, it can differentiate from precursors found in WAT in response to stimuli as cold exposure (48–50).

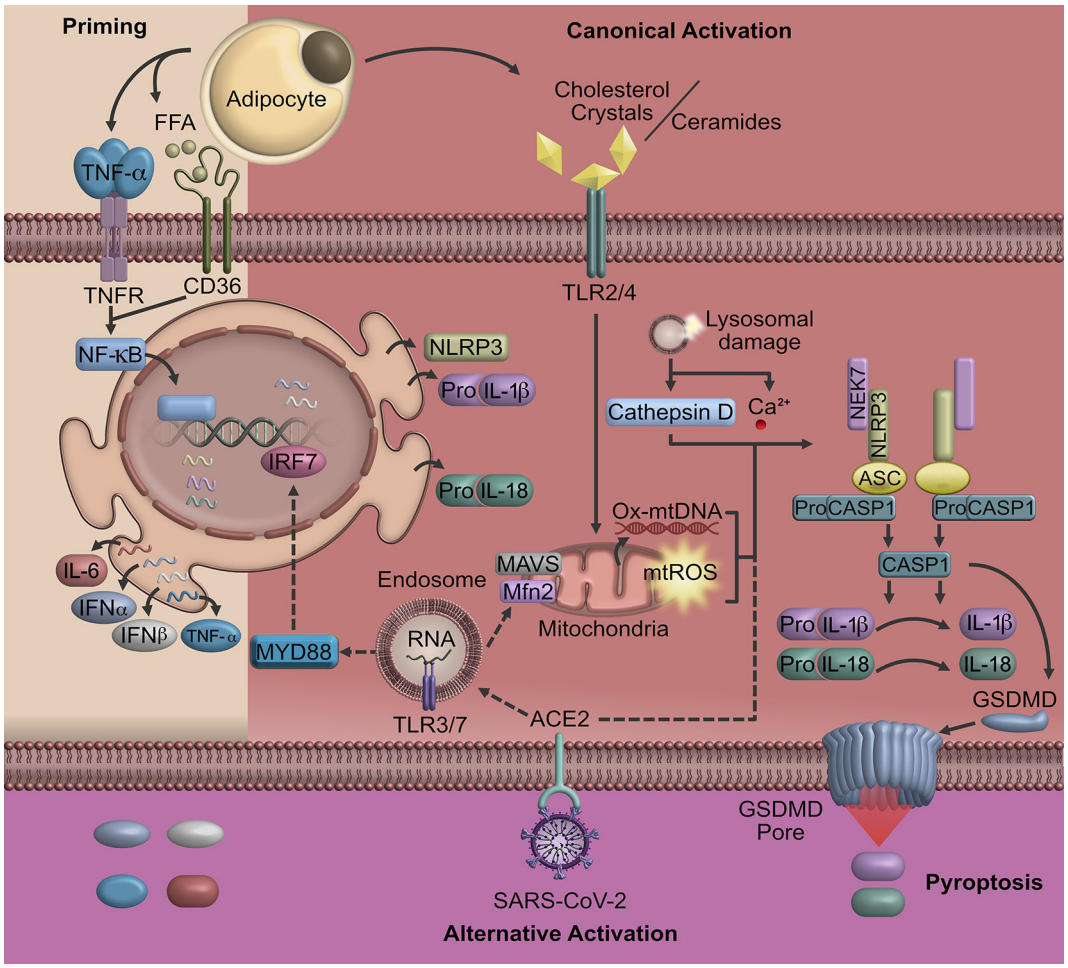


FIGURE 1 | Pyroptosis triggered in obesity and COVID-19. A possible signaling that triggers the activation of NLRP3 and consequently cell pyroptosis in COVID-19 may be linked to obesity. In individuals with obesity, pyroptosis is characterized by the activation of NOD-like receptors that induce the formation of cell membrane pores mediated by Gasdermin D and the release of inflammatory factors. The SARS-CoV-2 uses ACE2, a receptor highly express in AT, to entry human host cells promoting the expression of pro-inflammatory cytokines and oligomerization of NLRP3. Upon the NLRP3 inflammasome activation, Gasdermin-pores and cell membrane swelling promote cell pyroptosis, particularly in macrophages and lymphocytes. Cell signaling represented by straight lines show the canonical activation of NLRP3 in obesity. Dotted line represents the possible contribution of SARS-CoV-2 in NLRP3 activation. ACE2, angiotensin-converting enzyme 2; AT, adipose tissue; Mfn2, mitofusin-2; MAVS, mitochondrial antiviral signaling protein; GSDMD, Gasdermin D; IRF, interferon-regulatory factor; ROS, reactive oxygen species; TLR, toll-like receptor; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TRIF, protein-inducing interferon-β.

In obesity, white AT promotes cellular, molecular and biochemical alterations that cause local and systemic changes. Locally, adipocyte hyperplasia and hypertrophy modify the AT structure; at a systemic level, these alterations promote inflammation, insulin resistance, nonalcoholic fatty liver diseases, and dyslipidemias (42, 45, 51, 52).

The impaired adipocytes function caused by hyperplasia and hypertrophy induce an exacerbated lipolysis releasing fatty acids (such as palmitic and lauric acids), and triggers the formation of ceramides and cholesterol crystals that activate tissue-resident macrophages through the TLR4 signaling (53–55). This macrophage stimulation itself triggers the production of ROS, calcium accumulation, as well as the release of IL-6, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) (56–58). The chemokine MCP-1 leads to the recruitment of monocytes, while the interferon gamma (IFN-γ), secreted by T cells in AT stimulates a polarization process in macrophages, from the anti-inflammatory state (M2) to the pro-inflammatory (M1) phenotype thereby perpetuating a low-grade systemic inflammation (56, 57, 59, 60).

The inflammatory activity of M1 macrophages is traditionally mediated by the activation of TNF-αR, IL-1βR, and CD36, which is a priming signaling that activates NF-κB promoting the transcription of NLRP3, pro-IL-1β, pro-IL-18, and other inflammatory cytokines (61). However, for the NLRP3 inflammasome assembly, a second hit is required; this second hit is induced by the binding of ceramides, fatty acids, oxidized low-density lipoproteins and cholesterol crystals to TLR 2/4 (37, 58, 62–64). Finally, the sustained activation of NLRP3 will induce the assembly of GSDMD pores into the macrophage cell

membrane (40, 65). This process constitutes the pyroptotic cell death mechanism, disrupting the osmotic potential and pouring pro-inflammatory molecules to the system (65) (**Figure 1**).

COVID-19, INFLAMMASOME, AND PYROPTOSIS

The causal agent of COVID-19 is known as SARS-CoV-2. The viral infection is mediated by the attachment between a spike glycoprotein and the angiotensin-converting enzyme 2 (ACE2) in human host cells (66). The host target receptor mediates a virus-cell membrane fusion and a viral entry that could cause virus-linked pyroptosis (67, 68), leading to SARS-CoV-2-induced lymphopenia (22, 69).

The innate immune system cells detect the viral RNA by Pattern Recognition Receptors like TLR 3/7 in the endosome; then, cascades of signaling pathways are triggered by TRIF and MyD88 leading to the activation of transcription factors including NF-κB and interferon-regulatory factor 3/7 (IRF) (70). Not only TLR signaling can induce an excessive inflammatory response to SARS-CoV-2, the inflammasome activation stimulated by viral internalization can also induce it; presumably, this occurs through spike proteins binding to CD147 (71). The massive release of TNF-α, IFN-γ, IL-1β, IL-8, MCP-1, and IP-10 seen in acute phase of COVID-19 patients (22) may probably be linked to pyroptosis, especially in lymphocytes through the NLRP3 inflammasome activation.

The pyroptosis-mediated cell death has been described previously in another coronavirus infection (72, 73). Recent evidence suggests that Severe Acute Respiratory Syndrome-related Coronavirus (SARS-CoV) induces NLRP3-dependent pyroptosis in macrophages, which is triggered by the essential ion channel activity of viroporin 3a (72) as well as by a direct interaction of ORF8b with the LRR domain of NLRP3 (73). Moreover, it has been demonstrated that ORF3a and E protein can stimulate NF-κB signaling, resulting in the transcription of NLRP3, chemokines, and pro-inflammatory cytokine, including IL-1β, IL-18, and IL-8 (74–76). Additionally, ORF3a might also mediate NLRP3 inflammasome activation through the ubiquitination of ASC promoting maturation and secretion of IL-1β (75). In contrast, E protein induces the assembly of NLRP3 inflammasome *via* the formation of pores in Endoplasmic Reticulum-Golgi intermediate compartment membranes that triggers a massive calcium ion transportation to the cytosol (77–79). Finally, Chang et al. highlight the biological role of SARS-CoV unique domain (SUD) as a direct inductor of NLRP3 inflammasome activation in alveolar epithelial cells, as well as its activity modulating pulmonary inflammation mediated by CXCL10 *in vitro* and *in vivo* through NLRP3 inflammasome pathway (80). These molecular mechanisms have been linked to the induction of cytokine storm and cell death in SARS-CoV (72, 73, 81, 82).

In the particular case of SARS-CoV-2 strains, recent evidence suggests similar signaling pathways with SARS-CoV in modulating the inflammation by activating NLRP3. A novel study has revealed homology functional domains of ORF3a when compared with those reported in SARS-CoV strains, suggesting some hypothetical pathways of ORF3a linked to the NF-κB activation and NLRP3 inflammasome assembly (83). Considering SARS-CoV-2 has high nucleotide sequence homology to SARS-CoV and 94.7% amino acid identity of E protein (84, 85), it could be inferred that pyroptosis might play a central role in the pathogenesis of COVID-19. Individuals infected with SARS-CoV-2 often show high concentration of pro-inflammatory cytokines (22, 86), which is a downstream indicator of inflammatory programmed cell death (**Figure 1**) (65).

Different reports have shown that COVID-19 is characterized by a dysfunctional immune response, which exacerbates the disease progression as result of a persistent inflammation associated with high peripheral levels of IL-1β, IL-6, TNF-α, MCP-1, and IP10 (22, 87–89). This aggravated inflammatory response triggers a cytokine storm, contributing to the pathological inflammation and multi-organ injury seen in severely ill COVID-19 patients (23, 90–92). With regard to the exacerbated inflammation probably caused by aberrant activation of NLRP3 inflammasome in COVID-19, potential targets are being explored including host signaling proteins and effector molecules that lead cytokine storm (24, 93–95).

To date, some different drugs such as Acalabrutinib have shown their beneficial effects in COVID-19. This therapeutic strategy inhibits the Bruton tyrosine kinase (BTK) enzyme, which is a direct regulator in NLRP3 inflammasome activation (96, 97). The use of the drug in severe COVID-19 patients showed a decrease in serum inflammatory biomarkers (24). Furthermore, Colchicine (93–95) has been successfully tested as inhibitor of NLRP3 inflammasome, improving survival outcomes in COVID-19 patients since it suppresses caspase-1 activation and subsequent IL-1β and IL-18 processing (94, 98). In this sense, Hydroxychloroquine, another NLRP3 inhibitor, has shown a role affecting the NLRP3 inflammasome activation and assembly (99–101). In addition, different clinical trials registered to evaluate the efficacy of pharmacological inhibitors of the NLRP3 inflammasome in treating COVID-19; include Colchicine (NCT04326790, NCT04322565, NCT04328480, NCT04322682), Hydroxychloroquine in combination with Azithromycin (NCT04339816, NCT04336332), Melatonin (NCT04409522), and Tranilast (ChiCTR2000030002); versus standard care.

In other hand, recent studies have evaluated the use of Anakinra (102, 103) as a therapeutic strategy focused in signaling inhibition of IL-1β to treat COVID-19 related cytokine storm. This drug is a human IL-1β receptor antagonist that inhibits inflammation response. Moreover, there are different clinical trials registered to study the efficacy and safety of Canakinumab (anti-IL-1β monoclonal antibody) in COVID-19-induce pneumonia (NCT04362813, NCT04348448) and COVID-19 cardiac injury (NCT04365153). In addition, Tocilizumab (anti-IL-6 treatment) showed clinical improvement in COVID-19 patients (104–106). There are few drugs with mechanisms of action targeting NLRP3 such as Necrosulfonamide (107) and Disulfiram (108), by inhibiting the N-terminal GSDMD pores. The NLRP3 inhibition may represent an optimal strategy to

mitigate the impact of comorbidities associated with COVID-19 such as diabetes mellitus (109, 110), hypertension (111), and obesity (112, 113).

THE CLINICAL IMPACT OF OBESITY IN COVID-19

Different studies have reported fatal COVID-19 outcomes in individuals with at least one chronic disease such as hypertension, diabetes, cardiovascular disease, and obesity (87, 114–116). In individuals infected with SARS-CoV-2, overweight and obesity could be conditioning the critical outcome of COVID-19. The high number of young individuals with COVID-19 that have been hospitalized might be explained by the high obesity incidence found among them (117–119). Furthermore, data suggest that overweight and obesity determined by BMI are associated with the presence of severe pneumonia or increased incidence of ICU admission of individuals with COVID-19 in the USA (117, 120, 121), China (122, 123), Mexico (114, 124), and France (125).

A possible explanation is that tissue expression of ACE2 may play a key role in the progression of COVID-19 patients with obesity, since obese individuals have increased AT mass that leads to an elevated number of ACE2-expressing cells and therefore an increased risk of SARS-CoV-2 infection (126, 127). Other obesity-implicated conditions have been associated with a severe course of COVID-19 such as respiratory symptoms, impaired metabolic health, cardiac stress, dysfunctional host defense against viral infection, and multi-organ damage (120, 125).

Interestingly, obesity has been associated with a decrease in mortality in patients with acute respiratory distress syndrome (ARDS), and this is referred to as the "obesity paradox". However, the high mortality among patients with obesity who manifest SARS-CoV-2 infection has prompted the notion that SARS-CoV-2 has disproved the "obesity paradox" in ARDS (128, 129). The paradox fades if body composition (i.e., fat mass, lean mass, and skeletal muscle mass), body fat distribution (abdominal obesity carries a higher risk of developing metabolic disorders than peripheral or gluteofemoral obesity), AT functionality, and the differences between subcutaneous and visceral AT are considered (130–135). Moreover, the metabolically unhealthy obese phenotype seems to be associated with increased activation of the NLPR3 in macrophages infiltrating visceral AT and a less favorable inflammatory profile than the metabolically healthy phenotype (136). In obese individuals, the innate immune system might be already in a "primed state" due to chronic low-grade inflammation and this could promote an hyperinflammatory response (137, 138); under this scenario, we wonder if trained immunity mediated by NLRP3 in obese conditions to severe outcomes in COVID-19 patients, or normal weight patients infected by SARS-CoV-2 are developing trained immunity that accelerates and trigger short-term development of degenerative chronic comorbidities such as atherosclerosis, diabetes, osteoarthritis, gout, autoimmune diseases, and even obesity itself. This sustained activation state could induce poor clinical outcomes of COVID-19, amplifying the pro-inflammatory response to SARS-CoV-2 infection (**Figure 2**).

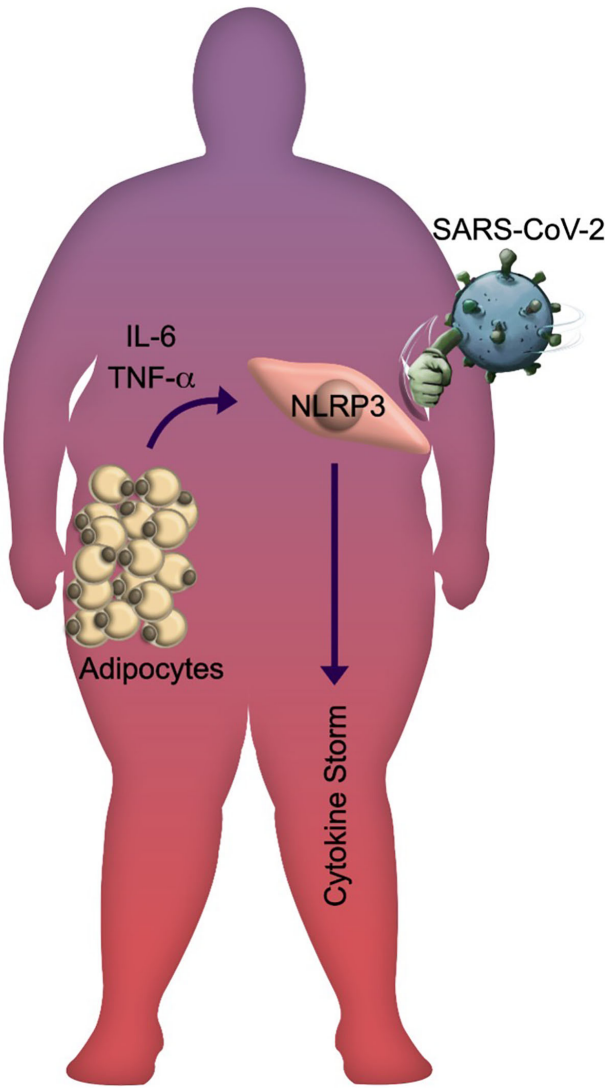


FIGURE 2 | The SARS-CoV-2 knockouts obese individuals. Schematic representation of the lethal impact generated by SARS-CoV-2 infection in obese individuals with metabolic and inflammatory stress. The released adipokines by hypertrophic and hyperplasic adipose tissue promote the NLRP3 inflammasome activation, leading to systemic inflammation that is amplified during viral infection.

DISCUSSION

Recent evidence suggests that 1) AT hypertrophy and hyperplasia promote the synthesis of triglycerides, oxidized phospholipids, IL-1β, TNF-α, and adipokines triggering the systemic inflammatory state regularly observed in individuals with obesity (53–55); 2) these molecules are responsible for increasing the vulnerability to infections in individuals with obesity because the priming phase of the inflammasome is already active (61); 3) the recognition of SARS-CoV-2 by endosomal TLRs 3/7 will trigger multiple signaling and cellular pathways that will allow inflammasome assembly and

consequent maturation of cytosolic pro-cytokines such as IL-1β and IL-18 as well as the activation of GSDMD (70, 83); 4) the Gasdermin-pore formation will start cell death by pyroptosis *via* the release of pro-inflammatory mediators in COVID-19 patients (22, 83, 86). To summarize the recognition of the molecular pathways involved in the inflammasome might explain the vulnerability of obese patients to develop severe cases of COVID-19 (**Figure 2**).

AUTHOR CONTRIBUTIONS

AL-R developed the concept for the review. MC-R, CM-A, and RE-V wrote the manuscript. PV-C, LG-Q, BP-G, and GN contributed to the editing and contextual design. All authors contributed to the article and approved the submitted version.

FUNDING

Financial support: CONACYT 312513 SARS-COV 2.

ACKNOWLEDGMENTS

We gratefully thank the extraordinary effort of healthcare workers who take care of individuals infected with SARS-CoV-2.

REFERENCES

1. World Health Organization. *Obesity and overweight* (2020). Available at: http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight (Accessed July 2, 2020).
2. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. *Metabolism* (2019) 92:6–10. doi: 10.1016/j.metabol.2018.09.005
3. Koliaki C, Liatis S, Kokkinos A. Obesity and cardiovascular disease: revisiting an old relationship. *Metabolism* (2019) 92:98–107. doi: 10.1016/j.metabol.2018.10.011
4. Medina-Luna D, Santamaria-Olmedo MG, Zamudio-Cuevas Y, Martinez-Flores K, Fernandez-Torres J, Martinez-Nava GA, et al. Hyperlipidemic microenvironment conditionates damage mechanisms in human chondrocytes by oxidative stress. *Lipids Health Dis* (2017) 16(1):114. doi: 10.1186/s12944-017-0510-x
5. Piche ME, Tchernof A, Despres JP. Obesity Phenotypes, Diabetes, and Cardiovascular Diseases. *Circ Res* (2020) 126(11):1477–500. doi: 10.1161/CIRCRESAHA.120.316101
6. Unamuno X, Gomez-Ambrosi J, Rodriguez A, Becerril S, Fruhbeck G, Catalan V. Adipokine dysregulation and adipose tissue inflammation in human obesity. *Eur J Clin Invest* (2018) 48(9):e12997. doi: 10.1111/eci.12997
7. Rheinheimer J, de Souza BM, Cardoso NS, Bauer AC, Crispim D. Current role of the NLRP3 inflammasome on obesity and insulin resistance: A systematic review. *Metabolism* (2017) 74:1–9. doi: 10.1016/j.metabol.2017.06.002
8. Farrell GC, Haczeyni F, Chitturi S. Pathogenesis of NASH: How Metabolic Complications of Overnutrition Favour Lipotoxicity and Pro-Inflammatory Fatty Liver Disease. *Adv Exp Med Biol* (2018) 1061:19–44. doi: 10.1007/978-981-10-8684-7_3
9. Oh YS, Bae GD, Baek DJ, Park EY, Jun HS. Fatty Acid-Induced Lipotoxicity in Pancreatic Beta-Cells During Development of Type 2 Diabetes. *Front Endocrinol (Lausanne)* (2018) 9:384. doi: 10.3389/fendo.2018.00384
10. Barra NG, Henriksbo BD, Anhe FF, Schertzer JD. The NLRP3 inflammasome regulates adipose tissue metabolism. *Biochem J* (2020) 477(6):1089–107. doi: 10.1042/BCJ20190472
11. Srinivasa S, Fitch KV, Torriani M, Zanni MV, Defilippi C, Christenson R, et al. Relationship of visceral and subcutaneous adipose depots to markers of arterial injury and inflammation among individuals with HIV. *AIDS* (2019) 33(2):229–36. doi: 10.1097/QAD.0000000000002060
12. Damouche A, Lazure T, Avettand-Fenoel V, Huot N, Dejucq-Rainsford N, Satie AP, et al. Adipose Tissue Is a Neglected Viral Reservoir and an Inflammatory Site during Chronic HIV and SIV Infection. *PLoS Pathog* (2015) 11(9):e1005153. doi: 10.1371/journal.ppat.1005153
13. Sun Y, Wang Q, Yang G, Lin C, Zhang Y, Yang P. Weight and prognosis for influenza A(H1N1)pdm09 infection during the pandemic period between 2009 and 2011: a systematic review of observational studies with meta-analysis. *Infect Dis (Lond)* (2016) 48(11-12):813–22. doi: 10.1080/23744235.2016.1201721
14. Zhou Y, Cowling BJ, Wu P, Chan WM, Lee SY, Lau EH, et al. Adiposity and influenza-associated respiratory mortality: a cohort study. *Clin Infect Dis* (2015) 60(10):e49–57. doi: 10.1093/cid/civ060
15. Neidich SD, Green WD, Rebeles J, Karlsson EA, Schultz-Cherry S, Noah TL, et al. Increased risk of influenza among vaccinated adults who are obese. *Int J Obes (Lond)* (2017) 41(9):1324–30. doi: 10.1038/ijo.2017.131
16. Chiappetta S, Sharma AM, Bottino V, Stier C. COVID-19 and the role of chronic inflammation in patients withobesity. *Int J Obes (Lond)* (2020) 44:1790–2. doi: 10.1038/s41366-020-0597-4
17. Worldmeters. *COVID-19 Coronavirus Pandemic* (2020). Available at: https://www.worldometers.info/coronavirus/ (Accessed August 14, 2020).
18. World Health Organization. *Coronavirus disease 2019 (COVID-19) pandemic* (2020). Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (Accessed July 2, 2020).
19. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome andDeath in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* (2020) 180(7):934–43. doi: 10.1001/jamainternmed.2020.0994
20. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* (2020) 8(4):420–2. doi: 10.1016/S2213-2600(20)30076-X
21. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* (2020) 395(10229):1033–4. doi: 10.1016/S0140-6736(20)30628-0
22. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* (2020) 395(10223):497–506. doi: 10.1016/S0140-6736(20)30183-5
23. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* (2020) 130(5):2620–9. doi: 10.1172/JCI137244
24. Roschewski M, Lionakis MS, Sharman JP, Roswarski J, Goy A, Monticelli MA, et al. Inhibition of Bruton tyrosine kinase in patients with severeCOVID-19. *Sci Immunol* (2020) 5(48):eabd0110. doi: 10.1126/sciimmunol.abd0110
25. Gurung P, Lukens JR, Kanneganti TD. Mitochondria: diversity in the regulation of the NLRP3 inflammasome. *Trends Mol Med* (2015) 21(3):193–201. doi: 10.1016/j.molmed.2014.11.008
26. Liu H, Xu R, Kong Q, Liu J, Yu Z, Zhao C. Downregulated NLRP3 and NLRP1 inflammasomes signaling pathways in the development and progression of type 1 diabetes mellitus. *BioMed Pharmacother* (2017) 94:619–26. doi: 10.1016/j.biopha.2017.07.102
27. Wan X, Xu C, Lin Y, Lu C, Li D, Sang J, et al. Uric acid regulates hepatic steatosis and insulin resistance through the NLRP3 inflammasome-dependent mechanism. *J Hepatol* (2016) 64(4):925–32. doi: 10.1016/j.jhep.2015.11.022
28. Sun X, Hao H, Han Q, Song X, Liu J, Dong L, et al. Human umbilical cord-derived mesenchymal stem cells ameliorate insulin resistance by suppressing NLRP3 inflammasome-mediated inflammation in type 2 diabetes rats. *Stem Cell Res Ther* (2017) 8(1):241. doi: 10.1186/s13287-017-0668-1
29. Clavijo-Cornejo D, Martinez-Flores K, Silva-Luna K, Martinez-Nava GA, Fernandez-Torres J, Zamudio-Cuevas Y, et al. The Overexpression of NALP3 Inflammasome in Knee Osteoarthritis Is Associated with Synovial

Membrane Prolidase and NADPH Oxidase 2. *Oxid Med Cell Longev* (2016) 2016:1472567. doi: 10.1155/2016/1472567
30. Guey B, Bodnar M, Manie SN, Tardivel A, Petrilli V. Caspase-1 autoproteolysis is differentially required for NLRP1b and NLRP3 inflammasome function. *Proc Natl Acad Sci U S A* (2014) 111(48):17254–9. doi: 10.1073/pnas.1415756111
31. Swanson KV, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol* (2019) 19 (8):477–89. doi: 10.1038/s41577-019-0165-0
32. Ketelut-Carneiro N, Silva GK, Rocha FA, Milanezi CM, Cavalcanti-Neto FF, Zamboni DS, et al. IL-18 triggered by the Nlrp3 inflammasome induces host innate resistance in a pulmonary model of fungal infection. *J Immunol* (2015) 194(9):4507–17. doi: 10.4049/jimmunol.1402321
33. Zhao X, Zhang C, Hua M, Wang R, Zhong C, Yu J, et al. NLRP3 inflammasome activation plays a carcinogenic role through effector cytokine IL-18 in lymphoma. *Oncotarget* (2017) 8(65):108571–83. doi: 10.18632/oncotarget.21010
34. Boriushkin E, Wang JJ, Li J, Bhatta M, Zhang SX. p58(IPK) suppresses NLRP3 inflammasome activation and IL-1beta production via inhibition of PKR in macrophages. *Sci Rep* (2016) 6:25013. doi: 10.1038/srep25013
35. Thompson JK, MacPherson MB, Beuschel SL, Shukla A. Asbestos-Induced Mesothelial to Fibroblastic Transition Is Modulated by the Inflammasome. *Am J Pathol* (2017) 187(3):665–78. doi: 10.1016/j.ajpath.2016.11.008
36. Zheng SC, Zhu XX, Xue Y, Zhang LH, Zou HJ, Qiu JH, et al. Role of the NLRP3 inflammasome in the transient release of IL-1beta induced by monosodium urate crystals in human fibroblast-like synoviocytes. *J Inflammation (Lond)* (2015) 12:30. doi: 10.1186/s12950-015-0070-7
37. Rajamaki K, Lappalainen J, Oorni K, Valimaki E, Matikainen S, Kovanen PT, et al. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PLoS One* (2010) 5(7):e11765. doi: 10.1371/journal.pone.0011765
38. Zhao C, Zhao W. NLRP3 Inflammasome-A Key Player in Antiviral Responses. *Front Immunol* (2020) 11:211. doi: 10.3389/fimmu.2020.00211
39. He WT, Wan H, Hu L, Chen P, Wang X, Huang Z, et al. Gasdermin D is an executor of pyroptosis and required for interleukin-1beta secretion. *Cell Res* (2015) 25(12):1285–98. doi: 10.1038/cr.2015.139
40. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* (2016) 535(7610):153–8. doi: 10.1038/nature18629
41. He Y, Hara H, Nunez G. Mechanism and Regulation of NLRP3 Inflammasome Activation. *Trends Biochem Sci* (2016) 41(12):1012–21. doi: 10.1016/j.tibs.2016.09.002
42. Caer C, Rouault C, Le Roy T, Poitou C, Aron-Wisnewsky J, Torcivia A, et al. Immune cell-derived cytokines contribute to obesity-related inflammation, fibrogenesis and metabolic deregulation in human adipose tissue. *Sci Rep* (2017) 7(1):3000. doi: 10.1038/s41598-017-02660-w
43. McLaughlin T, Liu LF, Lamendola C, Shen L, Morton J, Rivas H, et al. T-cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. *Arterioscler Thromb Vasc Biol* (2014) 34 (12):2637–43. doi: 10.1161/ATVBAHA.114.304636
44. Mohiuddin OA, Campbell B, Poche JN, Thomas-Porch C, Hayes DA, Bunnell BA, et al. Decellularized Adipose Tissue: Biochemical Composition, in vivo Analysis and Potential Clinical Applications. *Adv Exp Med Biol* (2020) 1212:57–70. doi: 10.1007/5584_2019_371
45. Hoffstedt J, Arner E, Wahrenberg H, Andersson DP, Qvisth V, Lofgren P, et al. Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. *Diabetologia* (2010) 53(12):2496–503. doi: 10.1007/s00125-010-1889-3
46. Zhang C, Wang JJ, He X, Wang C, Zhang B, Xu J, et al. Characterization and Beige Adipogenic Potential of Human Embryo White Adipose Tissue-Derived Stem Cells. *Cell Physiol Biochem* (2018) 51(6):2900–15. doi: 10.1159/000496042
47. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Front Endocrinol (Lausanne)* (2016) 7:30. doi: 10.3389/fendo.2016.00030
48. Garcia-Ruiz E, Reynes B, Diaz-Rua R, Ceresi E, Oliver P, Palou A. The intake of high-fat diets induces the acquisition of brown adipocyte gene expression features in white adipose tissue. *Int J Obes (Lond)* (2015) 39(11):1619–29. doi: 10.1038/ijo.2015.112
49. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* (2009) 58(7):1526–31. doi: 10.2337/db09-0530
50. Xu Z, You W, Zhou Y, Chen W, Wang Y, Shan T. Cold-induced lipid dynamics and transcriptional programs in white adipose tissue. *BMC Biol* (2019) 17(1):74. doi: 10.1186/s12915-019-0693-x
51. Belligoli A, Compagnin C, Sanna M, Favaretto F, Fabris R, Busetto L, et al. Characterization of subcutaneous and omental adipose tissue in patients with obesity and with different degrees of glucose impairment. *Sci Rep* (2019) 9(1):11333. doi: 10.1038/s41598-019-47719-y
52. Mulder P, Morrison MC, Verschuren L, Liang W, van Bockel JH, Kooistra T, et al. Reduction of obesity-associated white adipose tissue inflammation by rosiglitazone is associated with reduced non-alcoholic fatty liver disease in LDLr-deficient mice. *Sci Rep* (2016) 6:31542. doi: 10.1038/srep31542
53. Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. *J Biol Chem* (2001) 276(20):16683–9. doi: 10.1074/jbc.M011695200
54. Lee JY, Ye J, Gao Z, Youn HS, Lee WH, Zhao L, et al. Reciprocal modulation of Toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. *J Biol Chem* (2003) 278(39):37041–51. doi: 10.1074/jbc.M305213200
55. Giordano A, Murano I, Mondini E, Perugini J, Smorlesi A, Severi I, et al. Obese adipocytes show ultrastructural features of stressed cells and die of pyroptosis. *J Lipid Res* (2013) 54(9):2423–36. doi: 10.1194/jlr.M038638
56. Fujisaka S, Usui I, Ikutani M, Aminuddin A, Takikawa A, Tsuneyama K, et al. Adipose tissue hypoxia induces inflammatory M1 polarity of macrophages in an HIF-1alpha-dependent and HIF-1alpha-independent manner in obese mice. *Diabetologia* (2013) 56(6):1403–12. doi: 10.1007/s00125-013-2885-1
57. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med* (2009) 15(8):914–20. doi: 10.1038/nm.1964
58. Okla M, Zaher W, Alfayez M, Chung S. Inhibitory Effects of Toll-Like Receptor 4, NLRP3 Inflammasome, and Interleukin-1beta on White Adipocyte Browning. *Inflammation* (2018) 41(2):626–42. doi: 10.1007/s10753-017-0718-y
59. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* (2007) 117(1):175–84. doi: 10.1172/JCI29881
60. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* (2006) 116(6):1494–505. doi: 10.1172/JCI26498
61. Russo L, Lumeng CN. Properties and functions of adipose tissue macrophages in obesity. *Immunology* (2018) 155(4):407–17. doi: 10.1111/imm.13002
62. Karasawa T, Kawashima A, Usui-Kawanishi F, Watanabe S, Kimura H, Kamata R, et al. Saturated Fatty Acids Undergo Intracellular Crystallization and Activate the NLRP3 Inflammasome in Macrophages. *Arterioscler Thromb Vasc Biol* (2018) 38(4):744–56. doi: 10.1161/ATVBAHA.117.310581
63. Wen H, Gris D, Lei Y, Jha S, Zhang L, Huang MT, et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat Immunol* (2011) 12(5):408–15. doi: 10.1038/ni.2022
64. Camell CD, Nguyen KY, Jurczak MJ, Christian BE, Shulman GI, Shadel GS, et al. Macrophage-specific de Novo Synthesis of Ceramide Is Dispensable for Inflammasome-driven Inflammation and Insulin Resistance in Obesity. *J Biol Chem* (2015) 290(49):29402–13. doi: 10.1074/jbc.M115.680199
65. Evavold CL, Ruan J, Tan Y, Xia S, Wu H, Kagan JC. The Pore-Forming Protein Gasdermin D Regulates Interleukin-1 Secretion from Living Macrophages. *Immunity* (2018) 48(1):35–44e6. doi: 10.1016/j.immuni.2017.11.013
66. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020) 579(7798):270–3. doi: 10.1038/s41586-020-2012-7
67. Yang M. Cell pyroptosis, a potential pathogenic mechanism of 2019-nCoV infection. *SSRN* (2020). doi: 10.2139/ssrn.3527420
68. Yang Y, Peng F, Wang R, Yange M, Guan K, Jiang T, et al. The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus

epidemic in China. *J Autoimmun* (2020) 109:102434. doi: 10.1016/j.jaut.2020.102434
69. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* (2020) 382(18):1708–20. doi: 10.1056/NEJMoa2002032
70. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. *J Med Virol* (2020) 92(4):424–32. doi: 10.1002/jmv.25685
71. Wang K, Chen W, Zhou Y, Lian J, Zhang Z, Du P, et al. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. *bioRxiv [Preprint]* (2020). doi: 10.1101/2020.03.14.988345
72. Chen IY, Moriyama M, Chang MF, Ichinohe T. Severe Acute Respiratory Syndrome Coronavirus Viroporin 3a Activates the NLRP3 Inflammasome. *Front Microbiol* (2019) 10:50. doi: 10.3389/fmicb.2019.00050
73. Shi CS, Nabar NR, Huang NN, Kehrl JH. SARS-Coronavirus Open Reading Frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. *Cell Death Discov* (2019) 5:101. doi: 10.1038/s41420-019-0181-7
74. DeDiego ML, Nieto-Torres JL, Regla-Nava JA, Jimenez-Guardeno JM, Fernandez-Delgado R, Fett C, et al. Inhibition of NF-kappaB-mediated inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. *J Virol* (2014) 88(2):913–24. doi: 10.1128/JVI.02576-13
75. Siu KL, Yuen KS, Castano-Rodriguez C, Ye ZW, Yeung ML, Fung SY, et al. Severe acute respiratory syndrome coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC. *FASEB J* (2019) 33(8):8865–77. doi: 10.1096/fj.201802418R
76. Kanzawa N, Nishigaki K, Hayashi T, Ishii Y, Furukawa S, Niiro A, et al. Augmentation of chemokine production by severe acute respiratory syndrome coronavirus 3a/X1 and 7a/X4 proteins through NF-kappaB activation. *FEBS Lett* (2006) 580(30):6807–12. doi: 10.1016/j.febslet.2006.11.046
77. Nieto-Torres JL, Verdia-Baguena C, Jimenez-Guardeno JM, Regla-Nava JA, Castano-Rodriguez C, Fernandez-Delgado R, et al. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. *Virology* (2015) 485:330–9. doi: 10.1016/j.virol.2015.08.010
78. Nieto-Torres JL, DeDiego ML, Verdia-Baguena C, Jimenez-Guardeno JM, Regla-Nava JA, Fernandez-Delgado R, et al. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLoS Pathog* (2014) 10(5):e1004077. doi: 10.1371/journal.ppat.1004077
79. Jimenez-Guardeno JM, Nieto-Torres JL, DeDiego ML, Regla-Nava JA, Fernandez-Delgado R, Castano-Rodriguez C, et al. The PDZ-binding motif of severe acute respiratory syndrome coronavirus envelope protein is a determinant of viral pathogenesis. *PLoS Pathog* (2014) 10(8):e1004320. doi: 10.1371/journal.ppat.1004320
80. Chang YS, Ko BH, Ju JC, Chang HH, Huang SH, Lin CW. SARS Unique Domain (SUD) of Severe Acute Respiratory SyndromeCoronavirus Induces NLRP3 Inflammasome-Dependent CXCL10-Mediated Pulmonary Inflammation. *Int J Mol Sci* (2020) 21(9):3179. doi: 10.3390/ijms21093179
81. He L, Ding Y, Zhang Q, Che X, He Y, Shen H, et al. Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. *J Pathol* (2006) 210(3):288–97. doi: 10.1002/path.2067
82. Yue Y, Nabar NR, Shi CS, Kamenyeva O, Xiao X, Hwang IY, et al. SARS-Coronavirus Open Reading Frame-3a drives multimodal necrotic cell death. *Cell Death Dis* (2018) 9(9):904. doi: 10.1038/s41419-018-0917-y
83. Issa E, Merhi G, Panossian B, Salloum T, Tokajian S. SARS-CoV-2 and ORF3a: Nonsynonymous Mutations, Functional Domains,and Viral Pathogenesis. *mSystems* (2020) 5(3):e00266–20. doi: 10.1128/mSystems.00266-20
84. Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg Microbes Infect* (2020) 9(1):221–36. doi: 10.1080/22221751.2020.1719902
85. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* (2020) 395(10224):565–74. doi: 10.1016/S0140-6736(20)30251-8
86. Li S, Jiang L, Li X, Lin F, Wang Y, Li B, et al. Clinical and pathological investigation of patients with severeCOVID-19. *JCI Insight* (2020) 5(12): e138070. doi: 10.1172/jci.insight.138070
87. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan,China. *Clin Infect Dis* (2020) 71(15):762–8. doi: 10.1093/cid/ciaa248
88. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine* (2020) 55:102763. doi: 10.1016/j.ebiom.2020.102763
89. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of Peripheral Lymphocyte Subset Alteration in COVID-19 Pneumonia. *J Infect Dis* (2020) 221(11):1762–9. doi: 10.1093/infdis/jiaa150
90. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* (2020) 46(5):846–8. doi: 10.1007/s00134-020-05991-x
91. Yang Y, Shen C, Li J, Yuan J, Wei J, Huang F, et al. Plasma IP-10 and MCP-3 levels are highly associated with diseaseseverity and predict the progression of COVID-19. *J Allergy ClinImmunol* (2020) 146(1):119–27. doi: 10.1016/j.jaci.2020.04.027
92. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* (2020) 395(10234):1417–8. doi: 10.1016/S0140-6736(20)30937-5
93. Deftereos SG, Giannopoulos G, Vrachatis DA, Siasos GD, Giotaki SG, Gargalianos P, et al. Effect of Colchicine vs Standard Care on Cardiac and Inflammatory Biomarkers and Clinical Outcomes in Patients Hospitalized With Coronavirus Disease 2019: The GRECCO-19 Randomized Clinical Trial. *JAMA Netw Open* (2020) 3(6):e2013136. doi: 10.1001/jamanetworkopen.2020.13136
94. Montealegre-Gomez G, Garavito E, Gomez-Lopez A, Rojas-Villarraga A, Parra-Medina R. [Colchicine: a potential therapeutic tool against COVID-19. Experience of 5 patients]. *Reumatol Clin* (2020). doi: 10.1016/j.reuma.2020.05.001
95. Della-Torre E, Della-Torre F, Kusanovic M, Scotti R, Ramirez GA, Dagna L, et al. Treating COVID-19 with colchicine in community healthcare setting. *Clin Immunol* (2020) 217:108490. doi: 10.1016/j.clim.2020.108490
96. Ito M, Shichita T, Okada M, Komine R, Noguchi Y, Yoshimura A, et al. Bruton's tyrosine kinase is essential for NLRP3 inflammasome activation and contributes to ischaemic brain injury. *Nat Commun* (2015) 6:7360. doi: 10.1038/ncomms8360
97. Purvis GSD, Collino M, Aranda-Tavio H, Chiazza F, O'Riordan CE, Zeboudj L, et al. Inhibition of Bruton's tyrosine kinase regulates macrophageNF-kappaB and NLRP3 inflammasome activation in metabolic inflammation. *Br JPharmacol* (2020) 177(19):4416–32. doi: 10.1111/bph.15182
98. Scarsi M, Piantoni S, Colombo E, Airo P, Richini D, Miclini M, et al. Association between treatment with colchicine and improved survivalin a single-centre cohort of adult hospitalised patients with COVID-19 pneumonia and acuterespiratory distress syndrome. *Ann Rheum Dis* (2020)79:1286–9. doi: 10.1136/annrheumdis-2020-217712
99. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* (2020) 56(1):105949. doi: 10.1016/j.ijantimicag.2020.105949
100. Fujita Y, Matsuoka N, Temmoku J, Furuya MY, Asano T, Sato S, et al. Hydroxychloroquine inhibits IL-1beta production from amyloid-stimulated human neutrophils. *Arthritis Res Ther* (2019) 21(1):250. doi: 10.1186/s13075-019-2040-6
101. Lucchesi A, Silimbani P, Musuraca G, Cerchione C, Martinelli G, Di Carlo P, et al. Clinical and biological data on the use of hydroxychloroquine against SARS-CoV-2 could support the role of the NLRP3 inflammasome in the pathogenesis of respiratory disease. *J Med Virol* (2020). doi: 10.1002/jmv.26217
102. Cavalli G, De Luca G, Campochiaro C, Della-Torre E, Ripa M, Canetti D, et al. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol* (2020) 2(6):e325–e31. doi: 10.1016/S2665-9913(20)30127-2
103. Day JW, Fox TA, Halsey R, Carpenter B, Kottaridis PD. Interleukin-1 blockade with anakinra in acute leukaemia patientswith severe COVID-19 pneumonia appears safe and may result in clinical improvement. *Br J Haematol.* (2020) 190(2):e80–e83. doi: 10.1111/bjh.16873
104. Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci U S A* (2020) 117 (20):10970–5. doi: 10.1073/pnas.2005615117

105. Toniati P, Piva S, Cattalini M, Garrafa E, Regola F, Castelli F, et al. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in Brescia, Italy. *Autoimmun Rev* (2020) 19(7):102568. doi: 10.1016/j.autrev.2020.102568
106. Capra R, De Rossi N, Mattioli F, Romanelli G, Scarpazza C, Sormani MP, et al. Impact of low dose tocilizumab on mortality rate in patients with COVID-19 related pneumonia. *Eur J Intern Med* (2020) 76:31–5. doi: 10.1016/j.ejim.2020.05.009
107. Rathkey JK, Zhao J, Liu Z, Chen Y, Yang J, Kondolf HC, et al. Chemical disruption of the pyroptotic pore-forming protein gasderminD inhibits inflammatory cell death and sepsis. *Sci Immunol* (2018) 3(26):eaat2738. doi: 10.1126/sciimmunol.aat2738
108. Hu JJ, Liu X, Xia S, Zhang Z, Zhang Y, Zhao J, et al. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. *Nat Immunol* (2020) 21(7):736–45. doi: 10.1038/s41590-020-0669-6
109. Lee HM, Kim JJ, Kim HJ, Shong M, Ku BJ, Jo EK. Upregulated NLRP3 inflammasome activation in patients with type 2 diabetes. *Diabetes* (2013) 62 (1):194–204. doi: 10.2337/db12-0420
110. Pereira CA, Carlos D, Ferreira NS, Silva JF, Zanotto CZ, Zamboni DS, et al. Mitochondrial DNA Promotes NLRP3 Inflammasome Activation and Contributes to Endothelial Dysfunction and Inflammation in Type 1 Diabetes. *Front Physiol* (2019) 10:1557. doi: 10.3389/fphys.2019.01557
111. Sun HJ, Ren XS, Xiong XQ, Chen YZ, Zhao MX, Wang JJ, et al. NLRP3 inflammasome activation contributes to VSMC phenotypic transformation and proliferation in hypertension. *Cell Death Dis* (2017) 8(10):e3074. doi: 10.1038/cddis.2017.470
112. Guo DH, Yamamoto M, Hernandez CM, Khodadadi H, Baban B, Stranahan AM. Visceral adipose NLRP3 impairs cognition in obesity via IL-1R1 on CX3CR1+ cells. *J Clin Invest* (2020) 130(4):1961–76. doi: 10.1172/JCI126078
113. Canadas-Lozano D, Marin-Aguilar F, Castejon-Vega B, Ryffel B, Navarro-Pando JM, Ruiz-Cabello J, et al. Blockade of the NLRP3 inflammasome improves metabolic health and lifespan in obese mice. *Geroscience* (2020) 42(2):715–25. doi: 10.1007/s11357-019-00151-6
114. Hernandez-Garduno E. Obesity is the comorbidity more strongly associated for Covid-19 inMexico. A case-control study. *Obes Res Clin Pract* (2020) 14 (4):375–9. doi: 10.1016/j.orcp.2020.06.001
115. Deng Y, Liu W, Liu K, Fang YY, Shang J, Zhou L, et al. Clinical characteristics of fatal and recovered cases of coronavirus disease 2019 in Wuhan, China: a retrospective study. *Chin Med J (Engl)* (2020) 133(11):1261–7. doi: 10.1097/CM9.0000000000000824
116. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline Characteristics and Outcomes of 1591 Patients Infected WithSARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. *JAMA* (2020) 323 (16):1574–81. doi: 10.1001/jama.2020.5394
117. Cummings MJ, Baldwin MR, Abrams D, Jacobson SD, Meyer BJ, Balough EM, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. *Lancet* (2020) 395(10239):1763–70. doi: 10.1016/S0140-6736(20)31189-2
118. Kass DA, Duggal P, Cingolani O. Obesity could shift severe COVID-19 disease to younger ages. *Lancet* (2020) 395(10236):1544–5. doi: 10.1016/S0140-6736(20)31024-2
119. Klang E, Kassim G, Soffer S, Freeman R, Levin MA, Reich DL. Morbid Obesity as an Independent Risk Factor for COVID-19 Mortalityin Hospitalized Patients Younger than 50. *Obesity (Silver Spring)* (2020) 28 (9):1595–9. doi: 10.1002/oby.22913
120. Bhatraju PK, Ghassemieh BJ, Nichols M, Kim R, Jerome KR, Nalla AK, et al. Covid-19 in Critically Ill Patients in the Seattle Region - Case Series. *N Engl J Med* (2020) 382(21):2012–22. doi: 10.1056/NEJMoa2004500
121. Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell L, Chernyak Y, et al. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. *BMJ* (2020) 369:m1966. doi: 10.1136/bmj.m1966
122. Cai Q, Chen F, Wang T, Luo F, Liu X, Wu Q, et al. Obesity and COVID-19 Severity in a Designated Hospital in Shenzhen, China. *Diabetes Care* (2020) 43(7):1392–8. doi: 10.2337/dc20-0576
123. Zhang F, Xiong Y, Wei Y, Hu Y, Wang F, Li G, et al. Obesity predisposes to the risk of higher mortality in youngCOVID-19 patients. *J Med Virol* (2020) 92(11):2536–42. doi: 10.1002/jmv.26039
124. Valente-Acosta B, Hoyo-Ulloa I, Espinosa-Aguilar L, Mendoza-Aguilar R, Garcia-Guerrero J, Ontañon-Zurita D, et al. COVID-19 severe pneumonia in Mexico City – First experience in a Mexican hospital. *medRxiv [Preprint]* (2020). doi: 10.1101/2020.04.26.20080796
125. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation. *Obesity (Silver Spring)* (2020) 28(7):1195–9. doi: 10.1002/oby.22831
126. Kassir R. Risk of COVID-19 for patients with obesity. *Obes Rev* (2020) 21(6): e13034. doi: 10.1111/obr.13034
127. Jia X, Yin C, Lu S, Chen Y, Liu Q, Bai J, et al. Two Things About COVID-19 Might Need Attention. *Preprints* (2020) 2020020315. doi: 10.20944/preprints202002.0315.v1
128. Jose RJ, Manuel A. Does Coronavirus Disease 2019 Disprove the Obesity Paradox in Acute Respiratory Distress Syndrome? *Obesity (Silver Spring)* (2020) 28(6):1007. doi: 10.1002/oby.22835
129. Petrakis D, Margina D, Tsarouhas K, Tekos F, Stan M, Nikitovic D, et al. Obesity a risk factor for increased COVID19 prevalence, severity and lethality (Review). *Mol Med Rep* (2020) 22(1):9–19. doi: 10.3892/mmr.2020.11127
130. Wang HE, Griffin R, Judd S, Shapiro NI, Safford MM. Obesity and risk of sepsis: a population-based cohort study. *Obesity (Silver Spring)* (2013) 21 (12):E762–9. doi: 10.1002/oby.20468
131. Yeoh AJ, Pedley A, Rosenquist KJ, Hoffmann U, Fox CS. The Association Between Subcutaneous Fat Density and the Propensity to Store Fat Viscerally. *J Clin Endocrinol Metab* (2015) 100(8):E1056–64. doi: 10.1210/jc.2014-4032
132. Carbone S, Lavie CJ, Arena R. Obesity and Heart Failure: Focus on the Obesity Paradox. *Mayo Clin Proc* (2017) 92(2):266–79. doi: 10.1016/j.mayocp.2016.11.001
133. Kruglikov IL, Scherer PE. The Role of Adipocytes and Adipocyte-Like Cells in the Severity of COVID-19 Infections. *Obesity (Silver Spring)* (2020) 28 (7):1187–90. doi: 10.1002/oby.22856
134. Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, et al. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). *Arch Intern Med* (2008) 168(15):1617–24. doi: 10.1001/archinte.168.15.1617
135. Donini LM, Pinto A, Giusti AM, Lenzi A, Poggiogalle E. Obesity or BMI Paradox? Beneath the Tip of the Iceberg. *Front Nutr* (2020) 7:53. doi: 10.3389/fnut.2020.00053
136. Esser N, L'Homme L, De Roover A, Kohnen L, Scheen AJ, Moutschen M, et al. Obesity phenotype is related to NLRP3 inflammasome activity and immunological profile of visceral adipose tissue. *Diabetologia* (2013) 56 (11):2487–97. doi: 10.1007/s00125-013-3023-9
137. Christ A, Gunther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, et al. Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. *Cell* (2018) 172(1-2):162–75.e14. doi: 10.1016/j.cell.2017.12.013
138. Bekkering S, Saner C, Riksen NP, Netea MG, Sabin MA, Saffery R, et al. Trained Immunity: Linking Obesity and Cardiovascular Disease across the Life-Course? *Trends Endocrinol Metab* (2020) 31(5):378–89. doi: 10.1016/j.tem.2020.01.008

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 López-Reyes, Martinez-Armenta, Espinosa-Velázquez, Vázquez-Cárdenas, Cruz-Ramos, Palacios-Gonzalez, Gomez-Quiroz and Martínez-Nava. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

REVIEW
published: 19 November 2020
doi: 10.3389/fnut.2020.597600

The Impact of Obesity and Lifestyle on the Immune System and Susceptibility to Infections Such as COVID-19

Daan L. de Frel[1], Douwe E. Atsma[1], Hanno Pijl[2], Jacob C. Seidell[3], Pieter J. M. Leenen[4], Willem A. Dik[5,6] and Elisabeth F. C. van Rossum[7,8]*

[1] Department of Cardiology, Leiden University Medical Center, Leiden, Netherlands, [2] Department of Endocrinology, Leiden University Medical Center, Leiden, Netherlands, [3] Department of Health Sciences, VU Medical Center, Amsterdam, Netherlands, [4] Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [5] Laboratory Medical Immunology, Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [6] Division of Clinical Immunology, Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [7] Division of Endocrinology, Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [8] Obesity Center CGG, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands

Background: COVID-19 is a global challenge to healthcare. Obesity is common in patients with COVID-19 and seems to aggravate disease prognosis. In this review we explore the link between obesity, chronic disease, lifestyle factors and the immune system, and propose societal interventions to enhance global immunity.

Search Strategy and Selection Criteria: We performed three literature searches using the keywords (1) coronavirus AND comorbidities, (2) comorbidities AND immune system, and (3) lifestyle factors AND immune system. Results were screened for relevance by the main author and a total of 215 articles were thoroughly analyzed.

Results: The relationship between obesity and unfavorable COVID-19 prognosis is discussed in light of the impact of chronic disease and lifestyle on the immune system. Several modifiable lifestyle factors render us susceptible to viral infections. In this context, we make a case for fostering a healthy lifestyle on a global scale.

Conclusions: Obesity, additional chronic disease and an unhealthy lifestyle interactively impair immune function and increase the risk of severe infectious disease. In adverse metabolic and endocrine conditions, the immune system is geared toward inflammation. Collective effort is needed to ameliorate modifiable risk factors for obesity and chronic disease on a global scale and increase resistance to viruses like SARS-CoV-2.

Keywords: obesity, diet, COVID-19, lifestyle, immune system

OPEN ACCESS

Edited by:
Ioannis Zabetakis,
University of Limerick, Ireland

Reviewed by:
Evangeline Mantzioris,
University of South Australia, Australia
Liam O'Mahony,
University College Cork, Ireland

***Correspondence:**
Douwe E. Atsma
d.e.atsma@lumc.nl

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Nutrition

Received: *21 August 2020*
Accepted: *03 November 2020*
Published: *19 November 2020*

Citation:
de Frel DL, Atsma DE, Pijl H, Seidell JC, Leenen PJM, Dik WA and van Rossum EFC (2020) The Impact of Obesity and Lifestyle on the Immune System and Susceptibility to Infections Such as COVID-19. Front. Nutr. 7:597600. doi: 10.3389/fnut.2020.597600

INTRODUCTION

In recent months the entire globe has been affected by coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). Worldwide more than 11 million cases of COVID-19 have been detected until July 2020 and over 500,000 cases have proved fatal (2) Despite serious efforts, no effective vaccine has been developed yet. But, even without a vaccine, the impact of subsequent waves of SARS-CoV-2 spreading can

most likely be decreased. Among patients with COVID-19, obesity, hypertension, cardiovascular disease (CVD) and type 2 diabetes (T2D) are noticeable risk factors for hospitalization, ICU admission, and death. Apparently, chronic disease is linked with unfavorable outcome in COVID-19. As appropriate lifestyle intervention effectively ameliorates cardiometabolic conditions, public health could play a pivotal role in the battle against SARS-CoV-2 (3). The aim of this review is to summarize the current understanding of the links between lifestyle, metabolic syndrome, obesity, and increased susceptibility to viral infections. In addition, we aim to investigate whether public health interventions can affect resistance to viral infections.

SEARCH STRATEGY AND SELECTION CRITERIA

Three separate literature searches were performed in PubMed, EMBASE, and Google Scholar through April 2020 to May 2020. We considered English-language (systematic) review articles and meta-analyses found using the following term combinations: (1) "Coronavirus" and "diabetes/hypertension/obesity/cardiovascular disease/metabolic syndrome" yielding 1,132 articles on April 29, 2020. (2) "Immune system" and "diabetes/hypertension/obesity/cardiovascular disease/metabolic syndrome" yielding 1,364 articles on May 17, 2020. (3) "Immune system" and "lifestyle" yielding 753 results on June 4, 2020. The complete search strategies can be found in **Supplementary Material**. References lists of identified publications were also screened for relevant studies. If multiple articles were found per subject, preference went to clarity, relevance, and novelty. A total of 215 articles (17, 151, and 47 for search strategies 1, 2, and 3, respectively) were scrutinized for this review.

OBESITY AND CARDIOMETABOLIC DISEASES ARE RELATED TO WORSE OUTCOME OF COVID-19

The Chinese Center for Disease Control reports higher COVID-19 fatality rates in patients with comorbidities as compared with patients without (4). The fatality rate for patients with COVID-19 without comorbidities was 0.9%, whereas fatality rates in patients with diabetes (7.3%), CVD (10.5%), and hypertension (6.0%) are clearly higher (4). American and European studies confirm the higher chance of fatality and severe disease in patients with comorbidities and add obesity as another risk factor. According to the American Center for Disease Control and Prevention obesity was present in 48.3% of the hospitalized patients with laboratory-confirmed COVID-19 (5). It was also reported that 89.3% of patients with COVID-19 registered in COVID-NET had other underlying conditions, most of them obesity-related: hypertension (49.7%), chronic lung disease (34.6%), diabetes mellitus (28.3%), and CVD (27.8%). Notably, among patients aged 50–64 years, obesity was most prevalent, followed by hypertension and diabetes mellitus. Among those aged >65 years, hypertension was most prevalent, followed by CVD and diabetes mellitus (5).

TABLE 1 | Overview of mechanisms by which obesity contributes to worse prognosis of COVID-19.

Immune system	Chronic innate immune system activation
	Adaptive immune system dysregulation
	Lymphopenia
Endocrine and metabolic dysregulation in adipose tissue	Increased leptin secretion (pro-inflammatory)
	Decreased adiponectin secretion (anti-inflammatory)
	Hypoxia leading to low grade inflammation
Respiratory impairment	Reduced compliance, increased resistance
	Airway narrowing and collapse
	Reduced functional residual capacity
	Airway hyperresponsiveness
	Ventilation perfusion mismatch
Hemostasis	Enhanced prothrombotic state
Viral shedding	ACE2 expression on adipocytes
	Adipose tissue as potential reservoir for viruses
Comorbidities	Metabolic syndrome
	Type 2 Diabetes
	Cardiovascular disease, including hypertension
	Obstructive sleep apnea

Furthermore, a study of 4,103 positively tested patients with COVID-19 in New York revealed more frequent hospitalization of patients with obesity (39.8 vs. 14.5%), CVD (44.6 vs. 16.4%), and diabetes (31.8 vs. 5.4%) than in patients without comorbidities (6).

THE RELATIONSHIP BETWEEN OBESITY AND UNFAVORABLE OUTCOME OF COVID-19

Obesity is evidently associated with COVID-19 in terms of prevalence of symptomatic disease, disease severity and mortality (7, 8). Individuals with obesity have an approximately 3-fold increased risk of severe COVID-19 (7). Each unit increase in BMI was associated with a 12% increase in the risk of severe disease and obesity increased the need for invasive mechanical ventilation (7, 8). Furthermore, in patients below 60 years of age, who are generally considered to have a lower risk for severe disease, obesity appears to be a risk factor for hospital admission and need for critical care (9). Interestingly, similar associations were observed in other (viral) infections. During the Influenza A (H1N1) pandemic of 2009–2011, obesity was strongly associated with a worse disease outcome and death (10). Obesity also increases the duration of Influenza A virus shedding in adults, indicative of an impaired immune response that subsequently facilitates influenza transmission (11). Multiple mechanisms in obesity aggravate prognosis in COVID-19 (**Table 1**). Below we discuss the most important mechanisms known to date.

The Immune System

The immune system uses two sequential responses to react to disturbances in homeostasis, as seen in tissues under

physiological or infectious stress. An initial rapid innate immune response, which has only broad specificity for the trigger is followed by a delayed, highly specific adaptive immune response (12). The latter generates long term target-specific immune memory. Both types of responses require specialized white blood cell types, such as granulocytes, macrophages, and natural killer cells that are innate immune populations, while different types of T- and B-lymphocytes constitute the adaptive immune cells. Although often designated distinct immune responses it should be realized that innate and adaptive responses are highly interdependent.

In obesity, both local and systemic immune alterations occur due to metabolic stress (13). In adipose tissue, the anti-inflammatory/immune regulatory primed immune cells (e.g., M2-type macrophages, regulatory T cells (Treg), T-helper (Th)2, type 2 innate lymphoid cells (ILC2) normally present in lean adipose tissue are replaced by increased numbers of pro-inflammatory primed immune cells (e.g., M1 macrophages, Th1, Th17, $CD8^+$ T-cells) that secrete pro-inflammatory cytokines such as IL-1β, IL-6, IL-17, and IFN-γ. This may even be further enhanced by obesity-associated gut inflammation (13). In addition to local adipose tissue immune alterations, systemic immune adaptations are observed in obesity as well, including increased numbers of circulating (inflammatory) monocytes, neutrophils, Th1, Th17, Th22, decreased circulating Treg and elevated pro-inflammatory cytokine levels (14, 15). In concert, these changes lead to a pro-inflammatory state of the immune system in individuals with obesity, characterized by elevated cytokine levels, both locally in the adipose tissue as well as systemically, as depicted in **Figure 1**. This chronically elevated inflammatory state is thought to stimulate regulatory pathways, which in turn limit the response to an acute trigger such as SARS-CoV-2. This is exemplified by the hampered antiviral type I interferon response in individuals with obesity (16).

Besides systemic upregulation of pro-inflammatory cytokines, severe COVID-19 is associated with lymphopenia and T cell exhaustion (17). This may be related to functional depletion of the antigen-presenting dendritic cells due to viral infection, since they express both angiotensin-converting-enzyme 2 (ACE2) and the alternative viral receptor DC-SIGN (18). Substantial T-cell apoptotic death might then be caused by T-cell receptor engagement in the absence of appropriate co-stimulation, normally provided by dendritic cells. Moreover, SARS-CoV-2 hampers the function of $CD4^+$ T cells and promotes excessive activation and possibly subsequent exhaustion of $CD8^+$ T cells. Together, these perturbations of T cell subsets likely diminish host antiviral immunity (19). Obesity further worsens this condition as DC maturation appears to be hampered, which in itself already impacts the elicitation of appropriate T-cell responses (20).

Endocrine and Metabolic Dysregulation in Adipose Tissue

Adipose tissue changes play an important role in obesity associated chronic systemic inflammation. Changes in adipokine secretion, fatty acid induced inflammation, oxidative stress, endoplasmic reticulum (ER) stress, and adipose tissue hypoxia are proposed mechanisms (21). Adipocytes secrete adipokines such as leptin and adiponectin, that can modulate the immune system (21, 22). In obesity, leptin secretion is increased. Leptin is pro-inflammatory and stimulates monocyte proliferation and differentiation into macrophages, modulates the activation of natural killer cells, and induces the production of pro-inflammatory cytokines such as TNFα, IL-6, or IL-12 (21). However, leptin inhibits anti-viral type I interferons (16). At the same time, the secretion of adiponectin, which has opposite immunomodulatory effects, is diminished. Adiponectin inhibits phagocytic activity and production of TNFα production, the synthesis of adhesion molecules, the formation of foam cells and stimulates the release of anti-inflammatory cytokines (e.g., IL-10). Nutrient excess and cell expansion in obesity can induce ER stress. ER stress is linked to the production of reactive oxygen species and the activation of inflammatory pathways (21). In addition, adipose tissue expansion in obesity eventually reaches a point where the capacity of local vasculature is insufficient leading to hypoxia. This hypoxic state aggravates the pro-inflammatory profile through further upregulation of leptin, downregulation of adiponectin, and increased production of pro-inflammatory cytokines by resident macrophages (21).

Respiratory Impairment

Fat deposits in the mediastinum and abdominal cavity reduce compliance of the lungs, chest wall, and entire respiratory system. This likely contributes to respiratory symptoms of obesity such as wheeze, dyspnea, and orthopnea (23). The mechanical effects of obesity produce airway narrowing and closure, increased respiratory system resistance, reduced functional residual capacity, airway hyperresponsiveness, and a ventilation perfusion mismatch, all of which decrease lung function (23). Thus, altered lung function due to mechanical stress probably contributes to the worse prognosis in patients with obesity and COVID-19.

Hemostasis

Obesity is independently associated with both arterial and venous thrombotic events (24). Related mechanisms are increased levels of clotting factors (tissue factor, Factor VII, Factor VIII, and plasminogen activator inhibitor) inflammation and endothelial dysfunction (24). COVID-19 in its severe form is also associated with both serious systemic inflammation and a prothrombotic state, as reflected by a significant increase in fibrinogen and D-dimer levels and high rates of severe pulmonary embolism that forecast a poor prognosis (25). This could constitute another link between obesity and worse outcome in COVID-19 but more research is required to confirm this notion.

ACE2 Receptor, Viral Reservoir and Lymphopenia

Other potential mechanisms through which obesity may lead to worse COVID-19 prognosis are high ACE2 receptor expression and comorbidities. Expression of ACE2—the functional receptor for SARS-CoV-2 and other corona viruses—is upregulated in adipocytes of patients with obesity and diabetes, which turns

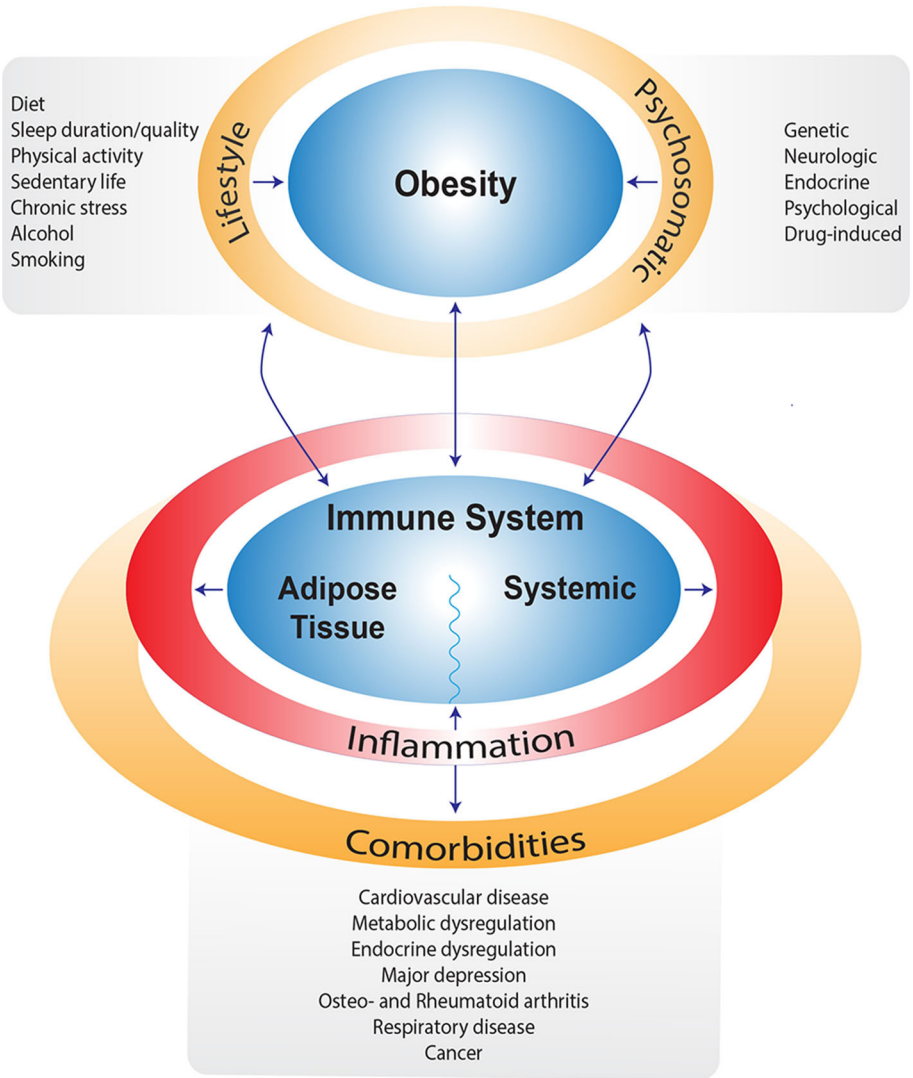


FIGURE 1 | Schematic overview of factors contributing to obesity and/or impairing weight loss. Next to obesity, most of these factors are also directly related to alterations in the immune system leading to a pro-inflammatory state and subsequent comorbidities.

adipose tissue into a potential target and viral reservoir (11). Potentially, this could result in prolonged viral shedding by adipose tissue, with extended activation of local "preactivated" immune responses and resident cytokine signaling pathways. However, currently, there is no evidence yet for direct SARS-CoV-2 infection of adipose tissue.

Comorbidities

Comorbidities associated with obesity have become major health problems in patients that have been admitted to the hospital (8). Below, we discuss the influence of metabolic syndrome, T2D, CVD, and obstructive sleep apnea (OSA) on the immune system. Obesity associated insulin resistance drives the metabolic syndrome, a condition characterized by a clustering of three or more of the following: central adiposity, elevated blood glucose, elevated plasma triglycerides, elevated blood pressure, and low plasma high density lipoprotein (26). Components of the metabolic syndrome are associated with low-grade chronic inflammation. In metabolic syndrome, levels of leptin are increased aggravating leptin resistance enhancing chronic inflammation. Also, circulating adiponectin levels are decreased in metabolic syndrome, which impairs the resolution of inflammation. In itself, metabolic syndrome increases the risk of T2D and CVD by 2- and 5-fold, respectively (26).

The presence of T2D aggravates the prognosis in patients with COVID-19 (27, 28). It is associated with an approximately 2-fold increase of severe/critical COVID-19 illness, after adjustment for age, sex, obesity, hypertension, and smoking (28). Also, higher ICU admission rates and mortality risk were reported in patients with diabetes and COVID-19 (29). The hazard ratio of death was 1.53 in patients with diabetes after adjustment for age, sex, hypertension, CVD, and cerebrovascular disease (27). Notably,

SARS-CoV-1-, MERS-, and influenza-infections are also marked by more severe symptoms in people with diabetes (27). Metabolic disruption, low grade systemic inflammation, and an unbalanced immune response may explain the more serious disease course of infections in T2D (28). This is further discussed in the Diet section.

CVD is a common comorbidity in COVID-19 (5). We did not find any literature that specifically explored the relationship between CVD and the capacity to respond to infectious challenges. Also, hypertension is often reported as a highly frequent comorbidity in severely ill patients with COVID-19 (5). Studies reveal higher ICU admission rates and higher mortality rates in patients with COVID-19 with hypertension (30). However, these data should be interpreted with caution as age and CVD may be important confounders (31). To date, there is no conclusive evidence for an independent causal link between hypertension and increased susceptibility for infectious diseases. Nonetheless, it should be realized that inflammatory conditions caused by innate and adaptive immune responses contribute to, or may even underlie alterations in vasculature, kidney, and the regulating sympathetic nervous system, which together cause hypertension (32).

It is believed that the underlying presence of OSA facilitates susceptibility to SARS-CoV-2 infection, and increases the risk for severe COVID-19 and the overall mortality of the disease (33). The CORONADO study shows that treated OSA is independently associated with risk of death by day 7 (34). Research suggests that when controlling for obesity, the presence of OSA is associated with decreased lung function, decreased lung-transfer factor for carbon monoxide, and, importantly, increased lung inflammation (35). Obesity plays a pivotal role in the pathogenesis of OSA and weight loss of approximately 7–11% leads to clinically significant and meaningful improvement of OSA (36). Lifestyle modification with reduced calorie intake and increased physical activity forms the foundation of all weight loss interventions and seems to have weight-independent benefits in OSA (36). Below we discuss the potential benefits of lifestyle adaptations in light of COVID-19 and its underlying risk factors.

THE INFLUENCE OF LIFESTYLE FACTORS ON THE IMMUNE SYSTEM

When discussing the relation between obesity and the severity of COVID-19, it is important to emphasize that obesity is a complex disease. Next to societal and lifestyle factors, numerous other factors contribute to weight gain or impair weight loss. For example, medication may promote weight gain, as well as endocrine or hypothalamic disease, genetic syndromes or psychiatric conditions (37). It is counterproductive to blame people for their obesity or for severe progression of COVID-19. The obesity stigma is a serious problem in this context, and guiding people in their attempt to lose weight in a respectful way is of crucial importance (38, 39). Furthermore, when intensive lifestyle intervention turns out to be insufficient to lose weight, other obesity treatments such as pharmacotherapy or bariatric surgery should be considered to attain a healthier weight (40).

The American Endocrine Society and the European Association for the Study of Obesity both include lifestyle interventions, bariatric surgery and pharmacotherapy in recommended weight reduction strategies for people with obesity (41, 42). Besides weight reduction effects, bariatric surgery and pharmacotherapy have also been shown to positively affect the immune system in obese patients (43–46). The rate of weight loss for optimal immune system function is yet unclear. It is known that relatively fast weight reduction through bariatric surgery as well as more gradual weight loss through lifestyle modification both improve immune system markers (44, 47). On the other hand, severe caloric restriction, as well as malnutrition, reduces immunity and renders one more susceptible for disease (48, 49). Nevertheless, the negative impact of obesity on general health and responses to infections, including COVID-19 as discussed above, warrants vigorous lifestyle intervention (47).

Lifestyle features (nutrition, physical activity, sleep, stress, alcohol, smoking) interact with genetics to disrupt physiology and cause disease (50). In this context, a low grade inflammatory process appears to play a central role in many distinct chronic non-communicable disorders (51). Since lifestyle is consequently at the root of these diseases, it follows that lifestyle intervention should be an integral part of their treatment. Accordingly, a recent study shows that lifestyle intervention can partly reverse the pro-inflammatory phenotype associated with obesity (47). Even though the decline in pro-inflammatory markers was correlated with a decline of waist circumference and markers of metabolic syndrome, metabolic improvement was not required for immunological recovery. This suggests that lifestyle factors can directly affect the function of the immune system. Below we discuss the influence of diet, physical activity, stress, smoking, alcohol and sleep on the immune system as depicted in **Figure 2**.

Diet

In affluent countries, chronic overeating of diets comprising a high proportion of ultra-processed energy-dense and nutrient-poor foods and drinks induces systemic low-grade inflammation, also referred to as "metaflammation" (52). These Western diet-components are thought to induce so-called "trained innate immunity," i.e., the functional reprogramming of innate immune cells by exogenous or endogenous stimuli. Training leads to a more vigorous response toward a second activating challenge, and this condition may be retained for a long period. This innate memory gives rise to an elevated inflammatory set point of innate immune cells, which is mediated through alterations in their progenitor cells (53). Exposure to adverse metabolic stimuli induces epigenetic changes during early development of these immature innate immune cells, which facilitate the expression of inflammatory genes. The mechanisms explaining inappropriate adaptive immune activation against pathogens in the presence of enhanced innate-driven inflammation are far from clear at present. They might include immune suppression by activated myeloid cells or regulatory T- and B-cells as well as inappropriate skewing of adaptive responses (54).

The enhanced inflammatory set point in obesity is reminiscent of changes occurring during aging, which is also characterized by increase of inflammatory parameters and designated as

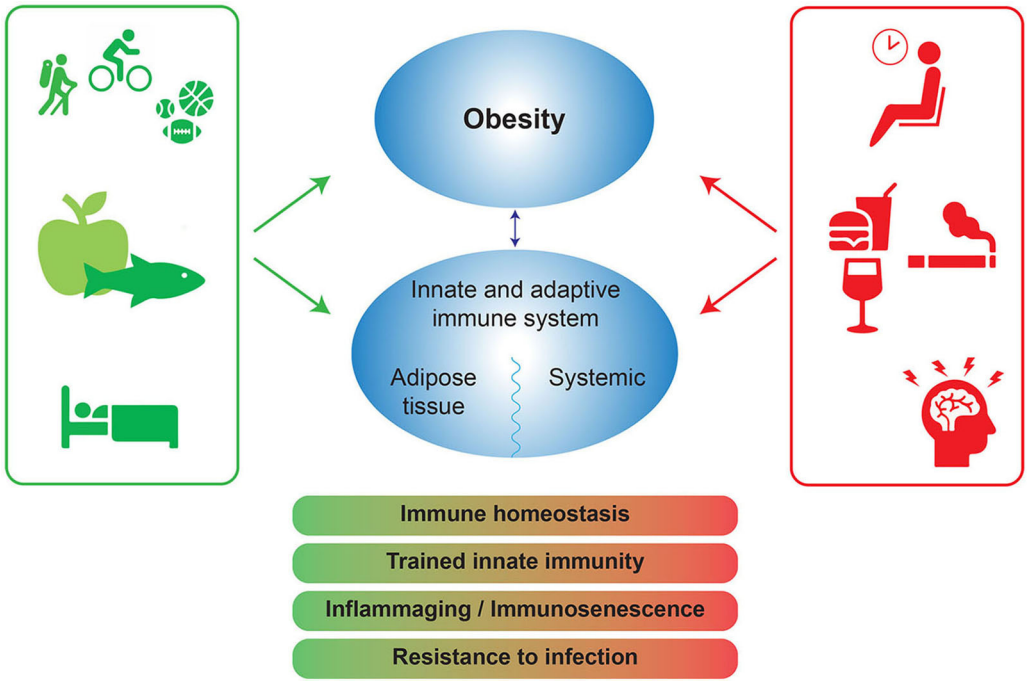


FIGURE 2 | Positive and negative lifestyle factors influence obesity and the immune system. The negative lifestyle factors (red) can lead to a disbalance in immune homeostasis, trained innate immunity, inflammaging, and a decrease in infection resistance. Positive factors (green) can protect immune homeostasis and potentially increase resistance to infection. Trained innate immunity: long term functional reprogramming of innate immune cells, which is evoked by exogenous or endogenous stimuli and which leads to a usually enhanced response toward a second challenge after return to a non-activated state. Inflammaging: chronic low-grade inflammation that occurs with aging and is characterized by dysregulated inflammatory responses, in general resulting in increased inflammatory responses and diminished responses to pathogens like influenza or SARS-CoV-2.

"inflammaging" (55). In fact, alterations in DNA methylation in blood cells and other tissues can be used as epigenetic marker of biological age, the 'epigenetic clock' (56). Lifestyle-related risk factors, including obesity and smoking, accelerate epigenetic aging (55). Moreover, obesity may promote thymic involution, leading to diminished output of naïve T-lymphocytes, which accelerates immunosenescence (57).

Dampening inflammation by adequate intake of fruit and vegetables is essential for prevention of chronic metabolic diseases, and sufficient consumption thereof is reflected by inhibition of epigenetic aging (55). Many fruits and vegetables, e.g., tomatoes, garlic, berries, apples, broccoli, grapes and olives, have anti-inflammatory effects (58). A number of vitamins (A, B6, B12, folate, C, D, and E) and trace elements (zinc, copper, selenium, iron) have been demonstrated to play important and complementary roles in supporting both the innate and adaptive immune systems (59). Foods such as nuts, meat, eggs, shellfish, and wholegrains are good sources of many of these trace elements. In addition, omega-3 fatty acids derived from fatty fish have anti-inflammatory action (59). There is evidence that omega-3 fatty acids can control the cytokine storm in acute respiratory distress syndrome (ARDS) (60). Such a diet is consistent with current healthy eating guidelines (60).

Foods containing probiotic and prebiotic substances can maintain a healthy microbiota, which can also benefit the immune system (60). The gut microbiota plays a role in educating and regulating the immune system. Gut dysbiosis is a feature of obesity, chronic disease and many infectious diseases, and has been described in COVID-19. Dietary approaches to achieve a healthy microbiota can also benefit the immune system. Indeed, optimal nutrient intake promotes optimal immune function and may limit the impact of novel, more virulent pathogenic viruses (59). It must be emphasized that severe caloric restriction, as well as malnutrition, reduces immunity and renders one more susceptible for disease (48, 49). Extreme and prolonged reduction of caloric intake or crash diets are therefore strongly discouraged.

Experimental evidence of the effects of diet in relation to COVID-19 is still lacking. However, it is generally accepted that a diet rich in vegetables, fruit, nuts, legumes, fish, and 'healthy' dietary fats is associated with a lower risk of chronic non-communicable diseases as well as improved immune function (61). A range of nutrients or bioactive compounds has been proposed to explain this association. Examples are polyphenols, vitamin D, n-3 PUFA in autoimmune and inflammatory disorders, and vitamin D, vitamin E, zinc, and probiotics in reduction of infection (62). A balanced whole foods version of a vegetarian or Mediterranean diet fits this description. Conversely, a diet that is rich in energy-dense, nutrient-poor foods (i.e., consisting of ultra-processed foods with high levels of refined carbohydrates, added sugar and fats) is related to an increased risk of non-communicable diseases and impaired immune function (63). Moreover, a recent study

found that ultra-processed food stimulated additional intake of approximately 500 kcal/day when compared to content-matched unprocessed diet (64).

Physical Activity

Similar to the Western diet, a sedentary lifestyle is associated with abdominal adiposity, a pro-inflammatory state and increased risk of infection (65). Besides facilitating weight loss, regular physical activity decreases the activation of the immune system and improves immunosurveillance and immunocompetence (65). Regular exercise may limit or delay inflammaging (66). Subsequently, a physically active lifestyle reduces the risk of contracting a range of communicable diseases including viral and bacterial infections (65, 66).

The near-daily practice of moderate intensity exercise (e.g., walking or cycling) reduces upper respiratory tract infection symptom days by 40–50% (67). There is a dose-response relationship between the intensity of regular exercise and the risk for infection (65). Exercise intensity is commonly expressed as the Metabolic Equivalent of Task (MET) which is the ratio of a person's working metabolic rate relatively to their resting metabolic rate. Light to moderate exercise (MET 2–5.9, e.g., walking, vacuum cleaning or yoga) is better than inactivity (MET <2, e.g., writing or desk work) but vigorous exercise (MET 6–8.7, e.g., running, basketball or swimming) is even better. On similar note, short periods of maximum intensity training (High-intensity interval training or HIIT) seem to be a valuable addition to moderate-intensity continuous training in terms of enhancing cardiorespiratory fitness, metabolic hemostasis, vascular function and immune system function (68, 69). However, prolonged and intensive or (near) maximal intensity exercise is associated with an increased illness risk (67).

Substantial reduction in acute respiratory infection illness is detectable as soon as after 8 weeks of moderate intensity exercise (70). Most studies used a moderate intensity program of 30–45 min/day for 5 days/ week (67). This is in line with the Global recommendations on Physical Activity for Health on which most national guidelines are based (71). Thus, adoption of a physically active lifestyle by a substantial percentage of the population would probably curtail infectious pandemics such as COVID-19 and non-infectious pandemics such as obesity (65).

Stress

Different emotional states, or exposure to psychological stressors, are associated with enhanced susceptibility to, or increased severity of disease through nervous system-induced alterations in innate and adaptive immunity (72). A main component of the stress response is cortisol, which production and secretion are regulated by the hypothalamus-pituitary-adrenal axis (73). Cortisol has many functions in the human body, such as mediating the stress response, and regulating metabolism as well as, inflammatory-, and immune function (73). Cortisol is ordinarily anti-inflammatory and confines the immune response, but chronic hypercortisolemia can lead to resistance of the immune system. The accumulation of stress hormones gives rise to an increased production of inflammatory cytokines that compromises the immune response (74). It has been demonstrated that individuals reporting higher levels of stress are more likely to develop clinical symptoms during experimental respiratory viral infection (72). Behavioral interventions targeted at alleviating stress, promoting heightened states of relaxation and encouraging moderate physical activity have been shown to bolster anti-viral immune responses and decrease markers of inflammation (75). A meta-analysis focusing on psychological interventions to alleviate stress showed positive effects on the immune system. It even suggested a positive relation between the frequency of self-reported of stress-reducing practice and a more adequate functioning of the immune system (76). Besides the impact on inflammatory markers, stress also promotes other negative lifestyle factors such as unhealthy eating and poor sleeping quality (77).

Smoking

Nicotine and other tobacco components have several pro- as well as anti-inflammatory effects, and thus contribute to an altered immune set point in smokers (78). Current or former smoking is a risk factor for severe disease in patients with COVID-19. Smokers appear to have a 40% higher chance of developing severe symptoms, and a 140% higher chance of being admitted to an ICU, need mechanical ventilation or die as compared to non-smokers (79). A similar observation was made in patients with influenza (80). Smokers were over 5 times more likely to develop laboratory-confirmed influenza, and 34% more likely to develop influenza-like illness than non-smokers. Importantly, smoking cessation recovers airway ciliary clearance and immune function as early as 1 month after the intervention (81). Thus, smoking cessation is strongly encouraged as a public health measure to limit the global impact of COVID-19 (81).

Alcohol

Alcohol consumption affects the number, survival and function of both innate and adaptive immune cells, thereby interfering with immune responses (82). Consumption of high doses can directly suppress a wide range of immune responses, and alcohol abuse is associated with an increased incidence of infectious disease (83). For instance, alcoholics are highly susceptible to respiratory pathogens and lung injury, and have a 2–4 fold increased risk of ARDS (84). Furthermore, alcohol consumption increases the risk of community-acquired pneumonia in adults (85). A dose-response relationship is observed, indicating that the risk for pneumonia increases by 8% for every 10–20 g (one unit of alcohol) higher daily alcohol intake. Surprisingly, even a single episode of binge drinking can have measurable effects on the immune system, inducing a transient pro-inflammatory state within the first 20 min after alcohol ingestion, followed by an anti-inflammatory state 2–5 h after alcohol ingestion (82). Also in animal models of pulmonary infections, alcohol administration is associated with adverse clinical parameters and increased lung damage (82). In many cultures, drinking alcohol is socially accepted and integrated in daily life. Increased awareness of the negative effects of alcohol and making moderation or abstinence the default choice are of paramount importance for public health.

Sleep

It is suggested that both circadian rhythm and sleep significantly modify the susceptibility to SARS-CoV-2 infection as well as the overall clinical manifestations of COVID-19 (86). This relates to an interdependent balance maintained between the circadian clock, the intestinal microbiota and immune system activity (87). Naturally occurring sleep acutely enhances the expression of cytokines such as IL-12 and IFN-γ that regulate anti-viral immune defense mechanisms (88). Also, sleep might improve immunological memory formation by boosting the number of antigen presenting cells and CD4+ T-cells (89). The central nervous system seems to use the metabolically quiescent period of sleep to regulate both innate and antiviral immune responses (88). Sleep deprivation leads to decreased lymphocyte blastogenesis and natural killer cell activity, and upregulates IL-1 and IL-2 (90). However, sleep also increases IL-2 production by T-cells (88). Short habitual sleep (<6 h) is associated in humans with reduced life span, increased vulnerability to viral infection and reduced antibody titers after vaccination (91). In addition, short-term sleep deprivation prior to vaccination appears to negatively impact antibody titers after influenza vaccination (92). In rodents, sleep disturbances reduces influenza vaccine efficacy (93). When attempting to improve on sleep quality to boost the immune system, paucity of evidence prevents strong recommendations. However, a key point is that in current times circadian rhythm and sleep disruption frequently occur through shift work, nighttime light exposure and social jet lag (91). Morin et al. provide a condense overview of recommendations to improve sleep quality, especially during the COVID-19 pandemic (**Table 2**) (94). Further research could explore the effects of these recommendations on immunity and susceptibility to infections.

TABLE 2 | Strategies to manage sleep disturbances during the COVID-19 pandemic.

1. Make sleep a priority and reserve at least 7–8 h per night for sleep.
2. Keep a regular schedule for sleep, meals, work, and social contacts. These activities are important time cues that help maintain entrainment of one's biological clock.
3. Get as much daylight exposure as possible (turn on the lights, open the curtains, go outside if possible) to regulate sleep-wake and circadian rhythms.
4. Avoid using electronics (cellular, tablets) in the bed or bedroom or near bedtime.
5. Allow at least 1 h to unwind before bedtime.
6. If sleep does not come within 15–20 min, go to another room and engage in quiet activities (reading), and return to bed only when sleep is imminent.
7. Reserve the bed and bedroom for sleep and sex only. This is not the place to worry, problem-solve, or plan the next day.
8. Get up at the same time every morning, regardless of the amount of sleep. While it may be tempting to sleep in, particularly when there is no obligation to be at work, it is best to arise at the same time to keep a regular sleep-wake schedule.
9. Although it is better to avoid napping for someone with insomnia, napping is beneficial for sleep-deprived people. Older adults, without sleep problems, may also benefit from a short catnap (15–20 min) around mid-day.
10. Keep in mind that short bouts of insomnia is a normal reaction to stressful life events, but when sleep difficulties occur several nights per week, take action and seek professional help to prevent acute insomnia from turning into chronic insomnia.

Adopted from Morin et al. (94).

TABLE 3 | Aspects of lifestyle to be addressed to improve immune function.

Diet	• Increase the intake of healthy foods (e.g., vegetables, fruits, nuts, legumes, whole grains, and fish)
	• Decrease the intake of unhealthy foods and drinks (e.g., ultra-processed, refined carbohydrates, added sugars, and fats)
Physical activity	• Increase adherence to (inter)national physical activity guidelines
	• Decrease sedentary behavior
Stress	• Increase active relaxation
	• Decrease chronic stress (e.g., in studies, jobs, and social life)
	• Increase skills and opportunities to cope with chronic stress
Smoking and alcohol	• Decrease the marketing, sale and use of (legal) addictive substances such as tobacco and alcohol
	• Provide help for those addicted
Sleep	• Increase sleeping quality

AN UPSTREAM ENVIRONMENTAL APPROACH TOWARD HEALTHIER LIFESTYLES IS NEEDED

Thus, we know that obesity, chronic disease and their underlying causes impair immune function and leave individuals more vulnerable to infection. These underlying causes include behavioral and modifiable factors such as an unhealthy diet, inadequate physical activity, chronic stress, use of alcohol and tobacco and suboptimal sleep quality (**Table 3**). To improve on all these aspects of health, multiple methods can be used. We make a case for health education, choice architecture, and regulation on local, national, and international levels.

Undeniably, widespread health education is an important aspect of reaching these lifestyle goals. This challenge is a shared responsibility of healthcare professionals, governments, the private sector, and civil society. Healthcare organizations and professionals should advocate the use of lifestyle as therapy in its own right, of equal importance to medication and interventions. Lifestyle modification should be an integral part of chronic disease management. National and local policies should address social determinants of health and health inequalities (95).

Extra attention should be given to personal health and lifestyle factors in periods of complete or partial lockdown. Lockdown periods lead to increases in sedentary behavior (unhealthy) food consumption, alcohol consumption, tobacco use, stress, and decreased well-being (96–98). Distressingly, increased food consumption and snacking are more prevalent in people with overweight or obesity. Even 2 weeks of physical inactivity and a positive energy balance can lead to reduced insulin sensitivity, higher total body fat, and a proinflammatory state (99). People should be aware of the influence of lockdown on their daily habits and be guided to eat healthy, exercise adequately, and find help for psychosocial adversities. We conclude that advocating

TABLE 4 | Facets of the campaign for healthier lifestyles.

Health education	• Public
	• Healthcare professionals
	• Policy makers
	• Extra attention during lockdown
Environment	• Supermarkets, Schools, Workplaces
	○ Food positioning (106)
	• Built environment
	○ Physical activity and active transport (108)
Regulation	• Taxation, pricing, ban, and restriction of advertising and sponsorship (109)

home-confinement should be accompanied by stressing the need to maintain a healthy lifestyle.

Besides health education, we need to realize that our environment influences our choices substantially. Choices are made by means of two cognitive processes: the reflective and the impulsive system. The former is rational and conscious, the latter is quick, automatic, and subconscious (100). In the context of the current environment, we make approximately 219 decisions in regard to food per day, of which 90% subconsciously (101). Default options such as easy availability of unhealthy foods significantly influence our choices in a negative way (102). Thus, in order to promote healthy choices we need to create an environment in which the default option is a healthy option.

In many parts of the world people currently live in an obesogenic environment (103). In recent decades most societies increasingly offer an overabundance of cheap and convenient energy-dense and nutrient-poor foods and drinks, which contribute to adverse dietary choices. Intensive marketing of ultra-processed foods only makes matters worse. Moreover, many of the current environments promote sedentary behavior and discourage physical activity (104). As a society, we need to promote a healthy lifestyle, and thereby decrease rates of obesity and improve immune function. To do so, we need to reconstruct our living environment so as to facilitate and promote healthy dietary choices, physical activity, stress relieve, and regular sleep (105). Changing choice architecture can be done by changing food positioning in supermarkets, schools, or workplaces (106, 107). Another example is using built environment to increase physical activity and active transport (108).

In addition to choice architecture, creating healthy communities requires regulation by governments aimed at protecting society from the commercial determinants of chronic non-communicable diseases (109). This includes regulating the sale and promotion of unhealthy commodities such as tobacco, alcohol, and ultra-processed food and drinks (109). Strong leadership is essential to resist attempts by powerful organizations with vested interests (e.g., the tobacco, food, and alcohol industries) to undermine the development and implementation of effective policies and laws (110). Evidence-based approaches such as legislation, regulation, taxation, pricing, ban, and restriction of advertising and sponsorship should be introduced to reduce consumption of unhealthy commodities (109). All in all, the campaign for healthier lifestyle includes, but is not limited to, aspects on health education, environmental changes, and regulatory actions (**Table 4**).

CONCLUSIONS

COVID-19 has significant impact on public health. Obesity, chronic disease, and various lifestyle components increase the severity of the disease course, the appeal to health care, and the mortality rate of SARS-CoV-2 infections. The harmful impact of these factors on the immune system probably explains the worse outcome to a large extent. Conditions associated with low-grade systemic inflammation, such as obesity, metabolic syndrome, and old age, hamper an optimal and timely response by the immune system to an infectious challenge like SARS-CoV-2. Several Western diet components train innate immune cells so as to adopt a more pro-inflammatory set point, which enigmatically diminishes their capacity to activate adequate adaptive immunity required for an effective battle against pathogens.

Adapting lifestyle on a global scale can most likely enhance population resistance to viral and other infections. However, making healthy choices is not as easy as knowing what to choose. In this context it is important to be aware of the existing obesity stigma and to counteract it. Obesity and related cardiometabolic diseases are not only a problem of the individual, but a burden for society as well. These metabolic disorders are primarily caused by environmental and societal factors, in concert with genetic and other individual (biological) characteristics. Reconstruction of our living environment so as to promote healthier lifestyles is of paramount importance to both prevent and treat obesity and related disorders. Obviously, a collective effort is required to get this done. Healthcare institutions, governments, the private sector, and (creative) citizens have to put their heads together to map out the route to a healthy future. Together we can change our lifestyle-choice architecture to make healthy living the norm, and thereby improve immune function to be better prepared for the next viral pandemic.

AUTHOR CONTRIBUTIONS

DF wrote and performed the literature searches, analyzed the results, selected relevant articles for this manuscript, and drafted the concept version of the manuscript. ER took the lead in supervising the creation process, provided content related to obesity, and gave feedback on every draft. DA, PL, WD, HP, and JS provided feedback during the creation process. Specifically on their fields of expertise but also on the manuscript as a whole. WD and PL designed both figures adopted in the manuscript. All authors provided them with feedback. All in all, DA, PL, WD, HP, and JS contributed roughly equally to the formation of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.597600/full#supplementary-material

REFERENCES

1. Harapan H, Itoh N, Yufika A, Winardi W, Keam S, Te H, et al. Coronavirus disease 2019 (COVID-19): a literature review. *J Infect Public Health*. (2020) 13:667–73. doi: 10.1016/j.jiph.2020.03.019
2. Worldometers.info. *Worldometer Coronavirus Dover, Delaware, U.S.A*. (2020). Available online at: https://www.worldometers.info/coronavirus/ (accessed August 20, 2020).
3. Kushner RF, Sorensen KW. Lifestyle medicine: the future of chronic disease management. *Curr Opin Endocrinol Diabetes Obes*. (2013) 20:389–95. doi: 10.1097/01.med.0000433056.76699.5d
4. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*. (2020) 323:1239–42. doi: 10.1001/jama.2020.2648
5. Garg S, Kim L, Whitaker M, O'Halloran A, Cummings C, Holstein R, et al. Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019. COVID-NET, 14 states. *Morb Mortal Wkly Rep*. (2020) 69:458–64. doi: 10.15585/mmwr.mm6915e3
6. Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell LF, Chernyak Y, et al. Factors associated with hospitalization and critical illness among 4,103 patients with COVID-19 disease in New York City. *BMJ*. (2020) 369:m1966. doi: 10.1136/bmj.m1966
7. Gao F, Zheng KI, Wang XB, Sun QF, Pan KH, Wang TY, et al. Obesity is a risk factor for greater COVID-19 severity. *Diabetes Care*. (2020) 43:e72–4. doi: 10.2337/dc20-0682
8. Petrakis D, Margină D, Tsarouhas K, Tekos F, Stan M, Nikitovic D, et al. Obesity—a risk factor for increased COVID-19 prevalence, severity and lethality (Review). *Mol Med Rep*. (2020) 22:9–19. doi: 10.3892/mmr.2020.11127
9. Lighter J, Phillips M, Hochman S, Sterling S, Johnson D, Francois F, et al. Obesity in patients younger than 60 years is a risk factor for COVID-19 hospital admission. *Clin Infect Dis*. (2020) 71:896–7. doi: 10.1093/cid/ciaa415
10. Tsatsanis C, Margioris AN, Kontoyiannis DP. Association between H1N1 infection severity and obesity-adiponectin as a potential etiologic factor. *J Infect Dis*. (2010) 202:459–60. doi: 10.1086/653842
11. Ryan PM, Caplice NM. Is adipose tissue a reservoir for viral spread, immune activation, and cytokine amplification in coronavirus disease 2019? *Obesity (Silver Spring, Md)*. (2020) 28:1191–4. doi: 10.1002/oby.22843
12. Yatim KM, Lakkis FG. A brief journey through the immune system. *Clin J Am Soc Nephrol*. (2015) 10:1274–81. doi: 10.2215/CJN.10031014
13. Kane H, Lynch L. Innate immune control of adipose tissue homeostasis. *Trends Immunol*. (2019) 40:857–72. doi: 10.1016/j.it.2019.07.006
14. Zhao R, Tang D, Yi S, Li W, Wu C, Lu Y, et al. Elevated peripheral frequencies of Th22 cells: a novel potent participant in obesity and type 2 diabetes. *PLoS ONE*. (2014) 9:e85770. doi: 10.1371/journal.pone.0085770
15. Touch S, Clément K, André S. T cell populations and functions are altered in human obesity and type 2 diabetes. *Curr Diab Rep*. (2017) 17:81. doi: 10.1007/s11892-017-0900-5
16. Terán-Cabanillas E, Hernández J. Role of leptin and SOCS3 in inhibiting the type i interferon response during obesity. *Inflammation*. (2017) 40:58–67. doi: 10.1007/s10753-016-0452-x
17. Wilk AJ, Rustagi A, Zhao NQ, Roque J, Martínez-Colón GJ, McKechnie JL, et al. A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nat Med*. (2020) 26:1070–6. doi: 10.1038/s41591-020-0944-y
18. Brufsky A, Lotze MT. DC/L-SIGNs of hope in the COVID-19 pandemic. *J Med Virol*. (2020) 92:1396–8. doi: 10.1002/jmv.25980
19. Zheng H-Y, Zhang M, Yang C-X, Zhang N, Wang X-C, Yang X-P, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cell Mol Immunol*. (2020) 17:541–3. doi: 10.1038/s41423-020-0401-3
20. O'Shea D, Corrigan M, Dunne MR, Jackson R, Woods C, Gaoatswe G, et al. Changes in human dendritic cell number and function in severe obesity may contribute to increased susceptibility to viral infection. *Int J Obes (Lond)*. (2013) 37:1510–3. doi: 10.1038/ijo.2013.16
21. de Heredia FP, Gómez-Martínez S, Marcos A. Obesity, inflammation and the immune system. *Proc Nutr Soc*. (2012) 71:332–8. doi: 10.1017/S0029665112000092
22. Larabee CM, Neely OC, Domingos AI. Obesity: a neuroimmunometabolic perspective. *Nat Rev Endocrinol*. (2020) 16:30–43. doi: 10.1038/s41574-019-0283-6
23. Dixon AE, Peters U. The effect of obesity on lung function. *Expert Rev Respir Med*. (2018) 12:755–67. doi: 10.1080/17476348.2018.1506331
24. Darvall KA, Sam RC, Silverman SH, Bradbury AW, Adam DJ. Obesity and thrombosis. *Eur J Vasc Endovasc Surg*. (2007) 33:223–33. doi: 10.1016/j.ejvs.2006.10.006
25. Pineton de Chambrun M, Frere C, Miyara M, Amoura Z, Martin-Toutain I, Mathian A, et al. High frequency of antiphospholipid antibodies in critically-ill COVID-19 patients: a link with hypercoagulability? *J Intern Med*. (2020). doi: 10.1111/joim.13126. [Epub ahead of print].
26. Andersen CJ, Murphy KE, Fernandez ML. Impact of obesity and metabolic syndrome on immunity. *Adv Nutr*. (2016) 7:66–75. doi: 10.3945/an.115.010207
27. Yan Y, Yang Y, Wang F, Ren H, Zhang S, Shi X, et al. Clinical characteristics and outcomes of patients with severe COVID-19 with diabetes. *BMJ Open Diabetes Res Care*. (2020) 8:e001343. doi: 10.1136/bmjdrc-2020-001343
28. Targher G, Mantovani A, Wang XB, Yan HD, Sun QF, Pan KH, et al. Patients with diabetes are at higher risk for severe illness from COVID-19. *Diabetes Metab*. (2020) 46:335–7. doi: 10.1016/j.diabet.2020.05.001
29. Roncon L, Zuin M, Rigatelli G, Zuliani G. Diabetic patients with COVID-19 infection are at higher risk of ICU admission and poor short-term outcome. *J Clin Virol*. (2020) 127:104354. doi: 10.1016/j.jcv.2020.104354
30. Cook TM. The importance of hypertension as a risk factor for severe illness and mortality in COVID-19. *Anaesthesia*. (2020) 75:976–7. doi: 10.1111/anae.15103
31. Li G, Li H, Lu J. No adequate evidence indicating hypertension as an independent risk factor for COVID-19 severity. *Clin Res Cardiol*. (2020) 109:1–2. doi: 10.1007/s00392-020-01653-6
32. Norlander AE, Madhur MS, Harrison DG. The immunology of hypertension. *J Exp Med*. (2018) 215:21–33. doi: 10.1084/jem.20171773
33. Tufik S, Gozal D, Ishikura IA, Pires GN, Andersen ML. Does obstructive sleep apnea lead to increased risk of COVID-19 infection and severity? *J Clin Sleep Med*. (2020) 16:1425–6. doi: 10.5664/jcsm.8596
34. Scheen AJ, Marre M, Thivolet C. Prognostic factors in patients with diabetes hospitalized for COVID-19: Findings from the CORONADO study and other recent reports. *Diabetes Metab*. (2020) 46:265–71. doi: 10.1016/j.diabet.2020.05.008
35. Memtsoudis SG, Ivascu NS, Pryor KO, Goldstein PA. Obesity as a risk factor for poor outcome in COVID-19-induced lung injury: the potential role of undiagnosed obstructive sleep apnoea. *Br J Anaesth*. (2020) 125:e262–3. doi: 10.1016/j.bja.2020.04.078
36. Tham KW, Lee PC, Lim CH. Weight management in obstructive sleep apnea: medical and surgical options. *Sleep Med Clin*. (2019) 14:143–53. doi: 10.1016/j.jsmc.2018.10.002
37. van der Valk ES, van den Akker ELT, Savas M, Kleinendorst L, Visser JA, Van Haelst MM, et al. A comprehensive diagnostic approach to detect underlying causes of obesity in adults. *Obes Rev*. (2019) 20:795–804. doi: 10.1111/obr.12836
38. Tomiyama AJ, Carr D, Granberg EM, Major B, Robinson E, Sutin AR, et al. How and why weight stigma drives the obesity "epidemic" and harms health. *BMC Med*. (2018) 16:123. doi: 10.1186/s12916-018-1116-5
39. Alberga AS, Russell-Mayhew S, von Ranson KM, McLaren L. Weight bias: a call to action. *Journal of eating disorders*. (2016) 4:34. doi: 10.1186/s40337-016-0112-4
40. Kahan S, Manson JE. Obesity treatment, beyond the guidelines: practical suggestions for clinical practice. *JAMA*. (2019) 321:1349–50. doi: 10.1001/jama.2019.2352
41. Yumuk V, Tsigos C, Fried M, Schindler K, Busetto L, Micic D, et al. European guidelines for obesity management in adults. *Obes Facts*. (2015) 8:402–24. doi: 10.1159/000442721
42. Apovian CM, Aronne LJ, Bessesen DH, McDonnell ME, Murad MH, Pagotto U, et al. Pharmacological management of obesity: an endocrine society

clinical practice guideline. *J Clin Endocrinol Metabol*. (2015) 100:342–62. doi: 10.1210/jc.2014-3415
43. Jongbloed F, Meijers RWJ, IJzermans JNM, Klaassen RA, Dollé MET, van den Berg S, et al. Effects of bariatric surgery on telomere length and T-cell aging. *Int J Obes (Lond)*. (2019) 43:2189–99. doi: 10.1038/s41366-019-0351-y
44. Moulin CM, Rizzo LV, Halpern A. Effect of surgery-induced weight loss on immune function. *Expert Rev Gastroenterol Hepatol*. (2008) 2:617–9. doi: 10.1586/17474124.2.5.617
45. Jing Y, Wu F, Li D, Yang L, Li Q, Li R. Metformin improves obesity-associated inflammation by altering macrophages polarization. *Mol Cell Endocrinol*. (2018) 461:256–64. doi: 10.1016/j.mce.2017.09.025
46. Drucker DJ. The cardiovascular biology of glucagon-like peptide-1. *Cell Metab*. (2016) 24:15–30. doi: 10.1016/j.cmet.2016.06.009
47. van der Zalm IJB, van der Valk ES, Wester VL, Nagtzaam NMA, van Rossum EFC, Leenen PJM, et al. Obesity-associated T-cell and macrophage activation improve partly after a lifestyle intervention. *Int J Obes (Lond)*. (2020) 44:1838–50. doi: 10.1038/s41366-020-0615-6
48. Arthur SS, Nyide B, Soura AB, Kahn K, Weston M, Sankoh O. Tackling malnutrition: a systematic review of 15-year research evidence from INDEPTH health and demographic surveillance systems. *Glob Health Action*. (2015) 8:28298. doi: 10.3402/gha.v8.28298
49. Collins N. Dietary regulation of memory T cells. *Int J Mol Sci*. (2020) 21(12). doi: 10.3390/ijms21124363
50. Ahmad S, Fatima SS, Rukh G, Smith CE. Gene Lifestyle interactions with relation to obesity, cardiometabolic, and cardiovascular traits among South Asians. *Front Endocrinol (Lausanne)*. (2019) 10:221. doi: 10.3389/fendo.2019.00221
51. Gupta SC, Kunnumakkara AB, Aggarwal S, Aggarwal BB. Inflammation, a double-edge sword for cancer and other age-related diseases. *Front Immunol*. (2018) 9:2160. doi: 10.3389/fimmu.2018.02160
52. Christ A, Lauterbach M, Latz E. Western diet and the immune system: an inflammatory connection. *Immunity*. (2019) 51:794–811. doi: 10.1016/j.immuni.2019.09.020
53. Bekkering S, Saner C, Riksen NP, Netea MG, Sabin MA, Saffery R, et al. trained immunity: linking obesity and cardiovascular disease across the life-course? *Trends Endocrinol Metab*. (2020) 31:378–89. doi: 10.1016/j.tem.2020.01.008
54. Salminen A. Activation of immunosuppressive network in the aging process. *Ageing Res Rev*. (2020) 57:100998. doi: 10.1016/j.arr.2019.100998
55. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. *Aging*. (2018) 10:573–91. doi: 10.18632/aging.101414
56. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. (2018) 19:371–84. doi: 10.1038/s41576-018-0004-3
57. Yang H, Youm YH, Vandanmagsar B, Rood J, Kumar KG, Butler AA, et al. Obesity accelerates thymic aging. *Blood*. (2009) 114:3803–12. doi: 10.1182/blood-2009-03-213595
58. Lapuente M, Estruch R, Shahbaz M, Casas R. Relation of fruits and vegetables with major cardiometabolic risk factors, markers of oxidation, and inflammation. *Nutrients*. (2019) 11:2381. doi: 10.3390/nu11102381
59. Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients*. (2020) 12(4). doi: 10.3390/nu12041181
60. Calder PC. Nutrition, immunity and COVID-19. *BMJ NPH*. (2020) 3. doi: 10.1136/bmjnph-2020-000085
61. Childs CE, Calder PC, Miles EA. Diet and immune function. *Nutrients*. (2019) 11(8). doi: 10.3390/nu11081933
62. Wu D, Lewis ED, Pae M, Meydani SN. Nutritional modulation of immune function: analysis of evidence, mechanisms, and clinical relevance. *Front Immunol*. (2018) 9:3160. doi: 10.3389/fimmu.2018.03160
63. Myles IA. Fast food fever: reviewing the impacts of the Western diet on immunity. *Nutr J*. (2014) 13:61. doi: 10.1186/1475-2891-13-61
64. Hall KD, Ayuketah A, Brychta R, Cai H, Cassimatis T, Chen KY, et al. Ultra-processed diets cause excess calorie intake and weight gain: an inpatient randomized controlled trial of ad libitum food intake. *Cell Metab*. (2019) 30:67–77.e3. doi: 10.1016/j.cmet.2019.05.008
65. da Luz Scheffer D, Latini A. Exercise-induced immune system response: anti-inflammatory status on peripheral and central organs. *Biochim Biophys Acta*. (2020) 1866:165823. doi: 10.1016/j.bbadis.2020.165823
66. Campbell JP, Turner JE. Debunking the Myth of exercise-induced immune suppression: redefining the impact of exercise on immunological health across the lifespan. *Front Immunol*. (2018) 9:648. doi: 10.3389/fimmu.2018.00648
67. Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system. *J Sport Health Sci*. (2019) 8:201–17. doi: 10.1016/j.jshs.2018.09.009
68. Wang M, Baker JS, Quan W, Shen S, Fekete G, Gu Y. A preventive role of exercise across the coronavirus 2 (SARS-CoV-2) pandemic. *Front Physiol*. (2020) 11:572718. doi: 10.3389/fphys.2020.572718
69. de Matos MA, Garcia BCC, Vieira DV, de Oliveira MFA, Costa KB, Aguiar PF, et al. High-intensity interval training reduces monocyte activation in obese adults. *Brain Behav Immun*. (2019) 80:818–24. doi: 10.1016/j.bbi.2019.05.030
70. Barrett B, Hayney MS, Muller D, Rakel D, Ward A, Obasi CN, et al. Meditation or exercise for preventing acute respiratory infection: a randomized controlled trial. *Ann Fam Med*. (2012) 10:337–46. doi: 10.1370/afm.1376
71. WHO. *Global recommendations on Physical Activity for Health*. Geneva: WHO (2011).
72. Bailey MT. psychological stress, immunity, and the effects on indigenous microflora. *Adv Exp Med Biol*. (2016) 874:225–46. doi: 10.1007/978-3-319-20215-0_11
73. Thau L Jayashree G, Sharma S. *Physiology, Cortisol*. Treasure Island, FL: StatPearls Publishing (2020). Available online at: https://www.ncbi.nlm.nih.gov/books/NBK538239/ (accessed June 12, 2020).
74. Morey JN, Boggero IA, Scott AB, Segerstrom SC. Current directions in stress and human immune function. *Curr Opin Psychol*. (2015) 5:13–7. doi: 10.1016/j.copsyc.2015.03.007
75. Morgan N, Irwin MR, Chung M, Wang C. The effects of mind-body therapies on the immune system: meta-analysis. *PLoS ONE*. (2014) 9:e100903. doi: 10.1371/journal.pone.0100903
76. Schakel L, Veldhuijzen DS, Crompvoets PI, Bosch JA, Cohen S, van Middendorp H, et al. Effectiveness of stress-reducing interventions on the response to challenges to the immune system: a meta-analytic review. *Psychother Psychosom*. (2019) 88:274–86. doi: 10.1159/000501645
77. Ingram J, Maciejewski G, Hand CJ. Changes in diet, sleep, and physical activity are associated with differences in negative mood during COVID-19 lockdown. *Front Psychol*. (2020) 11:588604. doi: 10.3389/fpsyg.2020.588604
78. Qiu F, Liang CL, Liu H, Zeng YQ, Hou S, Huang S, et al. Impacts of cigarette smoking on immune responsiveness: Up and down or upside down? *Oncotarget*. (2017) 8:268–84. doi: 10.18632/oncotarget.13613
79. Vardavas CI, Nikitara K. COVID-19 and smoking: a systematic review of the evidence. *Tob Induc Dis*. (2020) 18:20. doi: 10.18332/tid/119324
80. Lawrence H, Hunter A, Murray R, Lim WS, McKeever T. Cigarette smoking and the occurrence of influenza—systematic review. *J Infect*. (2019) 79:401–6. doi: 10.1016/j.jinf.2019.08.014
81. Komiyama M, Hasegawa K. Smoking cessation as a public health measure to limit the coronavirus disease 2019 pandemic. *Eur Cardiol*. (2020) 15:e16. doi: 10.15420/ecr.2020.11
82. Szabo G, Saha B. Alcohol's effect on host defense. *Alcohol Res*. (2015) 37:159–70.
83. Romeo J, Wärnberg J, Nova E, Díaz LE, Gómez-Martinez S, Marcos A. Moderate alcohol consumption and the immune system: a review. *Br J Nutr*. (2007) 98(Suppl. 1):S111–5. doi: 10.1017/S0007114507838049
84. Yeligar SM, Chen MM, Kovacs EJ, Sisson JH, Burnham EL, Brown LA. Alcohol and lung injury and immunity. *Alcohol*. (2016) 55:51–9. doi: 10.1016/j.alcohol.2016.08.005
85. Simou E, Britton J, Leonardi-Bee J. Alcohol and the risk of pneumonia: a systematic review and meta-analysis. *BMJ Open*. (2018) 8:e022344. doi: 10.1136/bmjopen-2018-022344
86. Meira ECM, Miyazawa M, Gozal D. Putative contributions of circadian clock and sleep in the context of SARS-CoV-2 infection. *Eur Respir J*. (2020) 55:2001023. doi: 10.1183/13993003.01023-2020

87. Zheng D, Ratiner K, Elinav E. Circadian influences of diet on the microbiome and immunity. *Trends Immunol*. (2020) 41:512–30. doi: 10.1016/j.it.2020.04.005
88. Irwin MR. Sleep and inflammation: partners in sickness and in health. *Nat Rev Immunol*. (2019) 19:702–15. doi: 10.1038/s41577-019-0190-z
89. Lange T, Born J, Westermann J. Sleep matters: CD4(+) T cell memory formation and the central nervous system. *Trends Immunol*. (2019) 40:674–86. doi: 10.1016/j.it.2019.06.003
90. Asif N, Iqbal R, Nazir CF. Human immune system during sleep. *Am J Clin Exp Immunol*. (2017) 6:92–6.
91. Haspel JA, Anafi R, Brown MK, Cermakian N, Depner C, Desplats P, et al. Perfect timing: circadian rhythms, sleep, and immunity—an NIH workshop summary. *JCI Insight*. (2020) 5(1). doi: 10.1172/jci.insight.131487
92. Benedict C, Brytting M, Markström A, Broman JE, Schiöth HB. Acute sleep deprivation has no lasting effects on the human antibody titer response following a novel influenza A H1N1 virus vaccination. *BMC Immunol*. (2012) 13:1. doi: 10.1186/1471-2172-13-1
93. Brown R, Pang G, Husband AJ, King MG. Suppression of immunity to influenza virus infection in the respiratory tract following sleep disturbance. *Reg Immunol*. (1989) 2:321–5.
94. Morin CM, Carrier J, Bastien C, Godbout R. Sleep and circadian rhythm in response to the COVID-19 pandemic. *Can J Public Health*. (2020) 111:654–7. doi: 10.17269/s41997-020-00382-7
95. Marmot M. Social determinants of health inequalities. *Lancet*. (2005) 365:1099–104. doi: 10.1016/S0140-6736(05)71146-6
96. Sidor A, Rzymski P. Dietary choices and habits during COVID-19 lockdown: experience from Poland. *Nutrients*. (2020) 12:1657. doi: 10.3390/nu12061657
97. Rolland B, Haesebaert F, Zante E, Benyamina A, Haesebaert J, Franck N. Global changes and factors of increase in caloric/salty food intake, screen use, and substance use during the early COVID-19 containment phase in the general population in France: survey study. *JMIR Public Health Surveill*. (2020) 6:e19630. doi: 10.2196/19630
98. Ruíz-Roso MB, de Carvalho Padilha P, Matilla-Escalante DC, Brun P, Ulloa N, Acevedo-Correa D, et al. Changes of physical activity and ultra-processed food consumption in adolescents from different countries during COVID-19 pandemic: an observational study. *Nutrients*. (2020) 12:2289. doi: 10.3390/nu12082289
99. Martinez-Ferran M, de la Guía-Galipienso F, Sanchis-Gomar F, Pareja-Galeano H. Metabolic impacts of confinement during the COVID-19 pandemic due to modified diet and physical activity habits. *Nutrients*. (2020) 12:1549. doi: 10.3390/nu12061549
100. Kahneman D. *Thinking, Fast and Slow*. New York, NY: Farrar, Straus and Giroux (2011). 499 p.
101. Wansink B, Sobal J. Mindless eating: the 200 daily food decisions we overlook. *Environ Behav*. (2007) 39:106–23. doi: 10.1177/0013916506295573
102. Smith N, Goldstein D, Johnson E. Choice without awareness: ethical and policy implications of defaults. *J Public Policy Mark*. (2013) 32:159–72. doi: 10.1509/jppm.10.114
103. Jackson SE, Llewellyn CH, Smith L. The obesity epidemic—nature *via* nurture: a narrative review of high-income countries. *SAGE Open Med*. (2020) 8:2050312120918265. doi: 10.1177/2050312120918265
104. Guthold R, Stevens GA, Riley LM, Bull FC. Global trends in insufficient physical activity among adolescents: a pooled analysis of 298 population-based surveys with 1·6 million participants. *Lancet Child Adolesc Health*. (2020) 4:23–35. doi: 10.1016/S2352-4642(19)30323-2
105. WHO. *Controlling the Global Obesity Epidemic*. (2020). Available online at: https://www.who.int/nutrition/topics/obesity/en/ (accessed August 21, 2020).
106. Bucher T, Collins C, Rollo ME, McCaffrey TA, De Vlieger N, Van der Bend D, et al. Nudging consumers towards healthier choices: a systematic review of positional influences on food choice. *Br J Nutr*. (2016) 115:2252–63. doi: 10.1017/S0007114516001653
107. Broers VJV, De Breucker C, Van den Broucke S, Luminet O. A systematic review and meta-analysis of the effectiveness of nudging to increase fruit and vegetable choice. *Eur J Public Health*. (2017) 27:912–20. doi: 10.1093/eurpub/ckx085
108. Smith M, Hosking J, Woodward A, Witten K, MacMillan A, Field A, et al. Systematic literature review of built environment effects on physical activity and active transport—an update and new findings on health equity. *Int J Behav Nutr Phys Act*. (2017) 14:158. doi: 10.1186/s12966-017-0613-9
109. Moodie R, Stuckler D, Monteiro C, Sheron N, Neal B, Thamarangsi T, et al. Profits and pandemics: prevention of harmful effects of tobacco, alcohol, and ultra-processed food and drink industries. *Lancet*. (2013) 381:670–9. doi: 10.1016/S0140-6736(12)62089-3
110. Bonita R, Magnusson R, Bovet P, Zhao D, Malta DC, Geneau R, et al. Country actions to meet UN commitments on non-communicable diseases: a stepwise approach. *Lancet*. (2013) 381:575–84. doi: 10.1016/S0140-6736(12)61993-X

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 de Frel, Atsma, Pijl, Seidell, Leenen, Dik and van Rossum. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

REVIEW
published: 07 December 2020
doi: 10.3389/fnut.2020.606398

Zinc, Vitamin D and Vitamin C: Perspectives for COVID-19 With a Focus on Physical Tissue Barrier Integrity

José João Name[1]*, *Ana Carolina Remondi Souza*[1], *Andrea Rodrigues Vasconcelos*[2], *Pietra Sacramento Prado*[1] and *Carolina Parga Martins Pereira*[1]

[1] Kilyos Consultoria, Assessoria, Cursos e Palestras, São Paulo, Brazil, [2] Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Some nutrients play key roles in maintaining the integrity and function of the immune system, presenting synergistic actions in steps determinant for the immune response. Among these elements, zinc and vitamins C and D stand out for having immunomodulatory functions and for playing roles in preserving physical tissue barriers. Considering the COVID-19 pandemic, nutrients that can optimize the immune system to prevent or lower the risk of severe progression and prognosis of this viral infection become relevant. Thus, the present review aims to provide a comprehensive overview of the roles of zinc and vitamins C and D in the immune response to viral infections, focusing on the synergistic action of these nutrients in the maintenance of physical tissue barriers, such as the skin and mucous membranes. The evidence found in the literature shows that deficiency of one or more of these three elements compromises the immune response, making an individual more vulnerable to viral infections and to a worse disease prognosis. Thus, during the COVID-19 pandemic, the adequate intake of zinc and vitamins C and D may represent a promising pharmacological tool due to the high demand for these nutrients in the case of contact with the virus and onset of the inflammatory process. Ongoing clinical trials will help to clarify the role of these nutrients for COVID-19 management.

Keywords: vitamin C, vitamin D, zinc, COVID-19, junctional complex, immunity, nutrients

OPEN ACCESS

Edited by:
Ioannis Zabetakis,
University of Limerick, Ireland

Reviewed by:
Kuldeep Dhama,
Indian Veterinary Research Institute (IVRI), India
Ruchi Tiwari,
U.P. Pandit Deen Dayal Upadhyaya Veterinary University, India

***Correspondence:**
José João Name
namepucsp@gmail.com

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Nutrition

Received: *14 September 2020*
Accepted: *16 November 2020*
Published: *07 December 2020*

Citation:
Name JJ, Souza ACR, Vasconcelos AR, Prado PS and Pereira CPM (2020) Zinc, Vitamin D and Vitamin C: Perspectives for COVID-19 With a Focus on Physical Tissue Barrier Integrity. Front. Nutr. 7:606398. doi: 10.3389/fnut.2020.606398

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic highlights the importance of the use of essential nutrients, especially those with immunomodulatory effects that support an organism's natural immune defenses in the event of that or other viral infections (1–3).

Viruses classified as coronavirus belong to the family Coronaviridae, a name derived from the crown-shaped spikes on the pathogen surface, and are characterized by compromising the human respiratory system (4, 5). At the end of 2019, individuals in the city of Wuhan, China, were diagnosed with pneumonia caused by a new coronavirus: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (4, 6, 7). With the rapid increase in the number of positive SARS-CoV-2 infections in all regions of the world, the World Health Organization (WHO) declared COVID-19 a pandemic in March 2020.

The clinical manifestations of this disease have a broad spectrum, including asymptomatic infection, mild upper respiratory tract infection and severe pneumonia with respiratory failure, for which hospitalization with sub-intensive or intensive care is required (8, 9).

Nutrition is a determinant factor for the maintenance of homeostasis and the health of different organs and physiological systems of an organism, including immune function (10, 11). In the current context of the COVID-19 pandemic, the "nutritional status-immune response" dyad of an individual becomes even more significant because in the absence of a widely available vaccine or treatment supported by high-quality evidence, the main therapeutic/preventive measure for the disease lies in the individual response to the virus (2, 3, 12–16). Inadequate nutrition is considered a contributing factor to the emergence of viral infection due to its contribution to weakness of immune system, which increases the rate of infections and the risk of mortality and morbidity. Moreover, viral infections increase the demand for several micronutrients such as vitamin A, B, C, D, zinc, and selenium (17–20).

Dietary supplementation of micronutrients with recognized roles in immune function can optimize the modulation of the body's immune response, reducing the risk of infections (19, 21–25). In this context, zinc and vitamins C and D are the micronutrients for which there is robust evidence of their immunomodulating activity, such that their deficiency, even if marginal, can compromise metabolism and, consequently, their action on the immune system, a concept grounded by the triage theory[1] proposed by Ames (26) and the Law of the Minimum[2] proposed by Justus von Liebig in 1840 (10, 17, 24–30).

Considering the importance of adequate levels of nutrients for immune system activity, this review article presents, in a narrative form, a review of the main medical-scientific findings for the relationship between zinc, vitamin C and vitamin D and viral infections, in particular those caused by SARS-CoV-2, demonstrating a confluence of mechanisms in various immune functions, with an emphasis on the integrity of physical tissue barriers composed of epithelial cells and intercellular functional complexes.

NUTRIENTS AND VIRAL INFECTIONS

Zinc

The immunomodulatory and antiviral activities of zinc have made this mineral and its ionophores candidates against COVID-19 (31, 32). Zinc is essential for the integrity of the immune system (33), with an important role in the maintenance, development and activation of cells during innate and adaptive immune responses. It also plays a role in the integrity of epithelial barriers, which are essential for organism defense and prevention of pathogen entry (34–37). Zinc can modulate the development and activity of T cells, hence reducing the cytokine storm, characterized by high levels of pro-inflammatory cytokines and chemokines that lead to systemic immune response impairment, resulting in acute respiratory distress syndrome (ARDS) or in multiple organ failure (38–40). Zinc deficiency decreases the activity of natural killer (NK) cells and cytolytic T cells, both of which are involved in the destruction of viruses, bacteria and tumor cells (41, 42).

Another important function of zinc is its direct antiviral activity, which makes it essential for the immune response upon viral infection. Increased intracellular concentration of this mineral can reduce the replication of a variety of RNA viruses (43–48) and interfere with the viral proteolytic processing of polyproteins (49). Importantly, in Vero-E6 cell culture, the incubation with low concentrations of zinc (2 μM) repressed SARS coronavirus (SARS-CoV) replication by inhibiting its RNA polymerase (45). Moreover, zinc can enhance interferon (IFN) cytokine signaling against RNA viruses (50–53) and inhibit the activity of angiotensin-converting enzyme 2 (ACE2), which is critical for SARS-CoV-2 entry into host cells (54, 55).

Zinc deficiency affects approximately one-third of the population worldwide (56) and is considered a global nutritional problem, affecting population groups in both developed and developing countries. According to a WHO report, zinc deficiency is responsible for ∼1.4% (0.8 million) of annual deaths and 2.9% of loss of healthy life years (total of 28 million years) around the world (56). It is also considered one of the main causes of morbidity in developing countries (57), and it is estimated that 0.5 million women and children die per year in these countries due to zinc deficiency (58–60). Worldwide, about 16% of lower respiratory tract infections occur due to zinc deficiency (56), suggesting a possible link between this nutritional deficiency and the increased risk of SARS-CoV-2 infection and severe disease progression (61).

According to epidemiological data, most deaths from COVID-19 are concentrated in the elderly with common comorbidities such as hypertension, diabetes or obesity (62–64). In general, this group of individuals has a higher prevalence of zinc deficiency (65–69), given that aging is associated with a progressive decline in zinc status in the body due to several factors, including reduced food intake, decreased nutrient absorption efficiency and use of medications (70, 71). Similarly, obese individuals or those with chronic kidney disease often exhibit zinc deficiency (65–68, 72–74).

Increasing zinc deficiency and particularly reductions in intracellular zinc levels in immune cells are associated with greater difficulty in mobilizing rare zinc reserves in the body of elderly individuals and, consequently, with the progressive dysregulation of immune responses (immunosenescence), resulting in higher susceptibility to infectious diseases (71, 75, 76). In general, elderly individuals with chronic diseases or who are hospitalized have even lower levels of minerals than do healthy elderly individuals (71), which may be responsible for the high incidence of infections and age-related degenerative pathologies (70).

[1]The triage theory postulates that in the event of a nutrient deficiency, that nutrient is allocated primarily to functions related to immediate survival while long-term functions are impaired (26).

[2]The Law of the Minimum says that the nutrients that are in an organism in less quantity or deficient become the limiting factor of metabolic reactions even if the other nutrients are present at adequate levels (27–29).

During an infection, an organism can mobilize zinc reserves for priority functions, such as those associated with the immune system, leading to a decrease in zinc levels and, possibly, to the lack of zinc to other less essential functions, such as the maintenance of smell and taste, senses often affected in patients with COVID-19. This notion agrees with the triage theory cited above (26).

Zinc also plays an important role in intercellular junction proteins, structures that promote adhesion between epithelial cells and that are necessary for epithelial tissue structure and its function as a selective barrier (34–36). Under zinc deficiency conditions, disruption of intercellular junctions occurs, with a consequent reduction in tissue integrity and impairment of the control of paracellular permeability, allowing the passage of pathogens (34, 36, 77). Conversely, zinc supplementation improves the function of these junctions (34). Considering the important role of zinc in maintaining the integrity of the physical barriers of the skin and mucous membranes against the invasion of pathogens (37), it may also play a role in reducing the risk of contamination by SARS-CoV-2.

The intestinal mucosa is an important cellular barrier that prevents the entry of pathogens and is compromised after SARS-CoV-2 infection. Data from Hubei Province, China, shows that up to 79% of infected patients may present gastrointestinal symptoms, such as diarrhea, vomiting, abdominal pain, and gastrointestinal bleeding (78). The literature reports the occurrence of diarrhea in 2 to 50% of COVID-19 cases (78, 79). In some patients, it was the only symptom presented, which was associated with worse disease prognosis (80, 81) and can reduce zinc levels in the body due to malabsorption and loss during dehydration (82).

Supplementation with zinc was shown to be effective in the treatment of acute diarrhea, which may be due to viral infection (83). That approach is recommended by the WHO for the treatment of children with acute diarrhea to reduce its duration and severity in addition to the risk of new episodes in the following 2 to 3 months (84). Regarding zinc sources, it was found that supplementation with zinc amino acid chelate, compared with placebo and zinc sulfate, had a better effect in reducing the incidence of diarrhea and acute respiratory infection, in addition to resulting in a lower incidence of side effects, in preschool children (22). Zinc reduces the risk of diarrhea through its function in the intestinal barrier (77) and through several mechanisms that act directly on pathogens, including a reduction in the expression of virulence factors (85, 86).

Zinc-deficient individuals are prone to increased respiratory and diarrheal morbidities (87, 88). Furthermore, it was found that zinc supplementation in children with zinc deficiency may reduce the morbidity and mortality related to lower respiratory tract infections caused by the measles virus (89). Zinc administration is also associated with a 41% reduction in the prevalence of childhood pneumonia (23), a lower respiratory tract infection.

Clinical studies have shown that zinc supplementation can also reduce, by up to 54%, the severity and duration of various cold symptoms, such as fever, cough, sore throat, muscle pain and nasal congestion (90–92), which may also occur after SARS-CoV-2 infection. In a randomized double-blind study, 48 volunteers with colds received zinc acetate lozenges (80 mg of elemental Zn/day) supplementation or placebo within 24 h after the onset of symptoms. In comparison to placebo, zinc administration was associated with a significant reduction in the duration of cold symptoms and the total severity score of all symptoms ($p < 0.002$) (90).

In a case report series of four patients with COVID-19, administration of high doses of oral zinc (up to 207 mg/day) was possibly associated with improved oxygenation and fast resolution of shortness of breath after 1 day of treatment. No adverse effects were reported (93). Conversely, a prospective study with 242 patients did not find a significant correlation between zinc supplementation and reduced COVID-19-related mortality (RR = 0.66; 95% CI: 0, 41-1.07; $p = 0.09$) (94). In view of some limitations of the study (single-center retrospective design, possible presence of confounding variables, sample size and a higher proportion of patients treated with zinc), the authors highlighted the need for randomized clinical trials to investigate the potential of zinc in COVID-19 therapy (94).

By the time this review was completed, more than 10 clinical trials involving oral zinc as monotherapy or in association with other compounds have been registered at clinicaltrials.gov and have alrealy started subjects' enrollment (NCT04468139, NCT04472585, NCT04342728, NCT04446104, NCT04335084, NCT04370782, NCT04447534, NCT04326725, NCT04334512, NCT04412746), two of which have already been completed (NCT04485169, NCT04491994). The results of these studies will be important to validate the usefulness of zinc as an adjuvant therapy in COVID-19.

Vitamin C

Ascorbic acid is a water-soluble micronutrient with antioxidant properties that plays a crucial role in the immune system, supporting the epithelial barrier against the entry of pathogens and the cellular functions of the innate and adaptive immune systems (19, 95).

As an antioxidant, vitamin C prevents damage to biomolecules (nucleic acids, proteins, lipids and carbohydrates) resulting from exposure to oxidants generated by normal metabolism and exposure to pollutants and toxins (96). Furthermore, this vitamin is a cofactor of several enzymes that are involved in the stabilization of the collagen tertiary structure (97), in the biosynthesis of hormones such as norepinephrine, catecholamines and vasopressin (98), and in the methylation of DNA and histones and is therefore important for the occurrence of epigenetic events (99).

Vitamin C levels in the body may vary due to environmental conditions, such as air pollution, and the presence of pathologies, such as type 2 diabetes (95). The elderly population is particularly affected by vitamin C deficiency because chronic or acute diseases are prevalent in this group, and aging is related to reduced vitamin C levels (100–103). For example, low levels of vitamin C ($\leq$17 μmol/L) in a population of British elderly individuals were associated with all causes of mortality, including cardiovascular causes (104). In addition, hospitalized elderly patients with acute respiratory infections, when supplemented with 200 mg/day of vitamin C, showed reduced disease severity indices compared to

the placebo group (105). Recently, Arvinte et al. (106) conducted a pilot study that included 21 critical COVID-19 patients and observed low serum levels of vitamin C and vitamin D among the patients. In addition, older age and low vitamin C levels appeared to be co-dependent risk factors for mortality, suggesting that serum vitamin C levels contributed to the significance of age as a predictor of mortality (106).

A meta-analysis of 44 studies that used doses of vitamin C starting at 200 mg per day reported a reduction in the duration of the common cold in adults and children, which has been related to the role of this vitamin in supporting the immune system and in reducing the severity of symptoms (107) and is associated with its antihistaminic properties (108). Johnston et al. (21) evaluated the effect of vitamin C supplementation (1 g for 8 weeks) on the symptoms of respiratory tract infections in men with hypovitaminosis C ($\leq$45 μmol/L). Although not statistically significant, supplementation reduced the episodes of cold and shortened the duration of infection by 59% compared to the placebo group (−3.2 days; 95% CI: −7.0–0.6; $p = 0.06$) (21).

With respect to acute respiratory infections, it was found that the administration of vitamin C reduced the score for respiratory symptoms of pneumonia in critically ill patients (109). In addition, it was used as an adjuvant in two case reports of patients with ARDS, with an effective reduction in pulmonary edema (110, 111). A high dose of vitamin C in patients with ARDS is related to some beneficial outcomes, such as: reduction of inflammation and organ injuries, decreased pathogen infection and virulence, and optimization of immune defense (112).

In ARDS, liquid and proteins penetrate the alveoli, leading to pulmonary edema. This process occurs due to tight junction damage in the lung endothelium, resulting in increased permeability to fluids, neutrophils and erythrocytes and an excess of these components in the alveolar space (113). The presence of neutrophils in the intravascular and extravascular spaces during acute lung injury is frequently associated with platelets, forming aggregates that, due to their inflammatory thrombogenic activity, lead to inflammatory processes (113).

A study in mice with sepsis and acute pulmonary dysfunction showed that the parenteral infusion of 200 mg/kg vitamin C increased the removal of alveolar fluid, promoted an improvement in the structure and function of the alveolar epithelial barrier, and attenuated the pro-inflammatory response, thus reducing the consequences of sepsis in pulmonary dysfunction (114). These results were obtained by normalization of the constituent proteins of intercellular junctions and by the prevention of rearrangements of cytoskeletons promoted by ascorbic acid (114). In mice deficient in the enzyme responsible for the synthesis of vitamin C ($Gulo^{-/-}$), in a peritoneal inflammatory process, neutrophils were not able to enter into apoptosis and accumulated in the peritoneal cavity due to the absence of recognition and phagocytosis of neutrophils by macrophages, thus reducing the removal of these cells (115). Therefore, vitamin C, as previously mentioned for zinc, plays an important role in the protein components of intercellular junctions, acting in the prevention of the entry of pathogens and in the restructuring of epithelial tissue, in addition to being essential for the removal of neutrophils in damaged tissue, which leads to a reduction in the accumulation of these cells and in the inflammatory process.

In view of the current COVID-19 pandemic, patients with this disease have abnormal chest CT scan results, which reveal bilateral involvement of the lungs with ground-glass opacity, resulting from increased fluid in the lungs, which occurs in 98% of cases. In addition, complications such as ARDS (29%) and secondary infections (10%) may be common (116). Vitamin C may be an adjuvant to ARDS, reduce the deleterious consequences of sepsis associated with acute pulmonary dysfunction, and reduce the incidence of pneumonia by ∼80% (117). A meta-analysis of 18 controlled clinical trials showed that oral or intravenous vitamin C reduces both the length of stay in the intensive care unit (ICU) by 7.8–8.6% ($p \leq 0.003$) and the duration of mechanical ventilation by 18.2% ($p = 0.001$) (118).

Moreover, vitamin C may modulate the cytokine storm (19, 38, 119, 120), characterized by high levels of the pro-inflammatory cytokine interleukin (IL)-6, resulting in increased risk of respiratory failure requiring mechanical ventilation in patients with COVID-19 (121). According to an *in vivo* study with 12 healthy men, pretreatment with vitamin C can reduce the levels of IL-6, released by the vasoconstrictor endothelin-1 (ET-1), thus reducing vascular dysfunction (122). In addition, increased ET-1 expression is also associated with pneumonia, pulmonary hypertension, interstitial pulmonary fibrosis and ARDS (120).

As of the time of completing this review, more than 20 clinical trials with COVID-19 patients are in progress, seven of which have already started enrolling participants, five using vitamin C as an intravenous treatment (NCT04323514, NCT04401150, NCT04357782, NCT04264533, NCT02735707) and two as an oral treatment (NCT04468139, NCT04382040).

In the study by Hiedra et al. (123), 17 COVID-19 patients who needed 30% of oxygen or more received 1 g of vitamin C intravenously for 3 days (123). These patients were receiving hydroxychloroquine, methylprednisolone or tocilizumab as initial treatments. After vitamin C therapy, the levels of some anti-inflammatory markers, such as D-dimer and ferritin, were significantly reduced. However, this study had a small number of participants, had a short duration (only 3 days) and did not investigate the effects of using vitamin C alone. In a case report, a COVID-19 patient underwent intravenous administration of 11 g of vitamin C after developing ARDS and needing mechanical ventilation (124). The high-dose vitamin C treatment was associated with a reduction of both ICU stay and need of mechanical ventilation, as well as a faster recovery of the patient compared to those who did not receive intravenous vitamin C. Importantly, the patient was using the following medications: hydroxychloroquine, azithromycin, colchicine, and zinc sulfate (124).

Despite the limitations of these studies with COVID-19 patients, their results highlight the importance of a more detailed investigation of the use of vitamin C in this disease treatment, since this micronutrient plays a key role in the development, maintenance and expression of the immune response, factors that affect the risk and severity of viral infection, such as SARS-CoV-2.

Vitamin D

Despite the traditional name, vitamin D is actually a hormone, given that in addition to being endogenously produced, it acts on the regulation of more than 200 genes in different cell types (125–127). Only ∼10% of the vitamin D required is obtained from food, its main sources including (i) animals, such as cold deep-water fish, for example, tuna and salmon (D_3 or cholecalciferol) and (ii) plants, such as edible mushrooms (D_2 or ergosterol). The remaining 80 to 90% are endogenously synthesized (128), a process that begins in the deep layers of the epidermis, following exposure to ultraviolet (UV) solar radiation, and involves various hydroxylation processes in the liver and kidneys. The final metabolite, 1α,25-dihydroxy-vitamin D [1,25(OH)2 D or calcitriol], is the metabolically active molecule (127, 128).

Variables such as skin pigmentation, aging and topical application of sunscreen may reduce the synthesis of vitamin D by the body (129–131). Importantly, its endogenous production does not lead to toxic accumulation in the organism because there is a system responsible for controlling its levels. During prolonged exposure to UV-B rays, precholecalciferol (previtamin D_3) is transformed into lumisterol, an inert isomer that maintains the balance of vitamin D_3 production (132, 133).

In addition to its classic effects on calcium homeostasis and the maintenance of bone health, vitamin D_3 plays an important role in the body's immune function (17, 19, 134–136). In fact, calcitriol acts as a powerful modulator of the immune system, given that vitamin D (i) has receptors in all immune cells; (ii) is associated with the production of T lymphocytes and the differentiation of B lymphocytes; (iii) optimizes anti-inflammatory functions by altering IL-10 cytokine levels; (iv) induces maturation and differentiation of monocytes and macrophages; (v) is associated with the production of cytokines and chemokines via the nuclear factor-κB (NF-κB); and (vi) induces the secretion of the lysosomal enzymes acid phosphatase and hydrogen peroxide (134, 136, 137). This immunomodulating function of vitamin D is considered complex during viral infections and appears to vary according to the nature of the pathogen and the type of immune function responsible for the resolution of the disease (136, 137).

Besides immune cells, the vitamin D receptor (VDR) is also found in pulmonary epithelial cells. When activated, VDR results in the expression of defensins and catelicidins, peptides with antiviral activity through direct action or via immunological modulation (138–140). It has been speculated that, during vitamin D deficiency, the impaired antiviral immune response in COVID-19 patients may be due to the reduction in LL37 levels, an antimicrobial peptide derived from catelicidin (139).

Vitamin D may also attenuate exacerbated inflammatory responses by downregulating pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and IL-6, involved in the development of cytokine storm during COVID-19-related ARDS (136, 140, 141). In fact, preliminary data from more than 5,000 patients with COVID-19 suggested a relationship between vitamin D deficiency and severity of cytokine storm, indicated by high serum levels of the inflammatory marker C-reactive protein (CRP) (142).

A meta-analysis of 25 randomized controlled studies showed that vitamin D_3 supplementation was associated with a lower risk of developing acute respiratory infections (OR=0.88, 95% CI: 0.81–0.96; $p < 0.001$) and that the positive effect of supplementation was even more pronounced in individuals with vitamin D insufficiency (serum levels below 25 ng/mL) at the beginning of the intervention (143).

In addition to its role in cellular and humoral immunity, vitamin D, similar to zinc and vitamin C, plays an important role in the formation and maintenance of epithelial and endothelial barriers, including lung tissue (17, 19, 144–146). Using a vitamin D receptor knockout mouse model, Chen et al. (146) observed that in the absence of this hormone signaling, the animals exhibited lung inflammation and impaired lung function, which can be explained by the increased pulmonary permeability resulting from impairment of the integrity of the epithelial barrier. The authors demonstrated a reduction in the expression of essential proteins for the maintenance of tight junctions, such as claudins. Thus, in vitamin D deficiency, the lungs lose epithelial integrity, becoming more susceptible to inflammatory processes and pathologies such as asthma, chronic pneumonia and cancer (146).

According to the Endocrine Society, serum levels of 25(OH)D below 20 ng/mL indicate vitamin D deficiency, whereas levels between 21 and 29 ng/mL indicate its insufficiency (147), both of which compromise the immunomodulatory functions of this hormone and have been associated with increased susceptibility to viral and bacterial infections (17, 125, 134).

Some studies suggest that there is a correlation between vitamin D deficiency and susceptibility to SARS-CoV-2 infection and disease severity (24, 106, 140, 148–152). Ilie et al. (149) found a negative correlation between the average serum vitamin D levels in 20 European countries and the COVID-19 cases and mortality per million population. Severely low levels of vitamin D have been identified in the elderly, especially in Switzerland, Italy, and Spain (149).

A meta-analysis of seven retrospective studies reported an average serum vitamin D level of 22.9 nmol/L in 1,368 patients with COVID-19 (140). Significantly lower serum levels of vitamin D were associated with patients with poor disease prognosis compared to those with good outcome, representing a standardized mean difference (SMD) of −5.12 (95% CI: −9.14, −1.10, $p = 0.012$). The difference in vitamin D levels was also substantial between surviving patients and those who died (SMD = −14.6, 95% CI: −15.3, −13.8). The authors concluded that vitamin D deficiency plays an independent causal role in COVID-19 severity and that preventive or therapeutic supplementation in populations at risk can be useful to prevent poor disease outcome (140).

Similarly, a subsequent study with an Israeli cohort of 7,807 subjects identified significantly lower levels of vitamin D among those who tested positive for COVID-19 compared to those who tested negative. Low plasma level of vitamin D (<30 ng/mL) was considered an independent risk factor for COVID-19 infection (OR = 1.58; 95% CI: 1.24–2.01; $p < 0.001$) and hospitalization (OR = 2.09; 95% CI: 1.01–4.30, $p < 0.05$) (150).

As previously mentioned, most deaths from COVID-19 are concentrated in the elderly with comorbidities, and these individuals also have vitamin D deficiency (24). Studies demonstrate the correlation between low levels of calcitriol and pathologies such as cancer, diabetes, hypertension and heart disease (24, 106). Likewise, there is also a known relationship between aging and a reduction in endogenous vitamin D synthesis, which can be explained by the increased levels of parathyroid hormone (PTH) (153). Specifically regarding the elderly population, epidemiological studies indicate that hypovitaminosis D is also associated with increased morbidity and mortality in general (154), whereas a meta-analysis (75,927 participants and 38 studies) showed that vitamin D_3 supplementation significantly reduced mortality (RR = 0.94; 95% CI: 0.91–0.98; $p = 0.002$) (155).

Given the COVID-19 pandemic, the WHO suggested social isolation as a measure for restricting the spread of the virus. This measure may also contribute to the reduction in serum vitamin D levels because confinement and possible lack of exposure to the sun prevent the endogenous production of this hormone, reducing the body's capacity to fight COVID-19 (156). Alternatives to prevent serum vitamin D deficiency, such as drug supplementation or the ingestion of foods rich in vitamin D, such as fatty fish, cod liver oil and egg yolk, should be considered during this period of isolation (156).

Finally, another important characteristic of the disease is its pathophysiology. SARS-CoV-2 infection starts with the interaction of the virus with the enzyme ACE2, reducing its activity. Importantly, ACE2 has a key role in counteracting ARDS and acute lung injury. Xu et al. (157) demonstrated that calcitriol is able to upregulate ACE2 and downregulate renin and angiotensin II in the lung tissue of rats exposed to lipopolysaccharide as a model of ARDS (157). In this scenario, recent literature have suggested that vitamin D can act by targeting ACE2 down-regulation in SARS-CoV-2 infection, which could be a potential therapeutic approach to COVID-19 and induced ARDS (158–161). However, further studies must be performed to understand the impact of ACE2 modulation for COVID-19 (17, 24, 158–162).

Considering the observations presented, it is suggested that vitamin D deficiency may be the common variable among elderly individuals and patients with underlying diseases, populations more susceptible to complications and mortality by COVID-19. That may justify the use of vitamin D supplementation as a measure of maintenance and expression of the immune response, which are important for reducing the risk and severity of viral infection as well as for mitigating the symptoms of this disease (163–165).

Clinical studies are warranted to determine the effects of vitamin D supplementation in patients with COVID-19. As of the writing of this review, about 30 clinical studies involving oral vitamin D supplementation in COVID-19 patients are registered at clinicaltrials.gov, 12 of those have started enrolling patients (NCT04449718, NCT04487951, NCT04407286, NCT04411446, NCT04502667, NCT04459247, NCT04403932, NCT04335084, NCT04344041, NCT04334512, NCT04386850, NCT04482673) and two are already completed (NCT04407572, NCT04435119).

Next, the formation of the structural maintenance and activity pathways of intercellular junctions whose integrity is an important barrier to the penetration of viruses, bacteria and allergens will be addressed. In this particular context, the nutrients in question participate in metabolic pathways or constitute synergistic or even confluent structures, which reveals their interdependence, both to evidence a loss of selectivity of epithelial barriers in a deficient individual as well as for their repair when supplemented.

SYNERGISTIC ACTION OF NUTRIENTS ON PHYSICAL BARRIERS

The outer and inner surfaces of an organism, such as the skin and mucous membranes, are the first line of defense against pathogens, toxins and other foreign bodies, as they form physical barriers that prevent entry (17). SARS-CoV-2 is able to penetrate physical barriers when they are vulnerable. Thus, it can be transmitted directly, when there is physical contact with the infected person, or indirectly, when the infection occurs through droplets ($> 5\,\mu m$) and aerosols ($<5\,\mu m$) in suspension or through fomites, where steel and plastic are the materials in which this virus is more viable (166).

Droplets containing SARS-CoV-2 are transmitted when they come in contact with the oral, respiratory tract and intestinal mucosa and the conjunctiva, tissues that express ACE2 necessary for the entry of this virus into the body. However, whether the consumption of food contaminated with SARS-CoV-2 can cause its transmission through the digestive system remains to be confirmed (166–171). Thus, the physical and functional integrity of the physical barriers, especially epithelial cells, is important for reducing the risk of SARS-CoV-2 contagion.

The epithelial cells are joined by distinct intercellular junctions, known as the epithelial junctional complex, which is formed by tight junctions, adherens junctions and desmosomes (172, 173) (**Figure 1**). Because they act as a selective barrier, this complex prevents the entry of pathogens and toxins into the bloodstream.

Nutrient deficiency, the presence of specific pathologies, and interactions with pathogens are some of the possible causes of structural and functional dysfunctions of the junctional complex. Once the junctional complex is compromised, the organism loses its selective barrier and becomes susceptible to the entry of harmful agents, such as viruses, into the bloodstream (114, 172, 174–176). **Table 1** describes the constituent proteins of the junctional complex and the functions of each junction.

Tight junctions are essential for the establishment of the barrier between different compartments of the organism, and their main function is selective paracellular diffusion, which restricts the passage of molecules according to size and ionic charge (172, 173). The selective diffusion promoted by tight junctions is considered a key process for the maintenance of organ and tissue homeostasis (172).

Adherens junctions have an anchoring function, connecting actin filaments between cells and have two subcomplexes:

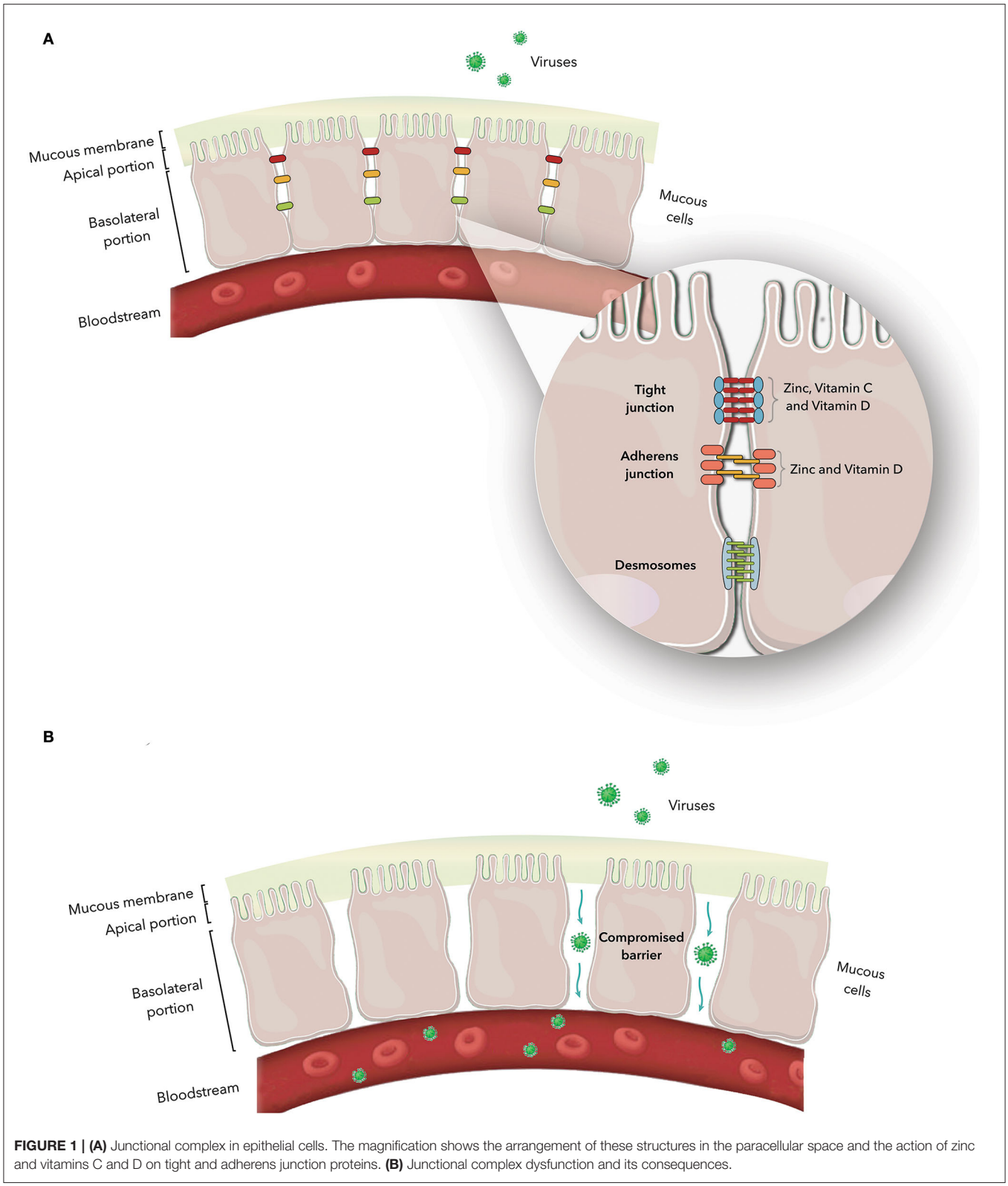


FIGURE 1 | (A) Junctional complex in epithelial cells. The magnification shows the arrangement of these structures in the paracellular space and the action of zinc and vitamins C and D on tight and adherens junction proteins. **(B)** Junctional complex dysfunction and its consequences.

nectin-based adhesions, which are important for the initial attachment between neighboring cells and for the establishment of apical-basolateral polarity, and cadherin-based adhesions, which strengthen intercellular binding. Evidence shows that these two junctions have an interconnection, which can be either physical or through signaling (177).

TABLE 1 | Functions and protein constituents of the junctional complex.

Junctional complex	Function	Constituent proteins	Cytoskeletal adaptor proteins
Tight junction	Seal intercellular spaces; selective paracellular diffusion	Claudins (26 members of this family); proteins of the MARVEL domain (occludin, tricellulin and MARVELD3)	Zonula occludens proteins (ZO): ZO1, ZO2, ZO3; cingulin; MAGI; PAR3; PAR6 PALS1 PATJ
Adherens junction	Connect the actin filament bundles between the adjacent cells	Classical cadherins (E-cadherin) Nectins	α-catenin, β-catenin, plakoglobin (γ-catenin), catenin p120, vinculin, α-actinin, FFA6
Desmosomes	They connect the intermediate filaments between adjacent cells	Cadherin (desmoglein, desmocollin)	Plakoglobin (γ-catenin), plakophilin, desmoplakin

MAGI, membrane-associated guanylate kinase inverted; PAR, partitioning defective; PALS1, protein associated with Lin-1; PATJ, PALS1-associated tight junction (PATJ); AF6, ALL1-fused gene from chromosome 6 protein; Adapted from (172, 173).

As with adherens junctions, desmosomes also have anchoring functions, connecting intermediate filaments between cells. This connection ensures the mechanical integrity of the tissues, which is essential for tissues susceptible to mechanical stress, such as skin and heart (178). Desmosomes also interact physically and through signaling pathways with tight and adherens junctions (178). Thus, the junctional complex interacts with each other dynamically to perform its functions effectively.

Some viruses and bacteria, such as the hepatitis C virus, influenza A virus, SARS-CoV, and the bacterium *Helicobacter pylori*, are known to interact with tight junction proteins, thus hindering the integrity and function of these barriers (172, 179, 180). Furthermore, diseases such as chronic inflammatory conditions and cancer can also lead to tight junction dysfunctions, but it is not known whether this dysregulation is the cause or consequence of these pathologies (172).

Zinc and vitamins C and D act in synergy, promoting the integrity and function of some of junctional complex proteins, as will be detailed below.

Zinc

Zinc is essential for the integrity and homeostasis of the intestinal barrier (181–183). In an experimental colitis model in rats, analysis by electron microscopy showed that zinc supplementation reduced intestinal permeability due to its action on tight junctions (34). In human intestinal carcinoma cells (Caco-2 line) and mouse colon, intracellular depletion of zinc increased the permeability of the intestinal barrier because tight junctions were impaired by a significant reduction in the protein levels of occludin and claudin-3 (176). Supplementation with 100 μM zinc reestablished barrier homeostasis, reducing the permeability of tight junctions (176).

Claudins, the constituent proteins of tight junctions, selectively control the size and charge of molecules that diffuse through the paracellular space. Two types of claudins can be differentiated based on their properties: those that seal the membrane (claudin-1, -3, -4, -5, -6, -8, -12, -18, and -19) and those that form pores (claudin-2 and−15); the latter allow the passage of molecules (184). An *in vitro* study with Caco-2 cells showed that zinc treatment increased transepithelial electrical resistance and mannitol flux, factors that are dependent on the integrity of tight junctions. Based on the results obtained, it was found that claudin-2 and claudin-7 protein levels were significantly reduced after zinc supplementation (77, 185–188). The reduction in these proteins with zinc supplementation influences the increased resistance of the epithelial barrier, which is associated with reduced electrolytic permeability. In turn, the observed increase in mannitol flux, which indicates increased non-electrolyte permeability, is an indication that zinc performs a fine adjustment in the junctional complex, acting differently according to the type of electrolyte (77).

In addition to altering the structure of tight junctions, zinc deficiency also compromised adherens junctions, leading to the delocalization of E-cadherin and β-catenin proteins as well as of the cytoskeleton in Caco-2 cells. This dysfunction in the organization of the intestinal epithelium led to increased permeability and, consequently, neutrophil infiltration in the paracellular space, inducing an inflammatory response (36). Accordingly, in zinc-deficient lung epithelial cells, exposure to cytokines increased cell death by apoptosis and barrier dysfunctions, with E-cadherin and β-catenin proteolysis (189). Apoptosis and barrier dysfunction were directly proportional to the level of intracellular zinc depletion and the time of exposure to depletion associated with acute inflammation (189). Conversely, zinc supplementation reversed the impairment of adherens junctions and was effective in preserving cellular integrity and barrier function (189). Based on these findings, the authors suggested that at the beginning of an inflammatory response in the lung, the mobilization of zinc to epithelial cells is an essential innate response both to increase immune function and to protect other cells from damage caused by inflammation (189). Likewise, zinc-treated Caco-2 cells did not show neutrophil infiltration, indicating that the replenishment of zinc levels

restored the integrity of the epithelial barrier and prevented the inflammatory process (36).

Vitamin C

Vitamin C, like zinc, alters the expression of some tight junction proteins. In a mouse model of induced abdominal peritonitis and subsequent acute lung tissue damage, intraperitoneal administration of 200 mg/kg ascorbic acid and dehydroascorbic acid in a mouse model of induced abdominal peritonitis and subsequent acute lung tissue damage resulted in downregulation of pro-inflammatory chemokines, reduced infiltration of neutrophil polymorphonuclears in the lung and lower severity of tissue damage. In the non-supplemented group, abdominal peritonitis induced in animals led to the loss of alveolar barrier function for small solutes, with a significant increase in the expression of claudin-2 and -4, and reduction in the levels of claudin-18, occludin and the cytoskeletal adaptor protein zonula occludens 1 (ZO1) (114). Supplementation with ascorbic acid significantly reduced pulmonary edema by preserving the epithelial barrier, preventing changes in the expression of these proteins and preserving the paracellular permeability of ions and small molecules, in addition to preventing the rearrangement of the cytoskeleton and of actin (114).

Vitamin C also preserves the integrity of occludins, preventing damage to tight junctions associated with endothelial barrier dysfunction (190, 191). This barrier dysfunction involves the production of the reactive species superoxide anion radical ($O_2^{\cdot -}$), which reacts with nitric oxide (NO), forming peroxynitrite ($ONOO^-$), also with high potential to induce damage to the endothelial barrier and dephosphorylation of occludins. Vitamin C inhibits the enzyme NADPH oxidase, responsible $O_2^{\cdot -}$ production, and eliminates $O_2^{\cdot -}$ and $ONOO^-$, thus avoiding the dephosphorylation of occludins and the consequent loosening of tight junctions (190–192).

Vitamin D

Vitamin D and its receptor exert an influence on tight junctions, participating in the expression and function of the proteins ZO1, occludin and claudin (19, 175). Fujita et al. (193) found that levels of claudin-2 and claudin-12 were low in the duodenum, jejunum, ileum and colon of vitamin D receptor knockout mice, thus compromising calcium uptake by the intestine (193). In addition, the expression of these two claudins was induced in Caco-2 cells in a dose-dependent and time-dependent manner by treatment with 1α,25-dihydroxy-vitamin D, showing that both are vitamin D signaling targets. Based on these findings, the authors suggest that claudin-2 and claudin-12 form paracellular Ca^{2+} channels in enterocytes and are important for the homeostasis of this cation (193). In human corneal epithelial cells, the metabolites $1,25(OH)2D_3$ and $25(OH)D_3$ increased barrier function, with increased expression of occludin proteins (194).

Other evidence of the function of vitamin D in the integrity of tight and adherens junctions is found in human colon cancer cell lines. Treatment with $1,25(OH)2D_3$ increased the expression of some proteins from these junctions, such as ZO1, ZO2, occludin, E-cadherin and vinculin, leading to decreased membrane permeability, as shown by transepithelial electrical resistance throughout treatment (195). E-cadherin transmembrane protein is important for maintaining the polarized epithelial cell phenotype as well as for adhesion between cells (196, 197). Its importance is revealed by the fact that the loss of E-cadherin expression, a common event during the transition from adenoma to carcinoma, promotes considerable epithelial morphological changes and the acquisition of invasive capacity (198–200). Treatment of colon cancer cells with vitamin D reestablished the compromised tissue morphology due to the increase in the expression of tight and adherens junction proteins.

CONCLUSION

Several minerals and vitamins have antioxidant, immunomodulatory and antimicrobial roles which could be helpful for the immune response against the SARS-CoV-2 virus. In the absence of a widely available treatment or a vaccine for COVID-19, supplementation of micronutrients emerges as an important measure to improve the immune system and to prevent the development of severe symptoms. Some of these micronutrients are the vitamins A, B, C, D and E, and minerals such as selenium, magnesium, and zinc (17, 19).

In this review, the role of zinc, vitamin C and vitamin D for immunity was explored since these micronutrients show the strongest evidence for immune support (17). In this scenario, the mentioned studies demonstrate that zinc and vitamins C and D are integral parts of the immune system and show synergistic functions at various stages of the host defenses, such as the maintenance of the integrity of biological barriers and the functionality of cells that make up the innate and adaptive systems. Therefore, the deficiency or insufficiency of these key nutrients, acting in synergy in tight and adherens junction proteins, can lead to impairment of mucosal epithelial cells, possibly making them more susceptible to pathogen entry, such as SARS-CoV-2.

Overall, the medical literature demonstrates that the supplementation with zinc, vitamin C and vitamin D can mitigate viral respiratory infections. Thus, in the context of the COVID-19 pandemic, the supplementation with such nutrients may be characterized as a widely available, safe and low cost measure that can be useful to cope with the increased demand for these nutrients in case of contact with the virus and onset of the immune responses, as well as to lower the risk of severe progression and prognosis of this viral infection.

Ongoing clinical trial will provide more information on their effect on COVID-19 patients.

AUTHOR CONTRIBUTIONS

JN contributed to the study concept. JN and CP critically reviewed the article. JN, AS, AV, and CP contributed to the design of the manuscript and figure preparation and edition. JN, AS, AV, PP, and CP contributed to the acquisition and analysis of data

and drafted the manuscript. All authors gave final approval for all aspects of the work, agreed to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

ACKNOWLEDGMENTS

We would like to thank Paula Mitie Hirata for the technical assistance in the figure's edition.

REFERENCES

1. Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients.* (2020) 12:1181. doi: 10.3390/nu12041181
2. Infusino F, Marazzato M, Mancone M, Fedele F, Mastroianni CM, Severino P, et al. Diet supplementation, probiotics, and nutraceuticals in SARS-CoV-2 infection: a scoping review. *Nutrients.* (2020) 12:1718. doi: 10.3390/nu12061718
3. Jaggers GK, Watkins BA, Rodriguez RL. COVID-19: repositioning nutrition research for the next pandemic. *Nutr Res.* (2020) 81:1–6. doi: 10.1016/j.nutres.2020.07.005
4. Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, et al. Coronavirus disease 2019-COVID-19. *Clin Microbiol Rev.* (2020) 33:e00028–20. doi: 10.1128/CMR.00028-20
5. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun.* (2020) 109:102433. doi: 10.1016/j.jaut.2020.102433
6. Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol.* (2020) 5:536–44. doi: 10.1038/s41564-020-0695-z
7. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* (2020) 579:270–3. doi: 10.1038/s41586-020-2012-7
8. Caccialanza R, Laviano A, Lobascio F, Montagna E, Bruno R, Ludovisi S, et al. Early nutritional supplementation in non-critically ill patients hospitalized for the 2019 novel coronavirus disease (COVID-19): Rationale and feasibility of a shared pragmatic protocol. *Nutrition.* (2020) 74:110835. doi: 10.1016/j.nut.2020.110835
9. Dhama K, Patel SK, Pathak M, Yatoo MI, Tiwari R, Malik YS, et al. An update on SARS-CoV-2/COVID-19 with particular reference to its clinical pathology, pathogenesis, immunopathology and mitigation strategies. *Travel Med Infect Dis.* (2020) 37:101755. doi: 10.1016/j.tmaid.2020.101755
10. Wu D, Lewis ED, Pae M, Meydani SN. Nutritional modulation of immune function: analysis of evidence, mechanisms, and clinical relevance. *Front Immunol.* (2018) 9:3160. doi: 10.3389/fimmu.2018.03160
11. Childs CE, Calder PC, Miles EA. Diet and immune function. *Nutrients.* (2019) 11:1933. doi: 10.3390/nu11081933
12. Cohen J. Saving lives without new drugs. *Science.* (2014) 346:911. doi: 10.1126/science.346.6212.911
13. Ferrara F, De Rosa F, Vitiello A. The central role of clinical nutrition in COVID-19 patients during and after hospitalization in intensive care unit. *SN Compr Clin Med.* (2020) 11:1–5. doi: 10.1007/s42399-020-00410-0
14. Laviano A, Koverech A, Zanetti M. *Nutrition* support in the time of SARS-CoV-2 (COVID-19). *Nutrition.* (2020) 74:110834. doi: 10.1016/j.nut.2020.110834
15. Naja F, Hamadeh R. Nutrition amid the COVID-19 pandemic: a multi-level framework for action. *Eur J Clin Nutr.* (2020) 74:1117–21. doi: 10.1038/s41430-020-0634-3
16. Romano L, Bilotta F, Dauri M, Macheda S, Pujia A, De Santis GL, et al. Short report - medical nutrition therapy for critically ill patients with COVID-19. *Eur Rev Med Pharmacol Sci.* (2020) 24:4035–9. doi: 10.26355/eurrev_202004_20874
17. Gombart AF, Pierre A, Maggini S. A review of micronutrients and the immune system-working in harmony to reduce the risk of infection. *Nutrients.* (2020) 12:236. doi: 10.3390/nu12010236
18. Jayawardena R, Sooriyaarachchi P, Chourdakis M, Jeewandara C, Ranasinghe P. Enhancing immunity in viral infections, with special emphasis on COVID-19: a review. *Diabetes Metab Syndr.* (2020) 14:367–82. doi: 10.1016/j.dsx.2020.04.015
19. Jovic TH, Ali SR, Ibrahim N, Jessop ZM, Tarassoli SP, Dobbs TD, et al. Could vitamins help in the fight against COVID-19? *Nutrients.* (2020) 12:2550. doi: 10.3390/nu12092550
20. Messina G, Polito R, Monda V, Cipolloni L, Di Nunno N, Di Mizio G, et al. Functional role of dietary intervention to improve the outcome of covid-19: a hypothesis of work. *Int J Mol Sci.* (2020) 21:3104. doi: 10.3390/ijms21093104
21. Johnston CS, Barkyoumb GM, Schumacher SS. Vitamin C supplementation slightly improves physical activity levels and reduces cold incidence in men with marginal vitamin C status: a randomized controlled trial. *Nutrients.* (2014) 6:2572–83. doi: 10.3390/nu6072572
22. Sanchez J, Villada OA, Rojas ML, Montoya L, Diaz A, Vargas C, et al. Effect of zinc amino acid chelate and zinc sulfate in the incidence of respiratory infection and diarrhea among preschool children in child daycare centers. *Biomedica.* (2014) 34:79–91. doi: 10.1590/S0120-41572014000100011
23. Lassi ZS, Moin A, Bhutta ZA. Zinc supplementation for the prevention of pneumonia in children aged 2 months to 59 months. *Cochrane Database Syst Rev.* (2016) 12:CD005978. doi: 10.1002/14651858.CD005978.pub3
24. Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, et al. Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. *Nutrients.* (2020) 12:988. doi: 10.3390/nu12040988
25. Name JJ, Vasconcelos AR, Souza ACR, Favaro WJ. *Vitamin D. Zinc Glutamine: Synergistic Action with OncoTherad Immunomodulator in Interferon Signaling COVID-19.* (2020). SSRN. Available online at: http://dx.doi.org/10.2139/ssrn.3650249 (accessed August 25, 2020).
26. Ames BN. Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc Natl Acad Sci USA.* (2006) 103:17589–94. doi: 10.1073/pnas.0608757103
27. Brock WH. *Justus von Liebig: The Chemical Gatekeeper*. Cambridge: Cambridge University Press. (2002).
28. Davidson EA, Howarth RW. Environmental science: nutrients in synergy. *Nature.* (2007) 449:1000–1. doi: 10.1038/4491000a
29. Kaspari M, Powers JS. Biogeochemistry and geographical ecology: embracing all twenty-five elements required to build organisms. *Am Nat.* (2016) 188(Suppl. 1):S62–73. doi: 10.1086/687576
30. Bauer SR, Kapoor A, Rath M, Thomas SA. What is the role of supplementation with ascorbic acid, zinc, vitamin D, or N-acetylcysteine for prevention or treatment of COVID-19? *Cleve Clin J Med.* (2020). doi: 10.3949/ccjm.87a.ccc046. [Epub ahead of print].
31. Brewer J, Gomez Marti JL, Brufsky A. Potential interventions for SARS-CoV-2 infections: zinc showing promise. *J Med Virol.* (2020). doi: 10.1002/jmv.26523. [Epub ahead of print].
32. Hoang BX, Han B. A possible application of hinokitiol as a natural zinc ionophore and anti-infective agent for the prevention and treatment of COVID-19 and viral infections. *Med Hypotheses.* (2020) 145:110333. doi: 10.1016/j.mehy.2020.110333
33. Maggini S, Wenzlaff S, Hornig D. Essential role of vitamin C and zinc in child immunity and health. *J Int Med Res.* (2010) 38:386–414. doi: 10.1177/147323001003800203
34. Sturniolo GC, Fries W, Mazzon E, Di Leo V, Barollo M, D'Inca R. Effect of zinc supplementation on intestinal permeability in experimental colitis. *J Lab Clin Med.* (2002) 139:311–5. doi: 10.1067/mlc.2002.123624
35. Shin K, Fogg VC, Margolis B. Tight junctions and cell polarity. *Annu Rev Cell Dev Biol.* (2006) 22:207–35. doi: 10.1146/annurev.cellbio.22.010305.104219
36. Finamore A, Massimi M, Conti Devirgiliis L, Mengheri E. Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration

in Caco-2 cells. *J Nutr.* (2008) 138:1664–70. doi: 10.1093/jn/138.9.1664
37. Maares M, Haase H. Zinc and immunity: An essential interrelation. *Arch Biochem Biophys.* (2016) 611:58–65. doi: 10.1016/j.abb.2016.03.022
38. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine Growth Factor Rev.* (2020) 53:25–32. doi: 10.1016/j.cytogfr.2020.05.003
39. Rahman MT, Idid SZ. Can Zn Be a Critical Element in COVID-19 Treatment? *Biol Trace Elem Res.* (2020). doi: 10.1007/s12011-020-02194-9. [Epub ahead of print].
40. Skalny AV, Rink L, Ajsuvakova OP, Aschner M, Gritsenko VA, Alekseenko SI, et al. Zinc and respiratory tract infections: perspectives for COVID19 (Review). *Int J Mol Med.* (2020) 46:17–26. doi: 10.3892/ijmm.2020.4575
41. Truong-Tran AQ, Carter J, Ruffin R, Zalewski PD. New insights into the role of zinc in the respiratory epithelium. *Immunol Cell Biol.* (2001) 79:170–7. doi: 10.1046/j.1440-1711.2001.00986.x
42. Prasad AS. Zinc: mechanisms of host defense. *J Nutr.* (2007) 137:1345–9. doi: 10.1093/jn/137.5.1345
43. Korant BD, Kauer JC, Butterworth BE. Zinc ions inhibit replication of rhinoviruses. *Nature.* (1974) 248:588–90. doi: 10.1038/248588a0
44. Suara RO, Crowe JE Jr. Effect of zinc salts on respiratory syncytial virus replication. *Antimicrob Agents Chemother.* (2004) 48:783–90. doi: 10.1128/aac.48.3.783-790.2004
45. te Velthuis AJ, van den Worm SH, Sims AC, Baric RS, Snijder EJ, van Hemert MJ. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity *in vitro* and zinc ionophores block the replication of these viruses in cell culture. *PLoS Pathog.* (2010) 6:e1001176. doi: 10.1371/journal.ppat.1001176
46. Wei Z, Burwinkel M, Palissa C, Ephraim E, Schmidt MF. Antiviral activity of zinc salts against transmissible gastroenteritis virus *in vitro*. *Vet Microbiol.* (2012) 160:468–72. doi: 10.1016/j.vetmic.2012.06.019
47. Kaushik N, Anang S, Ganti KP, Surjit M. Zinc: a potential antiviral against hepatitis E virus infection? *DNA Cell Biol.* (2018) 37:593–9. doi: 10.1089/dna.2018.4175
48. Read SA, Obeid S, Ahlenstiel C, Ahlenstiel G. The Role of Zinc in Antiviral Immunity. *Adv Nutr.* (2019) 10:696–710. doi: 10.1093/advances/nmz013
49. Lanke K, Krenn BM, Melchers WJ, Seipelt J, van Kuppeveld FJ. PDTC inhibits picornavirus polyprotein processing and RNA replication by transporting zinc ions into cells. *J Gen Virol.* (2007) 88(Pt 4):1206–17. doi: 10.1099/vir.0.82634-0
50. Cakman I, Kirchner H, Rink L. Zinc supplementation reconstitutes the production of interferon-alpha by leukocytes from elderly persons. *J Interferon Cytokine Res.* (1997) 17:469–72. doi: 10.1089/jir.1997.17.469
51. Berg K, Bolt G, Andersen H, Owen TC. Zinc potentiates the antiviral action of human IFN-alpha tenfold. *J Interferon Cytokine Res.* (2001) 21:471–4. doi: 10.1089/10799900152434330
52. Foster M, Samman S. Zinc and regulation of inflammatory cytokines: implications for cardiometabolic disease. *Nutrients.* (2012) 4:676–94. doi: 10.3390/nu4070676
53. McCarty MF, DiNicolantonio JJ. Nutraceuticals have potential for boosting the type 1 interferon response to RNA viruses including influenza and coronavirus. *Prog Cardiovasc Dis.* (2020) 63:383–5. doi: 10.1016/j.pcad.2020.02.007
54. Speth R, Carrera E, Jean-Baptiste M, Joachim A, Linares A. Concentration-dependent effects of zinc on angiotensin-converting enzyme-2 activity (1067.4). *FASEB J.* (2014) 28:1067.1064. doi: 10.1096/fasebj.28.1_supplement.1067.4
55. McPherson SW, Keunen JE, Bird AC, Chew EY, van Kuijk FJ. Investigate oral zinc as a prophylactic treatment for those at risk for COVID-19. *Am J Ophthalmol.* (2020) 216:A5–6. doi: 10.1016/j.ajo.2020.04.028
56. World Health Organization. *Childhood and Maternal Undernutrition*(2002). Chapter 4. Available online at: https://www.who.int/whr/2002/chapter4/en/index3.html (accessed April 17, 2020).
57. Bailey RL, West KP Jr, Black RE. The epidemiology of global micronutrient deficiencies. *Ann Nutr Metab.* (2015) 66(Suppl. 2):22–33. doi: 10.1159/000371618
58. Prasad AS. Discovery of human zinc deficiency and studies in an experimental human model. *Am J Clin Nutr.* (1991) 53:403–12.
59. do Nascimento Nogueira N, Parente JV, Cozzolino SMF. Mudanças na concentração plasmática de zinco e ácido fólico em adolescentes grávidas submetidas a diferentes esquemas de suplementação Changes in plasma zinc and folic acid. *Cad. Saúde pública.* (2003) 19:155–60. doi: 10.1590/S0102-311X2003000100017
60. Chaffee BW, King JC. Effect of zinc supplementation on pregnancy and infant outcomes: a systematic review. *Paediatr Perinat Epidemiol.* (2012) 26(Suppl. 1):118–37. doi: 10.1111/j.1365-3016.2012.01289.x
61. Wessels I, Rolles B, Rink L. The potential impact of zinc supplementation on COVID-19 pathogenesis. *Front Immunol.* (2020) 11:1712. doi: 10.3389/fimmu.2020.01712
62. Arumugam VA, Thangavelu S, Fathah Z. COVID-19 and the world with co-morbidities of heart disease, hypertension and diabetes. *J Pure Appl Microbiol.* (2020) 14:1623–38. doi: 10.22207/JPAM.14.3.01
63. Dhama K, Patel SK, Kumar R, Rana J, Yatoo MI, Kumar A, et al. Geriatric population during the COVID-19 pandemic: problems, considerations, exigencies, and beyond. *Front Public Health*. (2020) 8:574198. doi: 10.3389/fpubh.2020.574198
64. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
65. Chen MD, Lin PY, Lin WH, Cheng V. Zinc in hair and serum of obese individuals in Taiwan. *Am J Clin Nutr.* (1988) 48:1307–9. doi: 10.1093/ajcn/48.5.1307
66. Di Martino G, Matera MG, De Martino B, Vacca C, Di Martino S, Rossi F. Relationship between zinc and obesity. *J Med.* (1992) 24:177–83.
67. Marreiro DN, Fisberg M, Cozzolino SM. Zinc nutritional status in obese children and adolescents. *Biol Trace Elem Res.* (2002) 86:107–22. doi: 10.1385/bter:86:2:107
68. Ozata M, Mergen M, Oktenli C, Aydin A, Sanisoglu SY, Bolu E, et al. Increased oxidative stress and hypozincemia in male obesity. *Clin Biochem.* (2002) 35:627–31. doi: 10.1016/s0009-9120(02)00363-6
69. Pisano M, Hilas O. Zinc and taste disturbances in older adults: a review of the literature. *Consult Pharm.* (2016) 31:267–70. doi: 10.4140/TCP.n.2016.267
70. Meunier N, O'Connor JM, Maiani G, Cashman KD, Secker DL, Ferry M, et al. Importance of zinc in the elderly: the ZENITH study. *Eur J Clin Nutr.* (2005) 59(Suppl. 2):S1–4. doi: 10.1038/sj.ejcn.1602286
71. Haase H, Rink L. The immune system and the impact of zinc during aging. *Immun Ageing.* (2009) 6:9. doi: 10.1186/1742-4933-6-9
72. Mahajan SK, Prasad AS, Lambujon J, Abbasi AA, Briggs WA, McDonald FD. Improvement of uremic hypogeusia by zinc: a double-blind study. *Am J Clin Nutr.* (1980) 33:1517–21. doi: 10.1093/ajcn/33.7.1517
73. Eggert JV, Siegler RL, Edomkesmalee E. Zinc supplementation in chronic renal failure. *Int J Pediatr Nephrol.* (1982) 3:21–4.
74. Mahajan SK, Prasad AS, Rabbani P, Briggs WA, McDonald FD. Zinc deficiency: a reversible complication of uremia. *Am J Clin Nutr.* (1982) 36:1177–83. doi: 10.1093/ajcn/36.6.1177
75. Gruver AL, Hudson LL, Sempowski GD. Immunosenescence of ageing. *J Pathol.* (2007) 211:144–56. doi: 10.1002/path.2104
76. Wong CP, Magnusson KR, Ho E. Increased inflammatory response in aged mice is associated with age-related zinc deficiency and zinc transporter dysregulation. *J Nutr Biochem.* (2013) 24:353–9. doi: 10.1016/j.jnutbio.2012.07.005
77. Wang X, Valenzano MC, Mercado JM, Zurbach EP, Mullin JM. Zinc supplementation modifies tight junctions and alters barrier function of CACO-2 human intestinal epithelial layers. *Dig Dis Sci.* (2013) 58:77–87. doi: 10.1007/s10620-012-2328-8
78. Tian Y, Rong L, Nian W, He Y. Review article: gastrointestinal features in COVID-19 and the possibility of faecal transmission. *Aliment Pharmacol Ther.* (2020) 51:843–51. doi: 10.1111/apt.15731
79. D'Amico F, Baumgart DC, Danese S, Peyrin-Biroulet L. Diarrhea during COVID-19 infection: pathogenesis, epidemiology, prevention and management. *Clin Gastroenterol Hepatol.* (2020) 18:1663–72. doi: 10.1016/j.cgh.2020.04.001
80. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5

81. Pan L. Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study. *Am J Gastroenterol*. (2020) 115:766–73. doi: 10.14309/ajg.0000000000000620
82. Bhatnagar S, Natchu UC. Zinc in child health and disease. *Indian J Pediatr*. (2004) 71:991–5. doi: 10.1007/BF02828114
83. Lazzerini M. Oral zinc provision in acute diarrhea. *Curr Opin Clin Nutr Metab Care*. (2016) 19:239–43. doi: 10.1097/MCO.0000000000000276
84. World Health Organization. *The Treatment of Diarrhoea: A Manual for Physicians and Other Senior Health Workers*. Geneva: WHO. (2005).
85. Nataro JP, Yikang D, Yingkang D, Walker K. AggR, a transcriptional activator of aggregative adherence fimbria I expression in enteroaggregative *Escherichia coli*. *J Bacteriol*. (1994) 176:4691–9.
86. Medeiros P, Bolick DT, Roche JK, Noronha F, Pinheiro C, Kolling GL, et al. The micronutrient zinc inhibits EAEC strain 042 adherence, biofilm formation, virulence gene expression, and epithelial cytokine responses benefiting the infected host. *Virulence*. (2013) 4:624–33. doi: 10.4161/viru.26120
87. Aggarwal R, Sentz J, Miller MA. Role of zinc administration in prevention of childhood diarrhea and respiratory illnesses: a meta-analysis. *Pediatrics*. (2007) 119:1120–30. doi: 10.1542/peds.2006-3481
88. Roth DE, Richard SA, Black RE. Zinc supplementation for the prevention of acute lower respiratory infection in children in developing countries: meta-analysis and meta-regression of randomized trials. *Int J Epidemiol*. (2010) 39:795–808. doi: 10.1093/ije/dyp391
89. Awotiwon AA, Oduwole O, Sinha A, Okwundu CI. Zinc supplementation for the treatment of measles in children. *Cochrane Database Syst Rev*. (2017) 6:CD011177. doi: 10.1002/14651858.CD011177.pub3
90. Prasad AS, Fitzgerald JT, Bao B, Beck FW, Chandrasekar PH. Duration of symptoms and plasma cytokine levels in patients with the common cold treated with zinc acetate. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. (2000) 133:245–52. doi: 10.7326/0003-4819-133-4-200008150-00006
91. Hemila H, Chalker E. The effectiveness of high dose zinc acetate lozenges on various common cold symptoms: a meta-analysis. *BMC Fam Pract*. (2015) 16:24. doi: 10.1186/s12875-015-0237-6
92. Hemila H, Petrus EJ, Fitzgerald JT, Prasad A. Zinc acetate lozenges for treating the common cold: an individual patient data meta-analysis. *Br J Clin Pharmacol*. (2016) 82:1393–8. doi: 10.1111/bcp.13057
93. Finzi E. Treatment of SARS-CoV-2 with high dose oral zinc salts: A report on four patients. *Int J Infect Dis*. (2020) 99:307–9. doi: 10.1016/j.ijid.2020.06.006
94. Yao JS, Paguio JA, Dee EC, Tan HC, Moulick A, Milazzo C, et al. The minimal effect of zinc on the survival of hospitalized patients with COVID-19: an observational study. *Chest*. (2020). doi: 10.1016/j.chest.2020.06.082. [Epub ahead of print].
95. Carr AC, Maggini S. *Vitamin C and Immune Function*. *Nutrients*. (2017) 9:1211. doi: 10.3390/nu9111211
96. Carr A, Frei B. Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J*. (1999) 13:1007–24. doi: 10.1096/fasebj.13.9.1007
97. Englard S, Seifter S. The biochemical functions of ascorbic acid. *Annu Rev Nutr*. (1986) 6:365–406. doi: 10.1146/annurev.nu.06.070186.002053
98. Carr AC, Shaw GM, Fowler AA, Natarajan R. Ascorbate-dependent vasopressor synthesis: a rationale for vitamin C administration in severe sepsis and septic shock? *Crit Care*. (2015) 19:418. doi: 10.1186/s13054-015-1131-2
99. Young JI, Zuchner S, Wang G. Regulation of the Epigenome by Vitamin C. *Annu Rev Nutr*. (2015) 35:545–64. doi: 10.1146/annurev-nutr-071714-034228
100. Burr ML, Elwood PC, Hole DJ, Hurley RJ, Hughes RE. Plasma and leukocyte ascorbic acid levels in the elderly. *Am J Clin Nutr*. (1974) 27:144–51. doi: 10.1093/ajcn/27.2.144
101. Schorah CJ. The level of vitamin C reserves required in man: towards a solution to the controversy. *Proc Nutr Soc*. (1981) 40:147–54. doi: 10.1079/pns19810023
102. Hanck A. Vitamin C in the elderly. *Int J Vitam Nutr Res Suppl*. (1983) 24:257–69.
103. Thurman JE, Mooradian AD. Vitamin supplementation therapy in the elderly. *Drugs Aging*. (1997) 11:433–49. doi: 10.2165/00002512-199711060-00003
104. Fletcher AE, Breeze E, Shetty PS. Antioxidant vitamins and mortality in older persons: findings from the nutrition add-on study to the Medical Research Council Trial of Assessment and Management of Older People in the Community. *Am J Clin Nutr*. (2003) 78:999–1010. doi: 10.1093/ajcn/78.5.999
105. Hunt C, Chakravorty NK, Annan G. The clinical and biochemical effects of vitamin C supplementation in short-stay hospitalized geriatric patients. *Int J Vitam Nutr Res*. (1984) 54:65–74.
106. Arvinte C, Singh M, Marik PE. Serum levels of vitamin C and vitamin D in a Cohort of Critically Ill COVID-19 Patients of a North American Community Hospital Intensive Care Unit in May 2020: a pilot study. *Med Drug Discov*. (2020) 8:100064. doi: 10.1016/j.medidd.2020.100064
107. Hemila H, Chalker E. Vitamin C for preventing and treating the common cold. *Cochrane Database Syst Rev*. (2013) 31:CD000980. doi: 10.1002/14651858.CD000980.pub4
108. Uchida K, Mitsui M, Kawakishi S. Monooxygenation of N-acetylhistamine mediated by L-ascorbate. *Biochim Biophys Acta*. (1989) 991:377–9. doi: 10.1016/0304-4165(89)90131-1
109. Hunt C, Chakravorty NK, Annan G, Habibzadeh N, Schorah CJ. The clinical effects of vitamin C supplementation in elderly hospitalised patients with acute respiratory infections. *Int J Vitam Nutr Res*. (1994) 64:212–9.
110. Bharara A, Grossman C, Grinnan D, Syed A, Fisher B, DeWilde C, et al. Intravenous vitamin C administered as adjunctive therapy for recurrent acute respiratory distress syndrome. *Case Rep Crit Care*. (2016) 2016:8560871. doi: 10.1155/2016/8560871
111. Fowler AA, Kim C, Lepler L, Malhotra R, Debesa O, Natarajan R, et al. Intravenous vitamin C as adjunctive therapy for enterovirus/rhinovirus induced acute respiratory distress syndrome. *World J Crit Care Med*. (2017) 6:85–90. doi: 10.5492/wjccm.v6.i1.85
112. Hoang BX, Shaw DG, Fang W, Han B. A Possible application of high dose vitamin C in the prevention and therapy for Coronavirus Infections. *J Glob Antimicrob Resist*. (2020) 23:256–62. doi: 10.1016/j.jgar.2020.09.025
113. Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, et al. Acute respiratory distress syndrome. *Nat Rev Dis Primers*. (2019) 5:18. doi: 10.1038/s41572-019-0069-0
114. Fisher BJ, Kraskauskas D, Martin EJ, Farkas D, Wegelin JA, Brophy D, et al. Mechanisms of attenuation of abdominal sepsis induced acute lung injury by ascorbic acid. *Am J Physiol Lung Cell Mol Physiol*. (2012) 303:L20–32. doi: 10.1152/ajplung.00300.2011
115. Vissers MCM, Wilkie RP. Ascorbate deficiency results in impaired neutrophil apoptosis and clearance and is associated with up-regulation of hypoxia-inducible factor 1alpha. *J Leukoc Biol*. (2007) 81:1236–44. doi: 10.1189/jlb.0806541
116. Lake MA. What we know so far: COVID-19 current clinical knowledge and research. *Clin Med*. (2020) 20:124–7. doi: 10.7861/clinmed.2019-coron
117. Hemila H. Vitamin C intake and susceptibility to pneumonia. *Pediatr Infect Dis J*. (1997) 16:836–7. doi: 10.1097/00006454-199709000-00003
118. Hemila H, Chalker E. Vitamin C can shorten the length of stay in the ICU: a meta-analysis. *Nutrients*. (2019) 11:708. doi: 10.3390/nu11040708
119. de Melo AF, Homem-de-Mello M. High-dose intravenous vitamin C may help in cytokine storm in severe SARS-CoV-2 infection. *Crit Care*. (2020) 24:500. doi: 10.1186/s13054-020-03228-3
120. Feyaerts AF, Luyten W. Vitamin C as prophylaxis and adjunctive medical treatment for COVID-19? *Nutrition*. (2020) 79–80:110948. doi: 10.1016/j.nut.2020.110948
121. Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, von Bergwelt-Baildon M, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J Allergy Clin Immunol*. (2020) 146:128–36 e124. doi: 10.1016/j.jaci.2020.05.008
122. Bohm F, Settergren M, Pernow J. Vitamin C blocks vascular dysfunction and release of interleukin-6 induced by endothelin-1 in humans *in vivo*. *Atherosclerosis*. (2007) 190:408–15. doi: 10.1016/j.atherosclerosis.2006.02.018
123. Hiedra R, Lo KB, Elbashabsheh M, Gul F, Wright RM, Albano J, et al. The use of IV vitamin C for patients with COVID-19: a case series. *Expert Rev Anti Infect Ther*. (2020) 1–3. doi: 10.1080/14787210.2020.1794819
124. Waqas Khan HM, Parikh N, Megala SM, Predeteanu GS. Unusual early recovery of a critical COVID-19 patient after administration of intravenous

vitamin C. *Am J Case Rep.* (2020) 21:e925521. doi: 10.12659/AJCR.925521
125. Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, et al. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-a review of recent evidence. *Autoimmun Rev.* (2013) 12:976–89. doi: 10.1016/j.autrev.2013.02.004
126. Catarino AM, Claro C, Viana I. Vitamin D - current perspectives. *J Portuguese Soc Dermatol Venereol.* (2016) 74:345–53. doi: 10.29021/spdv.74.4.673
127. Peters BSE, Martini LA. Vitamina D, funções plenamente reconhecidas de nutrientes. *Int Life Sci Instit Brasil.* (2014) 2:1–23.
128. de Castro LCG. O sistema endocrinológico vitamina D. *Arq Bra Endocrinol Metabol.* (2011) 55:566–75. doi: 10.1590/S0004-27302011000800010
129. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. *Lancet.* (1982) 1:74–6. doi: 10.1016/s0140-6736(82)90214-8
130. Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF. Sunscreens suppress cutaneous vitamin D3 synthesis. *J Clin Endocrinol Metab.* (1987) 64:1165–8. doi: 10.1210/jcem-64-6-1165
131. Holick MF. Vitamin D deficiency. *N Engl J Med.* (2007) 357:266–81. doi: 10.1056/NEJMra070553
132. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. *Am J Clin Nutr.* (1995) 61(3 Suppl):638S−45S. doi: 10.1093/ajcn/61.3.638S
133. Goring H, Koshuchowa S. Vitamin D – the sun hormone. Life in environmental mismatch. *Biochemistry.* (2015) 80:8–20. doi: 10.1134/S0006297915010022
134. Vanherwegen AS, Gysemans C, Mathieu C. Regulation of immune function by vitamin D and its use in diseases of immunity. *Endocrinol Metab Clin North Am.* (2017) 46:1061–94. doi: 10.1016/j.ecl.2017.07.010
135. Chakhtoura M, Napoli N, El Hajj Fuleihan G. Commentary: myths and facts on vitamin D amidst the COVID-19 pandemic. *Metabolism.* (2020) 109:154276. doi: 10.1016/j.metabol.2020.154276
136. Xu Y, Baylink DJ, Chen CS, Reeves ME, Xiao J, Lacy C, et al. The importance of vitamin d metabolism as a potential prophylactic, immunoregulatory and neuroprotective treatment for COVID-19. *J Transl Med.* (2020) 18:322. doi: 10.1186/s12967-020-02488-5
137. Teymoori-Rad M, Shokri F, Salimi V, Marashi SM. The interplay between vitamin D and viral infections. *Rev Med Virol.* (2019) 29:e2032. doi: 10.1002/rmv.2032
138. Klotman ME, Chang TL. Defensins in innate antiviral immunity. *Nat Rev Immunol.* (2006) 6:447–56. doi: 10.1038/nri1860
139. Crane-Godreau MA, Clem KJ, Payne P, Fiering S. Vitamin D deficiency and air pollution exacerbate COVID-19 through suppression of antiviral peptide LL37. *Front Public Health.* (2020) 8:232. doi: 10.3389/fpubh.2020.00232
140. Munshi R, Hussein MH, Toraih EA, Elshazli RM, Jardak C, Sultana N, et al. Vitamin D insufficiency as a potential culprit in critical COVID-19 patients. *J Med Virol.* (2020). doi: 10.1002/jmv.26360. [Epub ahead of print].
141. Orru B, Szekeres-Bartho J, Bizzarri M, Spiga AM, Unfer V. Inhibitory effects of Vitamin D on inflammation and IL-6 release. A further support for COVID-19 management? *Eur Rev Med Pharmacol Sci.* (2020) 24:8187–93. doi: 10.26355/eurrev_202008_22507
142. Daneshkhah A, Agrawal V, Eshein A, Subramanian H, Roy HK, Backman V. The possible role of Vitamin D in suppressing cytokine storm and associated mortality in COVID-19 Patients. *medRxiv.* (2020) 2020.2004.2008.20058578. doi: 10.1101/2020.04.08.20058578
143. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ.* (2017) 356:i6583. doi: 10.1136/bmj.i6583
144. Clairmont A, Tessman D, Stock A, Nicolai S, Stahl W, Sies H. Induction of gap junctional intercellular communication by vitamin D in human skin fibroblasts is dependent on the nuclear Induction of gap junctional intercellular communication by vitamin D in human skin fibroblasts is dependent on the nuclear vitamin D receptor. *Carcinogenesis.* (1996) 17:1389–91. doi: 10.1093/carcin/17.6.1389
145. Gniadecki R, Gajkowska B, Hansen M. 1,25-dihydroxyvitamin D3 stimulates the assembly of adherens junctions in keratinocytes: involvement of protein kinase C. *Endocrinology.* (1997) 138:2241–8. doi: 10.1210/endo.138.6.5156
146. Chen H, Lu R, Zhang YG, Sun J. Vitamin D receptor deletion leads to the destruction of tight and adherens junctions in lungs. *Tissue Barriers.* (2018) 6:1–13. doi: 10.1080/21688370.2018.1540904
147. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* (2011) 96:1911–30. doi: 10.1210/jc.2011-0385
148. Braiman M. *Latitude Dependence of the COVID-19 Mortality Rate — A Possible Relationship to Vitamin D Deficiency?* New York, NY: Syracuse University Department of Chemistry. (2020). doi: 10.2139/ssrn.3561958
149. Ilie PC, Stefanescu S, Smith L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. *Aging Clin Exp Res.* (2020) 32:1195–8. doi: 10.1007/s40520-020-01570-8
150. Merzon E, Tworowski D, Gorohovski A, Vinker S, Golan Cohen A, Green I, et al. Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. *FEBS J.* (2020) 287:3693–702. doi: 10.1111/febs.15495
151. Panarese A, Shahini E. Letter: Covid-19, and vitamin D. *Aliment Pharmacol Ther.* (2020) 51:993–5. doi: 10.1111/apt.15752
152. Rhodes JM, Subramanian S, Laird E, Kenny RA. Editorial: low population mortality from COVID-19 in countries south of latitude 35 degrees North supports vitamin D as a factor determining severity. *Aliment Pharmacol Ther.* (2020) 51:1434–7. doi: 10.1111/apt.15777
153. Valcour A, Blocki F, Hawkins DM, Rao SD. Effects of age and serum 25-OH-vitamin D on serum parathyroid hormone levels. *J Clin Endocrinol Metab.* (2012) 97:3989–95. doi: 10.1210/jc.2012-2276
154. Amrein K, Venkatesh B. Vitamin D and the critically ill patient. *Curr Opin Clin Nutr Metab Care.* (2012) 15:188–93. doi: 10.1097/MCO.0b013e32834f0027
155. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev.* (2014) 10:CD007470. doi: 10.1002/14651858.CD007470.pub3
156. Lippi G, Henry BM, Bovo C, Sanchis-Gomar F. Health risks and potential remedies during prolonged lockdowns for coronavirus disease 2019 (COVID-19). *Diagnosis (Berl).* (2020) 7:85–90. doi: 10.1515/dx-2020-0041
157. Xu J, Yang J, Chen J, Luo Q, Zhang Q, Zhang H. Vitamin D alleviates lipopolysaccharideinduced acute lung injury via regulation of the reninangiotensin system. *Mol Med Rep.* (2017) 16:7432–8. doi: 10.3892/mmr.2017.7546
158. Kumar D, Gupta P, Banerjee D. Letter: does vitamin D have a potential role against COVID-19? *Aliment Pharmacol Ther.* (2020) 52:409–11. doi: 10.1111/apt.15801
159. Malek Mahdavi A. A brief review of interplay between vitamin D and angiotensin-converting enzyme 2: implications for a potential treatment for COVID-19. *Rev Med Virol.* (2020) 30:e2119. doi: 10.1002/rmv.2119
160. Musavi H, Abazari O, Barartabar Z, Kalaki-Jouybari F, Hemmati-Dinarvand M, Esmaeili P, et al. The benefits of Vitamin D in the COVID-19 pandemic: biochemical and immunological mechanisms. *Arch Physiol Biochem.* (2020). doi: 10.1080/13813455.2020.1826530. [Epub ahead of print].
161. Rhodes JM, Subramanian S, Laird E, Griffin G, Kenny RA. Perspective: Vitamin D deficiency and COVID-19 severity - plausibly linked by latitude, ethnicity, impacts on cytokines, ACE2 and thrombosis. *J Intern Med.* (2020). doi: 10.1111/joim.13149. [Epub ahead of print].
162. Patel AB, Verma A. COVID-19 Angiotensin-converting enzyme inhibitors angiotensin receptor blockers: what is the evidence? *JAMA.* (2020) 323:1769–70. doi: 10.1001/jama.2020.4812
163. Ebadi M, Montano-Loza AJ. Perspective: improving vitamin D status in the management of COVID-19. *Eur J Clin Nutr.* (2020) 74:856–9. doi: 10.1038/s41430-020-0661-0
164. Siuka D, Pfeifer M, Pinter B. Vitamin D supplementation during the COVID-19 pandemic. *Mayo Clin Proc.* (2020) 95:1804–5. doi: 10.1016/j.mayocp.2020.05.036

165. Weir EK, Thenappan T, Bhargava M, Chen Y. Does vitamin D deficiency increase the severity of COVID-19? *Clin Med.* (2020) 20:e107–8. doi: 10.7861/clinmed.2020-0301
166. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N Engl J Med.* (2020) 382:1564–7. doi: 10.1056/NEJMc2004973
167. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, et al. Virology, epidemiology, pathogenesis, and control of COVID-19. *Viruses*. (2020) 12:372. doi: 10.3390/v12040372
168. Li JO, Lam DSC, Chen Y, Ting DSW. Novel Coronavirus disease 2019 (COVID-19): The importance of recognising possible early ocular manifestation and using protective eyewear. *Br J Ophthalmol.* (2020) 104:297–8. doi: 10.1136/bjophthalmol-2020-315994
169. Li JY, You Z, Wang Q, Zhou ZJ, Qiu Y, Luo R, et al. The epidemic of 2019-novel-coronavirus (2019-nCoV) pneumonia and insights for emerging infectious diseases in the future. *Microbes Infect*. (2020) 22:80–5. doi: 10.1016/j.micinf.2020.02.002
170. Lu CW, Liu XF, Jia ZF. 2019-nCoV transmission through the ocular surface must not be ignored. *Lancet.* (2020) 395:e39. doi: 10.1016/S0140-6736(20)30313-5
171. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet*. (2020) 395:470–3. doi: 10.1016/S0140-6736(20)30185-9
172. Zihni C, Mills C, Matter K, Balda MS. Tight junctions: from simple barriers to multifunctional molecular gates. *Nat Rev Mol Cell Biol.* (2016) 17:564–80. doi: 10.1038/nrm.2016.80
173. Alberts B, Bray D, Watson J. *Biologia Molecular da Célula.* Artmed. (2017).
174. Laukoetter MG, Bruewer M, Nusrat A. Regulation of the intestinal epithelial barrier by the apical junctional complex. *Curr Opin Gastroenterol.* (2006) 22:85–9. doi: 10.1097/01.mog.0000203864.48255.4f
175. Zhang YG, Wu S, Sun J. Vitamin D. Vitamin D receptor, and tissue barriers. *Tissue Barriers*. (2013) 1:e23118. doi: 10.4161/tisb.23118
176. Miyoshi Y, Tanabe S, Suzuki T. Cellular zinc is required for intestinal epithelial barrier maintenance via the regulation of claudin-3 and occludin expression. *Am J Physiol Gastrointest Liver Physiol*. (2016) 311:105–16. doi: 10.1152/ajpgi.00405.2015
177. Campbell HK, Maiers JL, DeMali KA. Interplay between tight junctions & adherens junctions. *Exp Cell Res.* (2017) 358:39–44. doi: 10.1016/j.yexcr.2017.03.061
178. Green KJ, Jaiganesh A, Broussard JA. Desmosomes: essential contributors to an integrated intercellular junction network. *F1000Res.* (2019) 8. doi: 10.12688/f1000research.20942.1
179. Bergelson JM. Intercellular junctional proteins as receptors and barriers to virus infection and spread. *Cell Host Microbe.* (2009) 5:517–21. doi: 10.1016/j.chom.2009.05.009
180. Teoh KT, Siu YL, Chan WL, Schluter MA, Liu CJ, Peiris JS, et al. The SARS coronavirus E protein interacts with PALS1 and alters tight junction formation and epithelial morphogenesis. *Mol Biol Cell.* (2010) 21:3838–52. doi: 10.1091/mbc.E10-04-0338
181. Sturniolo GC, Di Leo V, Ferronato A, D'Odorico A, D'Inca R. Zinc supplementation tightens "leaky gut" in Crohn's disease. *Inflamm Bowel Dis.* (2001) 7:94–8. doi: 10.1097/00054725-200105000-00003
182. Tran CD, Ball JM, Sundar S, Coyle P, Howarth GS. The role of zinc and metallothionein in the dextran sulfate sodium-induced colitis mouse model. *Dig Dis Sci.* (2007) 52:2113–21. doi: 10.1007/s10620-007-9765-9
183. Zhang B, Guo Y. Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets. *Br J Nutr.* (2009) 102:687–93. doi: 10.1017/S0007114509289033
184. Garcia-Hernandez V, Quiros M, Nusrat A. Intestinal epithelial claudins: expression and regulation in homeostasis and inflammation. *Ann N Y Acad Sci.* (2017) 1397:66–79. doi: 10.1111/nyas.13360
185. Atisook K, Carlson S, Madara JL. Effects of phlorizin and sodium on glucose-elicited alterations of cell junctions in intestinal epithelia. *Am J Physiol.* (1990) 258:C77–85. doi: 10.1152/ajpcell.1990.258.1.C77
186. Reyes JL, Lamas M, Martin D, del Carmen Namorado M, Islas S, Luna J, et al. The renal segmental distribution of claudins changes with development. *Kidney Int.* (2002) 62:476–87. doi: 10.1046/j.1523-1755.2002.00479.x
187. Escaffit F, Boudreau F, Beaulieu JF. Differential expression of claudin-2 along the human intestine: implication of GATA-4 in the maintenance of claudin-2 in differentiating cells. *J Cell Physiol.* (2005) 203:15–26. doi: 10.1002/jcp.20189
188. Van Itallie CM, Anderson JM. Claudins and epithelial paracellular transport. *Annu Rev Physiol.* (2006) 68:403–29. doi: 10.1146/annurev.physiol.68.040104.131404
189. Bao S, Knoell DL. Zinc modulates cytokine-induced lung epithelial cell barrier permeability. *Am J Physiol Lung Cell Mol Physiol.* (2006) 291:L1132–1141. doi: 10.1152/ajplung.00207.2006
190. Oudemans-van Straaten HM, Spoelstra-de Man AM, de Waard MC. Vitamin C revisited. *Crit Care.* (2014) 18:460. doi: 10.1186/s13054-014-0460-x
191. Berger MM, Oudemans-van Straaten HM. Vitamin C supplementation in the critically ill patient. *Curr Opin Clin Nutr Metab Care.* (2015) 18:193–201. doi: 10.1097/MCO.0000000000000148
192. Rao R. Occludin phosphorylation in regulation of epithelial tight junctions. *Ann N Y Acad Sci.* (2009) 1165:62–8. doi: 10.1111/j.1749-6632.2009.04054.x
193. Fujita H, Sugimoto K, Inatomi S. Tight junction proteins claudin-2 and−12 are critical for Vitamin D-dependent Ca2 absorption between enterocytes. *Molecular Biol Cell.* (2008) 19:1912–21. doi: 10.1091/mbc.e07-09-0973
194. Yin Z, Pintea V, Lin Y, Hammock BD, Watsky MA. Vitamin D enhances corneal epithelial barrier function. *Invest Ophthalmol Vis Sci.* (2011) 52:7359–64. doi: 10.1167/iovs.11-7605
195. Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, et al. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. *J Cell Biol.* (2001) 154:369–87. doi: 10.1083/jcb.200102028
196. Takeichi M. Morphogenetic roles of classic cadherins. *Curr Opin Cell Biol.* (1995) 7:619–27. doi: 10.1016/0955-0674(95)80102-2
197. Gumbiner BM. *Cell* adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell.* (1996) 84:345–57. doi: 10.1016/s0092-8674(00)81279-9
198. Birchmeier W, Behrens J. Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. *Biochim Biophys Acta.* (1994) 1198:11–26. doi: 10.1016/0304-419x(94)90003-5
199. Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature.* (1998) 392:190–3. doi: 10.1038/32433
200. Christofori G, Semb H. The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. *Trends Biochem Sci.* (1999) 24:73–6. doi: 10.1016/s0968-0004(98)01343-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Name, Souza, Vasconcelos, Prado and Pereira. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 09 December 2020
doi: 10.3389/fimmu.2020.590459

A Network-Based Analysis Reveals the Mechanism Underlying Vitamin D in Suppressing Cytokine Storm and Virus in SARS-CoV-2 Infection

Firoz Ahmed [1,2]*

[1] Department of Biochemistry, College of Science, University of Jeddah, Jeddah, Saudi Arabia, [2] University of Jeddah Center for Scientific and Medical Research, University of Jeddah, Jeddah, Saudi Arabia

Background: SARS-CoV-2 causes ongoing pandemic coronavirus disease of 2019 (COVID-19), infects the cells of the lower respiratory tract that leads to a cytokine storm in a significant number of patients resulting in severe pneumonia, shortness of breathing, respiratory and organ failure. Extensive studies suggested the role of Vitamin D in suppressing cytokine storm in COVID-19 and reducing viral infection; however, the precise molecular mechanism is not clearly known. In this work, bioinformatics and systems biology approaches were used to understand SARS-CoV-2 induced cytokine pathways and the potential mechanism of Vitamin D in suppressing cytokine storm and enhancing antiviral response.

Results: This study used transcriptome data and identified 108 differentially expressed host genes (DEHGs) in SARS-CoV-2 infected normal human bronchial epithelial (NHBE) cells compared to control. Then, the DEHGs was integrated with the human protein-protein interaction data to generate a SARS-CoV-2 induced host gene regulatory network (*SiHgrn*). Analysis of *SiHgrn* identified a sub-network "*Cluster 1*" with the highest MCODE score, 31 up-regulated genes, and predominantly associated immune and inflammatory response. Interestingly, the iRegulone tool identified that "*Cluster 1*" is under the regulation of transcription factors STAT1, STAT2, STAT3, POU2F2, and NFkB1, collectively referred to as "*host response signature network*". Functional enrichment analysis with NDEx revealed that the "*host response signature network*" is predominantly associated with critical pathways, including "cytokines and inflammatory response", "non-genomic action of Vitamin D", "the human immune response to tuberculosis", and "lung fibrosis". Finally, in-depth analysis and literature mining revealed that Vitamin D binds with its receptor and could work through two different pathways: (i) it inhibits the expression of pro-inflammatory cytokines through blocking the TNF induced NFkB1 signaling pathway; and (ii) it initiates the expression of interferon-stimulating genes (ISGs) for antiviral defense program through activating the IFN-α induced Jak-STAT signaling pathway.

OPEN ACCESS

Edited by:
Christophe Matthys, KU Leuven, Belgium

Reviewed by:
Ran Wei, The State University of New Jersey, United States
Gerly Anne de Castro Brito, Federal University of Ceara, Brazil

***Correspondence:**
Firoz Ahmed
fahmed1@uj.edu.sa

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: *01 August 2020*
Accepted: *30 October 2020*
Published: *09 December 2020*

Citation:
Ahmed F (2020) A Network-Based Analysis Reveals the Mechanism Underlying Vitamin D in Suppressing Cytokine Storm and Virus in SARS-CoV-2 Infection. Front. Immunol. 11:590459. doi: 10.3389/fimmu.2020.590459

Conclusion: This comprehensive study identified the pathways associated with cytokine storm in SARS-CoV-2 infection. The proposed underlying mechanism of Vitamin D could be promising in suppressing the cytokine storm and inducing a robust antiviral response in severe COVID-19 patients. The finding in this study urgently needs further experimental validations for the suitability of Vitamin D in combination with IFN-α to control severe COVID-19.

Keywords: SARS-CoV-2, COVID-19, cytokine storm, vitamin D, lung fibrosis, bioinformatics, regulatory network, RNA sequencing

INTRODUCTION

The emergence and rapid spread of a highly pathogenic new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), created tremendous health, economic, and social crises worldwide. SARS-CoV-2 infects the cells of the lower respiratory tract and causes severe respiratory disease in humans, named coronavirus disease of 2019 (COVID-19). In late 2019, SARS-CoV-2 was reported in patients with severe pneumonia in Wuhan city of Hubei province, China. In a short period of time, the virus has infected a large number of people around the world, and this number is increasing exponentially (1). On March 11, 2020, the COVID-19 outbreak was declared a new pandemic by the World Health Organization (WHO). As of October 5, 2020, the number of SARS-CoV-2 infected people were 35,645,015; and among them, 1,044,898 cases of death and 26,791,820 cases of recovered were reported (https://www.worldometers.info/coronavirus/).

SARS-CoV-2 is closely related to previously identified pathogenic beta-coronavirus SARS-CoV, responsible for the epidemic in 2002 to 2003; and Middle East respiratory syndrome coronavirus (MERS-CoV), accountable for the epidemic in 2012 (1–4). The SARS-CoV-2 genome is about 29,000 nucleotides long positive-sense single-stranded RNA that encodes four structural proteins, sixteen non-structural proteins, and nine accessory proteins (1). These proteins help the virus to infect the host cell and hijack the host's cellular machinery for virus assembly, amplification, and pathogenesis. During infection, the spike (S) protein of SARS-CoV-2 binds with host receptor angiotensin-converting enzyme 2 (ACE2) (5). Subsequently, host serine protease TMPRSS2 cleaves the viral S protein to generate S1 and S2 subunits, which fuses the viral and host membrane resulting internalization of the virus (5).

SARS-CoV-2–infected patients showed a symptom of fever, cough, and dyspnea. However, severe COVID-19 patients with comorbidities of pneumonia lead to acute respiratory distress syndrome (ARDS), and multi-organ failure resulting in death (1, 6–9). After SARS-CoV-2 infection, the lung cells activate the innate immune response against viruses. The severe COVID-19 cases show an influx of pro-inflammatory cytokines in the surrounding environment resulting in recruitments and stimulation of immune cells, including macrophages, neutrophils, and natural killer (NK) cells to kill virus-infected cells. The activated immune system regulation is important to target only virus-infected cells without harming healthy cells. Clinical data suggest that most COVID-19 deaths are due to the influx of various pro-inflammatory cytokines, termed as cytokine storm or cytokine release syndrome (CRS), which are responsible for severe damage of the healthy cells, tissues, and organs (6, 10). A study found an increase in Th17 cell and inflammatory cytokines (IL2, IL6, IL10, and IFN-γ) in severe COVID-19 patients compared to mild cases (10, 11). In contrast, severe COVID-19 cases reported a decrease in $CD4^+$ cell, $CD8^+$ cell, and lymphocytes compared to mild cases.

Previous studies identified that only 20% of COVID-19 patients showed the severe symptom of cytokine storm. Several studies indicate the role of Vitamin D supplements in protecting against diseases and reducing the risk of bacterial and viral infections, including influenza and SARS-CoV-2 infections (12–15). Vitamin D is a fat-soluble vitamin that involves maintaining the calcium level in the body. Classically, Vitamin D interacts with the nuclear Vitamin D receptor (VDR) to form a complex, which binds to the promoter region and modulates the expression of target genes (16). Besides, Vitamin D could also work through non-genomic action where it binds to the VDR and activates several signaling pathways, and indirectly regulates the transcription of numerous genes, including genes associated with immune response (12, 16). Numerous studies indicated that vitamin D deficiency is associated with cytokine storm and causes high morbidity and mortality in COVID-19 patients (14, 17). Moreover, the studies suggested that Vitamin D supplementation reduces the risk of the cytokine storm. However, the underlying molecular mechanism of Vitamin D in suppressing the cytokine storm and reducing viral infection in COVID-19 is not clearly understood yet, which is crucial to develop better therapy and management to save the life of SARS-CoV-2 infected person.

With the advancement of high-throughput "*omics*" technology and computational methods, the gene expression and protein interaction data could be integrated at the systems biology level to identify the altered gene regulatory network, activated pathways, and the molecular mechanism underlying complex genetic and infectious diseases (18–26).

In this work, bioinformatics and systems biology approaches were used to understand SARS-CoV-2 induced altered host gene regulatory network, biological pathways of cytokine storm, and the potential regulatory mechanism of Vitamin D in suppressing cytokine storm and reducing viral infection. The study used publicly available transcriptome data to identify the differentially expressed host genes (DEHGs) in SARS-CoV-2–infected human

cells compared to mock-infected cells (control). Then, the biological role of DEHGs was analyzed with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Moreover, DEHGs were integrated with the human protein-protein interaction data (PPI). A gene regulatory network was created and analyzed to understand altered biological processes and their upstream master regulators. Our comprehensive analysis revealed a vital sub-network of highly interconnected 31 proteins predominantly responsible for the immune and inflammatory response and is under the regulation of transcription factors (TFs) STAT1, STAT2, STAT3, POU2F2, and NFkB1, which collectively referred to as "*host response signature network*".

Furthermore, the "*host response signature network*" is significantly associated with altered biological pathways, including "cytokines and inflammatory response", and "non-genomic action of Vitamin D". Interestingly, our study revealed a potential mechanism through which Vitamin D could suppress cytokine storm and reduce viral infection in COVID-19. Finally, differentially expressed host genes from COVID-19 patients ($DEHGs^{COVID\text{-}19}$) were taken to validate our findings. This study will contribute to a better understanding of the regulatory mechanism of cytokine storm and therapeutic intervention for severe COVID-19 patients.

MATERIALS AND METHODS

Transcriptome Data and Identification of DEHGs

This study used the RNA sequencing data of normal human bronchial epithelial (NHBE) cells infected with SARS-CoV-2 (USA-WA1/2020) and mock-infected NHBE cells as control. The transcriptome data were downloaded from the Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo/) with accession number GSE147507 (27). Each infected and controlled group has independent biological triplicate experimental data, and the reads were mapped on the human genome (hg19). The raw counts of expression data were used to identify the DEHGs between virus-infected cells compared to control with DESeq2 package version 1.22.2 in R version 3.5.2 (28). The DESeq2 takes two files as input: (a) a table of raw read counts from different samples where each row represents a gene and a column indicates the sample, and (b) an associated phenodata file describing the experimental group of each sample. DESeq2 performs internal normalization to adjust the differences in library size and library composition. For this, DESeq2 first calculates the scaling factor for each sample. Then the original read count is divided with sample scaling factor to get a normalized value. For each gene, DESeq2 uses a negative binomial distribution to model the counts and fit the normalized count data. DESeq2 uses shrinkage estimation for dispersions and fold changes and uses the Wald test to find out genes with significant differential expression between two sample groups.

A schematic diagram of our study is presented in **Figure 1**. The host gene is considered as differentially expressed if the log2 Fold Change |log2FC| is > 1 and adjusted p-value is < 0.05. The expression data of the significant DEHGs we selected and transformed into Z-score (row-wise of value), then a heatmap was created with pheatmap package version 1.0.12 in R.

Functional Enrichment of DEHGs

To understand the biological processes induced by SARS-CoV-2, the list of DEHGs were analyzed for functional enrichment, including GO of Biological Process (BP), Molecular Function (MF), and Cellular Component (CC); KEGG and BIOCARTA pathways; and DISEASE using DAVID 6.8 (https://david.ncifcrf.gov/home.jsp). The significantly enriched terms were considered when at least five genes were present, and the Benjamini adjusted p-value is less than 0.05 (p-adjust <0.05). Then the enrichment was visualized using dotplot with the ggplot2 in R.

Construction and Analysis of SARS-CoV-2–induced Host Gene Regulatory Network (SiHgrn)

The list of DEHGs, along with two additional host genes (ACE2 and TMPRSS2 involved in SARS-CoV-2 infection), was mapped to the STRING version 11 application (29). Although *ACE2* and *TMPRSS2* were not in the list of DEHGs, these two host proteins are nevertheless crucial for SARS-CoV-2 infection. Therefore, ACE2 and TMPRSS2 were taken into account during network construction to determine whether these proteins could interact with other proteins of DEHGs and influence the regulatory network. The human PPI data with medium confidence interaction score >0.400 was downloaded from STRING, and then a network was constructed and visualized in Cytoscape software version 3.7. In this network, each protein is represented by a node, while the interaction between two proteins is represented by an edge (30). Furthermore, the gene expression level (log2FC score) from DEHGs was integrated into the protein node to construct a PPI network called *SiHgrn*. After that, the topological structure of the *SiHgrn* was analyzed using Cytoscape plug-in "NetworkAnalyzer."

The highly connected local sub-networks modules in the *SiHgrn* were extracted using the Cytoscape plug-in MCODE clustering algorithm (31). The functional enrichment of the highest score MCODE (*Cluster 1*) was analyzed for GO, KEGG, and BIOCARTA pathways, and DISEASE with DAVID 6.8. Besides, the gene set of *Cluster 1* was analyzed with Molecular Signatures Database (MSigDB version 7.1) (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp), which is the most famous repository of biological gene sets for performing enrichment analysis (32). Each gene set contains a collection of genes that shared a specific-biological property. In this study, MSigBD was used to identify the common underlying biological process and pathways with "hallmark gene sets" and the "positional gene set" at default parameters. The "hallmark gene sets" is the collection of 50 gene sets in which each set contains a well-defined biological state or process with coordinate expression (33). The "positional gene sets" is the collection of 299 gene sets by chromosome and cytogenetic band, and thus, it can help identify effects related to the chromosomal position.

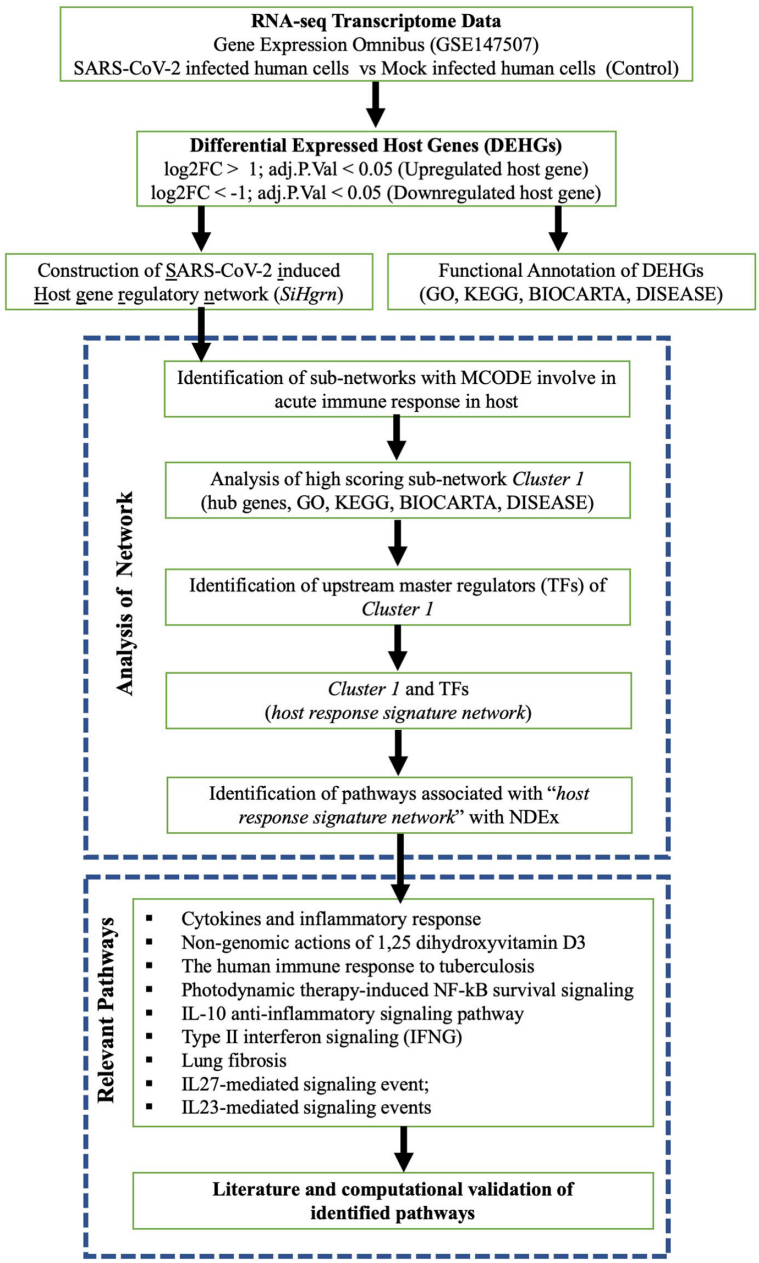


FIGURE 1 | Schematic diagram of present study workflow.

Furthermore, MSigDB was also used to determine the gene family according to their protein homology or biochemical activity.

Discovery of Upstream Master Regulators of Cluster 1

To find the upstream TFs regulators of genes of *Cluster 1*, Cytoscape plug-in iRegulone (version 1.3) was used at default parameters (34). The output result was further analyzed using the in house Perl program, and a matrix table was generated where each row indicates a TF, while a column indicates a target gene. The matrix was then visualized with a heatmap using the Morpheus tool (https://software.broadinstitute.org/morpheus/). The proteins of *Cluster 1* and upstream TFs are considered as a "*host response signature network*".

Biological Pathways of *"Host Response Signature Network"*

The gene set of "*host response signature network* (*Cluster 1* and its upstream regulators)" were analyzed to identify the biological pathways using the "Relevant Pathways" module of Network Data Exchange (NDEx version 2.4.5, 18 June 2020) (https://ndexbio.org/) (35, 36). NDEx is an open-source resource

for the collection and analysis of biological network knowledge. The output result of "Relevant Pathways" analysis gave a list of biological pathways enriched for query genes. The result was sorted according to the highest similarity score between our query set and the network's genes. Then, the top nine significantly enriched pathways having more than 5 overlapping genes were collected for further analysis. If required, figures from WikiPathways were modified with PathVisio version 3.3.0.

RESULTS

Identification of DEHGs

In order to understand how the host gene expression altered in response to SARS-CoV-2 infection, the raw read counts of host transcriptome data were preprocessed and analyzed to identify the DEHGs in SARS-CoV-2–infected cell compared to control cell with DESeq2. The boxplots showed the raw read counts, and normalized read counts across samples (**Figure S1**). A global view of differentially expressed genes in RNA-seq datasets was visualized with the MA-plot, where M indicates the $\log_2$ fold change on the y-axis, while A indicates the mean of the normalized counts on the x-axis (**Figure S2**). A total of 108 DEHGs including 93 up-regulated and 15 down-regulated genes were identified with |log2FC| > 1 and adjusted p-value < 0.05. Among them, the top 15 genes showing up-regulation are *CSF3, SPRR2E*, *CXCL5*, *CCL20*, *SPRR2D*, *CSF2*, *IL6*, *IFI27*, *IL36G*, *XAF1*, *MX1*, *PDZK1IP1*, *SAA2*, *IFI6, and CXCL8*; while 15 genes showing down-regulation are *RBM20*, *IFITM10*, *NANOS1*, *STON1*, *CXCL14*, *MYLK*, *MAP7D2*, *NID1*, *ZNF488*, *ATG9B*, *VTCN1*, *CYP4F3*, *MIR221*, *PPARGC1A*, and *METTL7A* (**Table 1**). The detailed information of up- and down-regulated genes is provided in **Tables S1** and **S2**, respectively. Furthermore, a heatmap of gene expression level was generated, which shows a distinct pattern of up-regulated and down-regulated genes in SARS-CoV-2–infected cells compared with mock across all biological samples (**Figure S3**).

Functional Enrichment of DEHGs

To understand the biological processes and key pathways altered in SARS-CoV-2–infected cell, the function and pathway enrichment analysis of the DEHGs were performed. The BP of GO analysis revealed that the up-regulated genes are primarily associated with inflammatory response, immune response, apoptotic process, type 1 interferon (IFN) signaling pathway, innate immune response, defense response to viruses, and positive regulation of NFkB transcription factor activity (**Figure 2A**). The MF of GO analysis revealed that the up-regulated genes are primarily associated with cytokine activity, growth factor activity, and chemokine activity (**Figure 2A**); while the CC of GO analysis revealed that the up-regulated genes are significantly related to extracellular space, extracellular region, and cell surface (**Figure 2A**). The KEGG pathway analysis showed the up-regulated genes are significantly enriched in TNF signaling pathway, cytokine-cytokine receptor interaction, measles, influenza A, Herpes simplex infection (**Figure 2A**). The BIOCARTA pathways showed that the up-regulated genes are significantly enriched in signal transduction through IL1R, cytokines and inflammatory response, and cells and molecules involved in the local acute inflammatory response (**Figure S4A**). The DISEASE annotation showed that the up-regulated genes are significantly enriched in type 2 diabetes, ovarian cancer, atherosclerosis, and respiratory syncytial virus bronchiolitis (**Figure S4B**). The BP, MF, and CC of GO, KEGG, BIOCARTA, and DISEASE analysis of down-regulated genes did not show any functional enrichment term. The complete results of GO, KEGG, BIOCARTA, and DISEASE annotation analyses of up-regulated host genes are available in **Table S3**.

Construction and Analysis of SiHgrn Network

We mapped the 110 genes (108 DEHGs; *ACE2* and *TMPRSS2* of host genes) to the STRING database and retrieved the PPI network having 88 nodes and 563 edges. Then, the PPI network was visualized in Cytoscape software, in which each node represents a protein, while an edge represents an interaction between proteins.

TABLE 1 | A list of the top 15 differentially expressed host genes (DEHGs) in SARS-CoV-2–infected NHBE cells.

Up-regulated			Down-regulated		
Gene	**Log2FC**	**Adj. p-value**	**Gene**	**Log2FC**	**Adj. p-value**
CSF3	4.85	3.65E-20	*RBM20*	−1.58	3.93E-02
SPRR2E	3.60	4.59E-14	*IFITM10*	−1.57	3.39E-10
CXCL5	3.49	3.78E-28	*NANOS1*	−1.52	6.21E-03
CCL20	3.15	1.38E-76	*STON1*	−1.51	8.89E-03
SPRR2D	2.98	2.14E-48	*CXCL14*	−1.45	7.56E-06
CSF2	2.98	9.98E-11	*MYLK*	−1.43	7.58E-06
IL6	2.93	7.71E-24	*MAP7D2*	−1.39	6.13E-04
IFI27	2.90	2.18E-12	*NID1*	−1.23	6.75E-04
IL36G	2.73	3.86E-50	*ZNF488*	−1.14	1.54E-02
XAF1	2.53	4.03E-13	*ATG9B*	−1.13	3.40E-03
MX1	2.51	6.73E-34	*VTCN1*	−1.07	7.25E-06
PDZK1IP1	2.43	8.68E-21	*CYP4F3*	−1.07	1.65E-02
SAA2	2.42	9.38E-76	*MIR221*	−1.05	4.95E-02
IFI6	2.35	1.94E-03	*PPARGC1A*	−1.05	2.17E-03
CXCL8(IL8)	2.34	5.24E-105	*METTL7A*	−1.03	1.57E-05

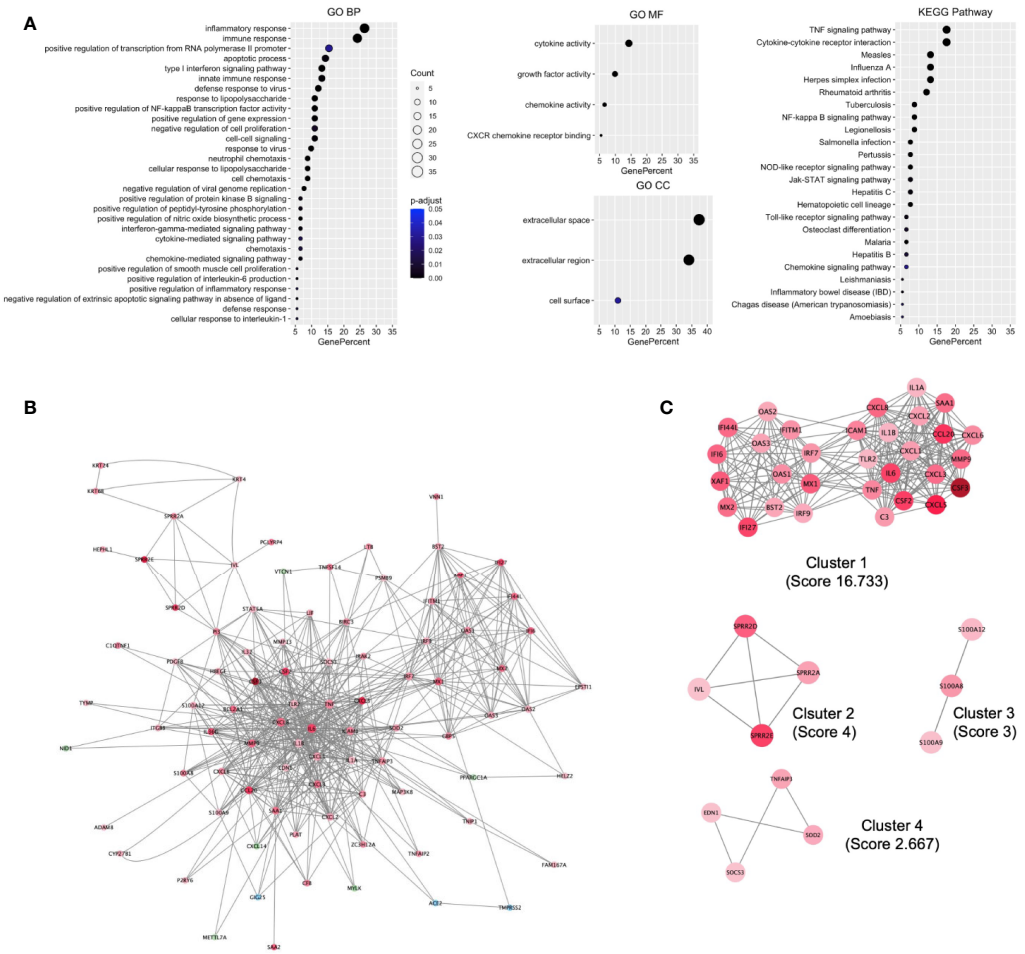


FIGURE 2 | Functional enrichment of host genes in cells infected with SARS-CoV-2 and their biological network. **(A)** GO and KEGG pathways functional enrichment of up-regulated host genes. **(B)** SARS-CoV-2 induced the Host gene regulatory network (*SiHgrn*). **(C)** MCODE clusters extracted from the *SiHgrn* network. MCODE score is given in the bracket. A red node and a green node represent an up-regulated and down-regulated gene, respectively, in DEHGs, while the blue node represents without expression value. GO, Gene Ontology; BP, Biological Processes; MF, Molecular Function; CC, Cell Component; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Furthermore, the log2FC expression level of DEHGs was integrated into the network, where the red node indicates an up-regulated gene, while the green node indicates a down-regulated gene in SARS-CoV-2–infected cell compared to control and the network is called as *SiHgrn* (**Figure 2B**). In the *SiHgrn network*, 79 nodes are up-regulated and 6 nodes are down-regulated while 3 nodes are not having gene expression level (shown in blue, identified through PPI interaction and not in the list of our DEHGs).

Though *ACE2* and *TMPRSS2* showed no differential expression in the SARS-CoV-2–infected cells compared to control. Interestingly, the *SiHgrn network* revealed that ACE2 interacts with three proteins: IL6 [log2FC=2.92], EDN1 [log2FC=1.06], and TMPRSS2; while TMPRSS2 interacts with two proteins: MX1 [log2FC=2.51] and ACE2. The structural topological of the *SiHgrn network*, including betweenness centrality, closeness centrality, clustering coefficient, and degree, was analyzed with Cytoscape plug-in "NetworkAnalyzer". The complete result of network topology is given in **Table S4**. The degree of node connectivity is used to determine the node size in the *SiHgrn network*.

Identification and Analysis of Sub-Network in SiHgrn

Detailed analysis of the *SiHgrn network* with Cytoscape plug-in MCODE identified four local sub-networks (**Figure 2C**; **Table 2**). Among them, only the highest MCODE score, 16.7, *Cluster 1* containing 31 genes, was selected for further study. Highly connected hub genes could influence the topology of a network and biological function. It was found that IL6 and TNF nodes have the highest degree of connectivity [degree=50] in the ***SiHgrn*** *network*. Other top-five hub nodes with degree of connectivity are CXCL8 [44], IL1B [40], MMP9 [35], TLR2 [34], and ICAM1 [32] (**Table S4**). Interestingly, these all top hub genes are highly interconnected, and belong to MCODE *Cluster 1* (**Figure 2C**; **Table 2**).

TABLE 2 | List of MCODE clusters and their associated proteins identified from *SiHgrn*.

Cluster	Score (Density*#Nodes)	# Nodes	# Edges	Node IDs
1	16.733	31	251	IL6, CXCL3, CXCL5, IRF9, SAA1, OAS3, CSF2, IFI6, OAS2, CSF3, IRF7, ICAM1, CXCL2, MX1, OAS1, MMP9, IL1A, IL1B, C3, TLR2, IFI27, CXCL6, CXCL1, CCL20, XAF1, TNF, IFI44L, MX2, BST2, IFITM1, CXCL8
2	4	4	6	SPRR2A, IVL, SPRR2D, SPRR2E
3	3	3	3	S100A8, S100A9, S100A12
4	2.667	4	4	SOD2, SOCS3, TNFAIP3, EDN1

Function enrichment analysis of *Cluster 1* genes with the BP of GO showed that they are primarily associated with immune response, inflammatory response, type 1 IFN signaling pathway, and defense response to viruses (**Figure 3A**). The MF of GO analysis revealed that *Cluster 1* genes are primarily associated with chemokine activity, cytokine activity, and CXCR chemokine receptor binding (**Figure 3A**). The CC of GO of *Cluster 1* genes are significantly related to extracellular space and extracellular region (**Figure 3A**). The KEGG pathway analysis showed that *Cluster 1* is significantly enriched in cytokine-cytokine receptor interaction, influenza A, rheumatoid arthritis, TNF signaling pathway, measles, Herpes simplex infection (**Figure 3A**). The BIOCARTA pathways showed that the *Cluster 1* genes are significantly enriched in cytokines and inflammatory response,

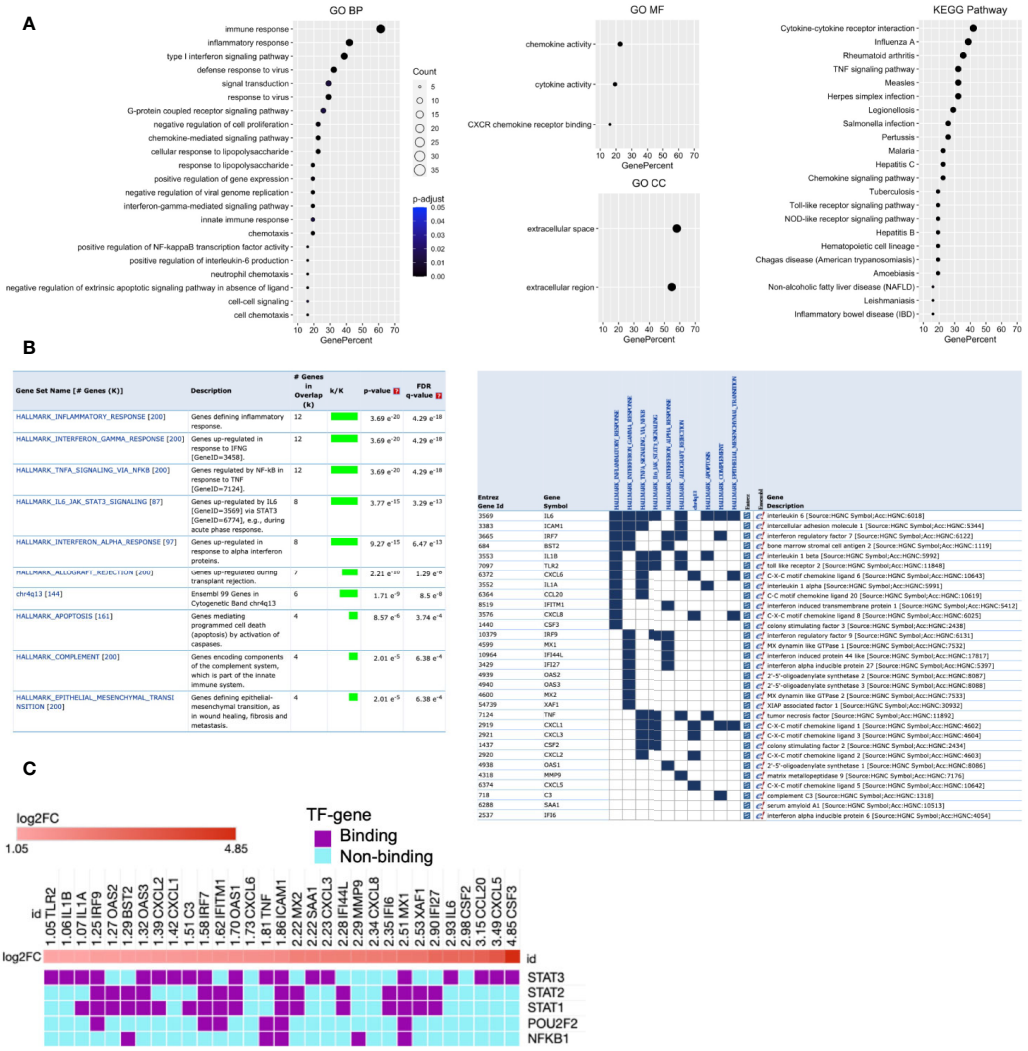


FIGURE 3 | Functional enrichment of *Cluster 1* and its potential upstream regulators. **(A)** GO and KEGG pathways functional enrichment. **(B)** MSigDB Hallmark and positional gene sets enrichment. **(C)** Potential upstream regulators of *Cluster 1* genes. Each column indicates the gene of *Cluster 1*, while each row indicates TF identified by iRegulone. Up-regulated DEHGs in the cluster are red with positive log2FC. TF binding with the mRNA is in purple, while non-binding in cyan.

cells and molecules involved in the local acute inflammatory response, and adhesion and diapedesis of granulocytes (**Figure S5A**). The DISEASE annotation showed that the *Cluster 1* genes are significantly enriched in ovarian cancer, respiratory syncytial virus bronchiolitis, asthma, and type 2 diabetes (**Figure S5B**). The complete results of GO, KEGG, BIOCARTA, and DISEASE annotation analyses of *Cluster 1* genes are available in **Table S5**.

Furthermore, enrichment analysis with MSigDB hallmark gene sets showed that *Cluster 1* genes are significantly enriched in several crucial pathways including, inflammatory response, genes up-regulated in response to IFN-γ and IFN-α, genes regulated by NFkB in response to TNF, genes up-regulated by IL6 *via* STAT3, genes up-regulated during transplant rejection and others (**Figure 3B**). While positional gene sets enrichment analysis showed that *Cluster 1* genes are enriched with the location of Cytogenetic Band chr4q13 (Ensembl 99). The detailed information with the statistically significant test associated overlap matrix is presented in the **Table S6**. In addition, the gene set of *Cluster 1* was investigated to identify the gene family according to their protein homology or biochemical activity with MSigDB. Out of 31 genes, 15 genes (*C3, CCL20, CSF2, CSF3, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, IL1A, IL1B, IL6, SAA1*, and *TNF*) belong to Cytokines and growth factors; 2 genes (*IRF7* and *IRF9*) belong to transcription factors; 4 genes (*BST2, ICAM1, IFITM1, TLR2*) belong to Cell differentiation markers (**Table S7**).

Analysis for Upstream Regulator of Cluster 1

Transcription factors rapidly modulate gene expression, especially in activating the host immune response against viral infection. Their identification will improve the understanding of the immunoregulatory mechanism, and a better approach to control the cytokine storm and viral defense in severe COVID-19. Therefore, we analyzed the *Cluster 1* to determine its potential upstream TFs regulators using the iRegulone tool. The output result showed that most of the *Cluster 1* genes, up-regulated (log2FC >1) in SARS-CoV-2 response, are under the control of TFs STAT1, STAT2, STAT3, POU2F2, and NFkB1 (**Figure 3C**).

Biological Pathways Enrichment of "*Host Response Signature Network*"

The genes of the "*host response signature network*" (consist of *Cluster 1* and its upstream regulators =36 query genes) were analyzed with NDEx, which returns a list of relevant pathways according to the highest similarity score. Among them, a list of top nine pathways with more than 5 overlapping genes with our query genes was selected (**Table 3**). It was observed that the "Cytokines and Inflammatory Response" pathway significantly matched with the "*host response signature network*" with the highest similarity score [similarity score=0.21; the number of overlap gene=8; p-value=2.70e-10] (**Table 3** and **Figure 4A**). On the other hand, "Non-genomic actions of 1,25 dihydroxyvitamin D3" pathway significantly matched with the "*host response signature network*" with the highest number of genes [similarity score=0.19; the number of overlap gene=11; p-value=1e-12] (**Table 3** and **Figure 4B**). **Figure 4B** showed that TNF induced NFkB1 signaling pathway is enriched with four proteins TNF, NFkB1, CXCL8, and IL6. While, IFN-α induced Jak-STAT signaling pathway is enriched with proteins STAT1, STAT2, ILI44L, OAS1, OAS2, and OAS3. It was also observed

TABLE 3 | Biological pathways enrichment of "*host response signature network*" using NDEx v2.4.5.

Pathway Name	Pathway Properties	Number of overlap gene	p-value <	Similarity score
WP530—Cytokines and Inflammatory Response - Homo sapiens http://identifiers.org/wikipathways/WP530_r96982 Source: wikipathways	Nodes:124; Egde:37	8 genes	2.70e-10	0.21
WP4341—Non-genomic actions of 1,25 dihydroxyvitamin D3 - Homo sapiens http://identifiers.org/wikipathways/WP4341_r107169 Source: WikiPathways	Nodes:166; Egde:68	11 genes	1e-12	0.19
WP4197—The human immune response to tuberculosis - Homo sapiens http://identifiers.org/wikipathways/WP4197_r105840 Source: wikipathways	Nodes:68; Egde:30	6 genes	1.57e-8	0.20
WP3617—Photodynamic therapy-induced NF-kB survival signaling - Homo sapiens http://identifiers.org/wikipathways/WP3617_r106541 Source WikiPathways	Nodes: 59; Egde:12	9 genes	1e-12	0.17
WP4495—IL-10 Anti-inflammatory Signaling Pathway - Homo sapiens http://identifiers.org/wikipathways/WP4495_r102692 Source WikiPathways	Nodes: 40; Egde:17	6 genes	4.02e-12	0.16
WP619—Type II interferon signaling (IFN-γ) - Homo sapiens http://identifiers.org/wikipathways/WP619_r106442 Source WikiPathways	Nodes: 127; Egde:83	6 genes	3.09e-7	0.13
WP3624—Lung fibrosis - Homo sapiens http://identifiers.org/wikipathways/WP3624_r106633 Source: WikiPathways	Nodes: 186; Egde:55	8 genes	9.35e-11	0.12
IL27-mediated signaling events Source: Pathway Interaction Database (PID) curated by NCI/Nature.	Node: 26; Edge: 101	6 genes	3.72e-10	0.11
IL23-mediated signaling events Source: Pathway Interaction Database (PID) curated by NCI/Nature.	Node: 37; Edge: 201	7 genes	8.21e-11	0.11

that the gene of TLR2 is over-expressed in our study, which involves inducing the expression of *CYP27B1* and *VDR* genes. In our study, *VDR* is not differentially expressed; but, *CYP27B1* is up-regulated [log2FC=1.21] in the SARS-CoV-2–induced cells (**Table S1**). Interestingly, the *SiHgrn network* shows interactions of CYP27B1 with IL6 and TLR2 (**Figure 2B**). Despite this, CYP27B1 is not associated with any of the MCODE clusters (**Figure 2C**). The genes of "*host response signature network*" are also showing enrichment with other pathways involve in immune response including "the human immune response to tuberculosis" [similarity score=0.20; the number of overlap gene=6; p-value=1.57e-8] (**Table 3** and **Figure 5A**); "Photodynamic therapy-induced NF-kB survival signaling" [similarity score=0.17; the number of overlap gene=9; p-value=1e-12] (**Table 3** and **Figure S6A**); "IL-10 Anti-inflammatory Signaling Pathway" [similarity score=0.16; number of overlap gene=6; p-value=4.02e-12] (**Table 3** and **Figure S6B**); "Type II interferon signaling (IFN-γ)" [similarity score=0.13; the number of overlap gene=6; p-value=3.09e-7] (**Table 3** and **Figure S6C**); "IL27-mediated signaling events" [similarity score=0.11; the number of overlap gene=6; p-value=3.72e-10] (**Table 3** and **Figure S7A**); and "IL23-mediated signaling events" [similarity score=0.11; the number of overlap gene=7; p-value=8.21e-11] (**Table 3** and **Figure S7B**).

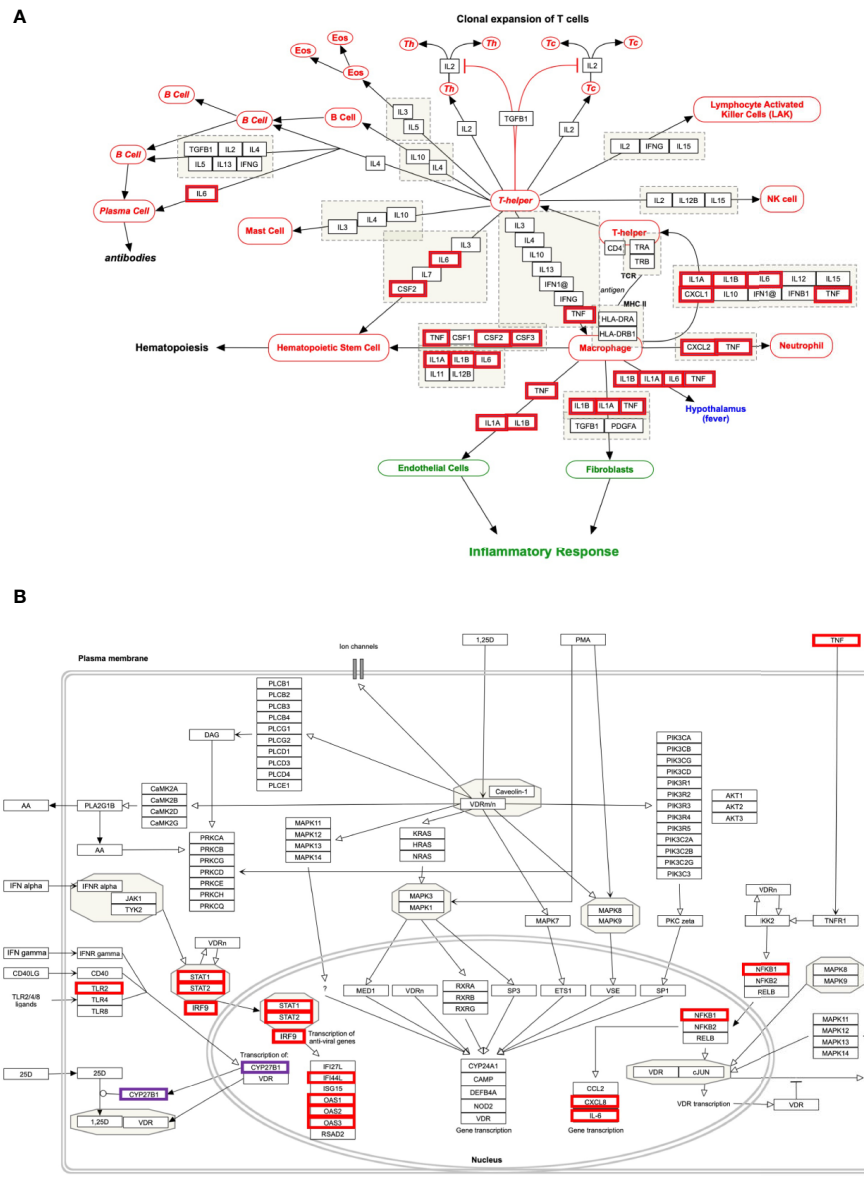


FIGURE 4 | Biological pathways enrichment of "*host response signature network*": **(A)** "Cytokines and Inflammatory Response" and **(B)** "Non-genomic actions of 1,25 dihydroxyvitamin D3". Gene from the "*host response signature network*" of the SARS-CoV-2 infected cell is in the red box. The IRF9 and CYP27B1 genes are up-regulated in DEHGs. CYP27B1 in the purple box is not associated with the "*host response signature network*".

Interestingly, genes of the "*host response signature network*" are also showing enrichment with the "lung fibrosis" pathway [similarity score=0.12; the number of overlap gene=8; p-value=9.35e-11], which could indicate the etiology of lung damage in COVID-19 patients (**Table 3** and **Figure 5B**).

Validation of Host Response Pathways Using Gene Expression Data

To validate the identified host response pathways, a list of differentially expressed host genes was taken from a recent study conducted in 24 patients who died from COVID-19 (37). The study used the lung autopsy specimens from the 25 samples from the high viral load and 21 samples from the low viral load patients. The study identified 338 up-regulated and 5,710 down-regulated host genes in the high viral load samples compared to low viral load samples (| log2FC| >0.3 and adjusted p-value <0.01), and we referred it to as $DEHGs^{COVID\text{-}19}$ (37). Different analytical techniques, including viral gene expression, were considered for the classification of COVID-19 patients into the high and low viral load. The SARS-CoV-2 gene *ORF1a* is over-expressed (log2FC=9.27, adjusted p-value= 2.68e-25) in the high viral load samples compared to the low viral load samples. With the NDEx tool (28 September 2020), the up-regulated genes of $DEHGs^{COVID\text{-}19}$ identifies the "response to interferon-alpha (GO:0035455)" as a

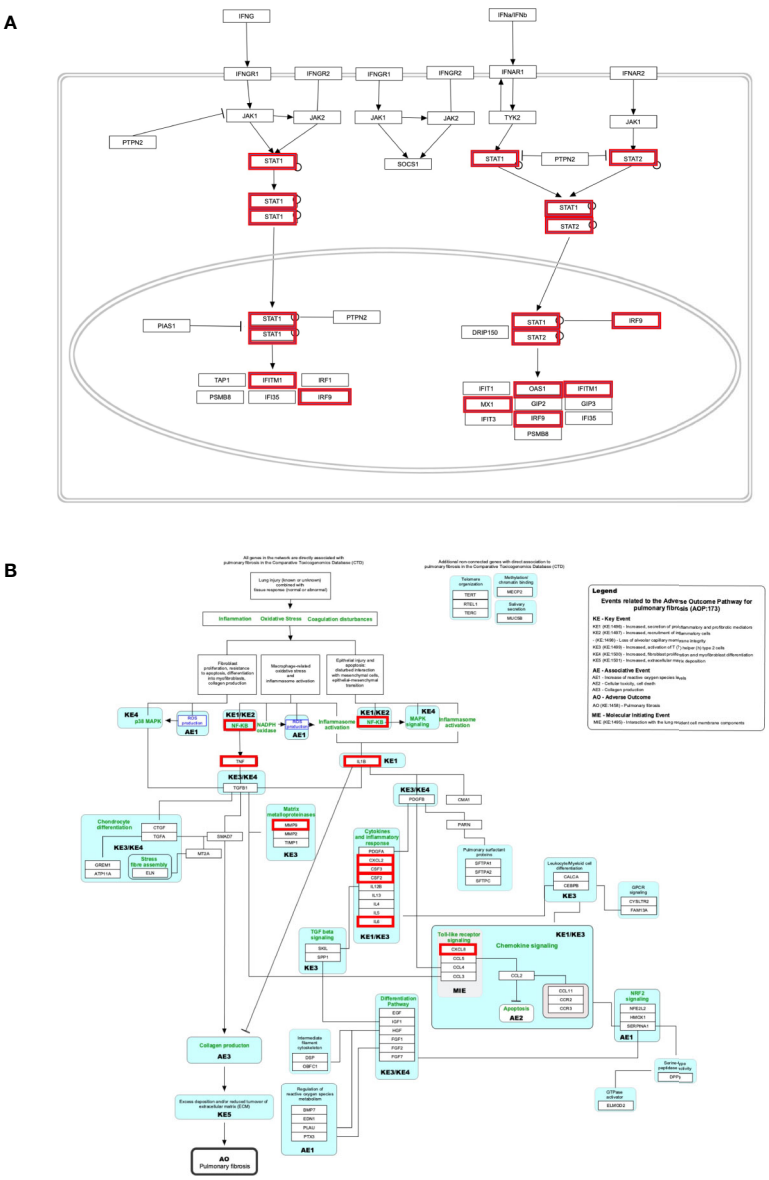


FIGURE 5 | Biological pathways enrichment of "host signature network": **(A)** "The human immune response to tuberculosis" and **(B)** "Lung fibrosis." Gene from the "*host response signature network*" of the SARS-CoV-2 infected cell is in the red box.

top scores relevant pathway with the similarity score=0.12; the number of overlap gene=8 (*ADAR*, *BST2*, *EIF2AK2*, *IFIT2*, *IFIT3*, *IFITM1*, *IFITM3*, *TPR*); and p-value=2.27e-6. After that, the DEHGs$^{COVID-19}$ was mapped to previously identified host response pathways with NDEx. The up-regulated and down-regulated genes with their associated pathways are given in **Table 4**, and the gene expression level is provided in **Table S8**.

Our analysis observed that only 4 down-regulated genes (*IL1B*, *IL12B*, *TNF*, and *IL6*) were associated with "Cytokines and Inflammatory Response" (**Table 4** and **Figure 6A**). However, 10 up-regulated genes (*STAT1*, *STAT2*, *IFI44L*, *ISG15*, *OAS1*, *OAS2*, *OAS3*, *RSAD2*, *PIK3R1*, *PIK3C2A*) and 12 down-regulated genes (*CAMK2B*, *VDR*, *TNF*, *CYP27B1*, *CYP24A1*, *CXCL8*, *IL6*, *CAMK2A*, *PRKCB*, *RXRA*, *PRKCA*, *PIK3C2B*, *MAPK13*) were associated with the "Non-genomic actions of 1,25 dihydroxyvitamin D3" pathway (**Table 4** and **Figure 6B**). The 11 up-regulated genes (*STAT1*, *STAT2, JAK2, PSMB8, TAP1, IFI35, IFIT1, IFITM1, IFIT3, OAS1, MX1*) were mapped to "the human immune response to tuberculosis" pathway (**Table 4** and **Figure S8A**). Furthermore, two up-regulated genes (*MT2A* and *EDN1*) and 15 down-regulated genes (*IL1B*, *CCR3*, *CXCL8*, *IL12B*, *TNF*, *CYSLTR2*, *FGF1*, *IL6*, *BMP7*, *MMP9*, *MUC5B*, *SFTPC*, *MECP2*, *TGFA*, *HMOX1*) were associated with "lung fibrosis" pathway (**Table 4** and **Figure S8B**). The DEHGs$^{COVID-19}$ associated with others pathways are also provided in the supplementary figures, including "Photodynamic therapy-induced NF-kB survival signaling" (**Table 4** and **Figure S9A**); "IL-10 Anti-inflammatory Signaling Pathway" pathway (**Table 4** and **Figure S9B**); "Type II interferon signaling (IFN-γ)" pathway (**Table 4** and **Figure S9C**); "IL27-mediated signaling events" (**Table 4** and **Figure S10A**); and "IL23-mediated signaling events" (**Table 4** and **Figure S10B**).

DISCUSSION

In the present work, *omics* data and computational methods were used to understand the underlying mechanisms of SARS-CoV-2 induced altered host gene regulatory networks, and the potential regulatory mechanism of Vitamin D in suppressing cytokine storm and reducing viral load. Using the gene expression data, this study identified 108 DEHGs, including 93 up-regulated and 15 down-regulated genes in SARS-CoV-2 infected NHBE cell compared to control. The up-regulated genes are significantly enriched in the inflammatory response, immune response that induces through TNF signaling pathways, and cytokine-cytokine receptor interactions (**Figure 2A**). In order to understand the regulatory network, the DEHGs were integrated with the human PPI to generate a *SiHgrn network* (**Figure 2B**), which contains a highly connected sub-network of *Cluster 1* (**Figure 2C**). The functional enrichment analysis of *Cluster 1* showed that its primary role in immune response and inflammatory response, which induces through the cytokine-cytokine receptor interactions pathway (**Figures 3A, B**). Besides, enriched gene percentages in *Cluster 1* was increased compared to that of the up-regulated genes of DEHGs, which indicates that *Cluster 1* plays a significant role in inducing an inflammatory response in COVID-19 (**Figures 2A**, **3A**; **Figures S4**, **S5**). Furthermore, about half of *Cluster 1* genes belong to the family of cytokines and growth factors, which trigger a severe inflammatory response (**Table S7**).

Additional analysis of *Cluster 1* with the iRegulone tool identified five potential upstream regulators: STAT1, STAT2, STAT3, POU2F2, and NFkB1 (**Figure 3C**). Furthermore, the up-regulation of *STAT1* and *STAT2* in the high viral load lung samples compared to the low viral load lung samples of DEHGs$^{COVID-19}$ supports our finding of *Cluster 1* upstream regulators (**Figures 3C**, **6B**).The *Cluster 1* and these five upstream regulators are collectively referred to as "*host response signature network*", which was used for the detailed understanding of the regulatory mechanism of cytokine storm, host immune response, and clinical manifestation in COVID-19. Functional enrichment analysis of the "*host response signature network*" with the NDEx tool revealed several alterations in crucial pathways associated with SARS-CoV-2 infection and pathogenesis (**Table 3**).

TABLE 4 | The differentially expressed host genes (DEHGs$^{COVID-19}$) shows the overlapping with host response pathways using NDEx v2.4.5.

Pathway Name	Up-regulated gene	Down-regulated gene
WP530—Cytokines and inflammatory response	No gene	*IL1B*, *IL12B*, *TNF*, *IL6*
WP4341—Non-genomic actions of 1,25 dihydroxyvitamin D3	*STAT1*, *STAT2*, *IFI44L*, *ISG15*, *OAS1*, *OAS2*, *OAS3*, *RSAD2*, *PIK3R1*, *PIK3C2A*	*CAMK2B*, *VDR*, *TNF*, *CYP27B1*, *CYP24A1*, *CXCL8*, *IL6*, *CAMK2A*, *PRKCB*, *RXRA*, *PRKCA*, *PIK3C2B*, *MAPK13*
WP4197—The human immune response to tuberculosis	*STAT1*, *STAT2, JAK2, PSMB8, TAP1, IFI35, IFIT1, IFITM1, IFIT3, OAS1, MX1*	No gene
WP3617—Photodynamic therapy-induced NF-kB survival signaling	No gene	*IL1B*, *TNF*, *MMP9*, *IL6*, *PTGS2*
WP4495—IL-10 anti-inflammatory signaling pathway	*STAT1, STAT2*	*TNF*, *IL6*, *HMOX1*
WP619—Type II interferon signaling (IFN-γ)	*IFIT2*, *STAT1*, *STAT2*, *JAK2*, *EIF2AK2*, *OAS1*, *HLA-B*, *GBP1*, *TAP1*	*IL1B*
WP3624—Lung fibrosis	*MT2A* and *EDN1*	*IL1B*, *CCR3*, *CXCL8*, *IL12B*, *TNF*, *CYSLTR2*, *FGF1*, *IL6*, *BMP7*, *MMP9*, *MUC5B*, *SFTPC*, *MECP2*, *TGFA*, *HMOX1*
IL27-mediated signaling events	*JAK2, STAT1, STAT2*	*IL1B*, *IL12B*, *TNF*, *IL6*
IL23-mediated signaling events	*JAK2, PIK3R1, STAT1*	*IL1B*, *IL12B*, *TNF*, *IL6*

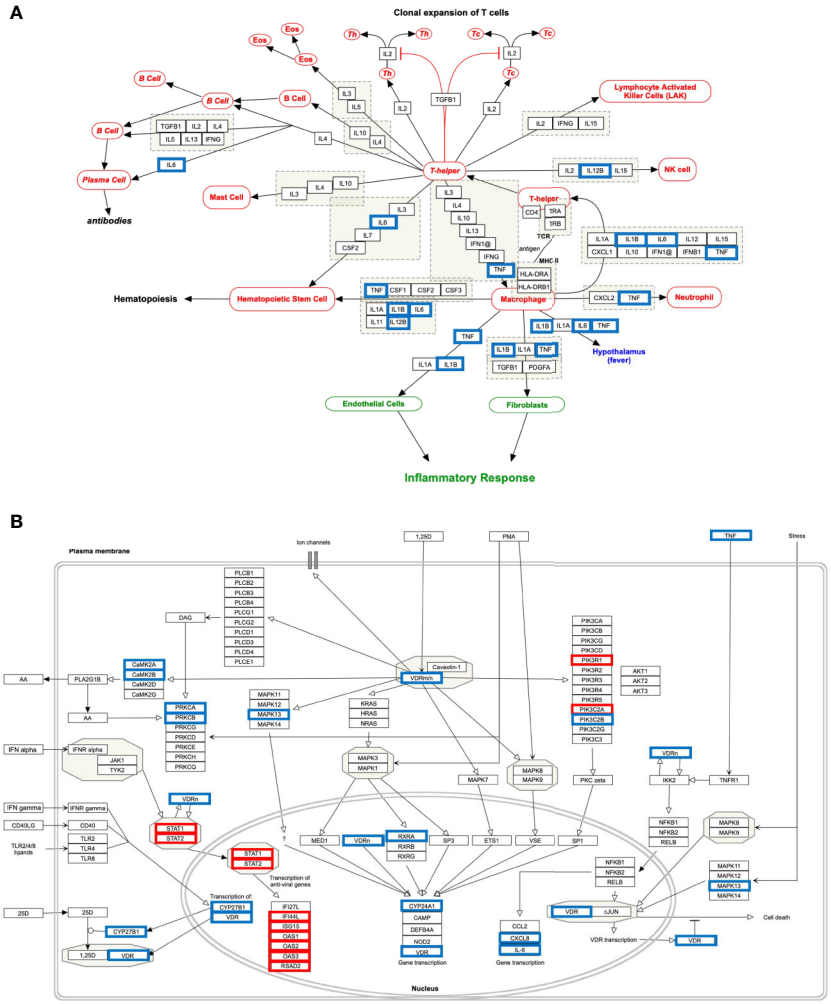


FIGURE 6 | Host response pathways using differentially expressed host genes ($DEHGs^{COVID-19}$) in the high viral load compared to the low viral load of lungs autopsy of COVID-19 patients. **(A)** "Cytokines and Inflammatory Response" and **(B)** "Non-genomic actions of 1,25 dihydroxyvitamin D3." A red box indicates an up-regulated gene, while a blue box represents a down-regulated gene in the high viral load compared to the low viral load of COVID-19 lung samples, respectively.

The "Cytokines and Inflammatory Response" Pathway

Our analysis showed that the "*host response signature network*" is significantly associated with "cytokines and inflammatory response" pathway (**Table 3** and **Figure 4A**). The gene of cytokine IL6, TNF, IL1A, IL1B, CSF2, and CSF3 were over-expressed in SARS-CoV-2 infected cells and could be responsible for proliferating hematopoietic stem cells to fight against the virus (**Figure 4A**). Other cytokine genes were also over-expressed in SARS-CoV-2 infected cells, including TNF, IL1A, and IL1B, which activate the fibroblasts and endothelial cells for inflammatory response. **Figure 4A** showed that CXCL2 and TNF induce neutrophil, while IL1A, IL1B, IL6, CXCL1, and TNF induce the T-helper cell. Enhance concentration of IL1A, IL1B, IL6, and TNF cause increase body temperature *via* the hypothalamus in COVID-19 (**Figure 4A**).

The binding of CSF2 to its receptor on the myeloid cell induces its differentiation and proliferation into monocytes and macrophages (38). However, another study showed that CSF2 required for normal pulmonary physiology, including surfactant homeostasis in the mice (39, 40). Disruption of CSF2 resulted in pulmonary alveolar proteinosis due to compromise in the development of alveolar macrophage, making lungs susceptible to infection (39, 40). During infection, CSF3 works along with IL3, IL6, and CSF2 to stimulate neutrophil granulopoiesis in the bone marrow to restore neutrophil homeostasis. The expression of CSF3 is higher in poorly controlled asthma, and inhibiting the signaling by neutralizing its receptor, CSF3R, decrease the production of mucus and hyperreactivity in the airway (41).

An effective immunological response against SARS-CoV-2 infection requires an appropriate cytokine level and an adequate number of activated T cells for reducing the viral load. A prior

study found that the number of T cells was drastically decreased, while the level of TNF, IL6, and IL10 were significantly increased in severe COVID-19 patients compared to healthy control (42). Besides, the authors observed that the concentration of TNF, IL6, and IL10 was negatively correlated with the number of T cells, including total T cell, CD4+ cell, and CD8+ cells. Hence, they suggested that these cytokines play a critical role in the acute inflammatory response and lower the survival of T cells. Furthermore, the survived T cells became functionally exhausted in COVID-19, as indicated by the expression of PD-1 and Tim-3 on the T cell surface (42). The cells of healthy mice, including epithelium, endothelium, and fibroblasts, release different cytokines and involve in the immune response against pathogens (43). Our study found that several cytokine genes are over-expressed in NHBE cells infected with SARS-CoV-2. Thus, suggesting that this NHBE cell is also involved in the immunoregulatory role and is strongly implicated in the immune response against SARS-CoV-2 infection (**Figure 2A** and **Table S3**), and therefore support previous findings (42, 43).

The "Non-genomic Actions of 1,25 Dihydroxyvitamin D3 (1,25 D)" Pathway

The 1,25 D is a biologically active form of vitamin D. Interestingly; prior studies observed that lower Vitamin D is associated with increased risk of autoimmune disease, inflammation, bacterial and viral infection including SARS-CoV-2 (12–15). Furthermore, an adequate amount of vitamin D could substantially reduce the risk of cytokine storm, other complications, and death in COVID-19 patients; however, its underlying mechanism is not clearly known (14, 17, 44).

Our analysis showed that the "*host response signature network*" is significantly associated with non-genomic actions of 1,25 D pathway with p-value 1e-12 (**Table 3** and **Figure 4B**). Viral infection recognized by innate immune systems triggers two signaling cascades: (a) stimulating the production of pro-inflammatory cytokine (e.g., IL1, IL6, TNF) through NFkB1-mediated pathway; (b) stimulating the production of type I and type III IFNs through interferon regulatory factor (IRF3 and IRF7) mediated pathways (45). Based upon data analysis and literature mining, our study proposed the following two pathways through which Vitamin D could reduce the cytokine storm and enhance the antiviral response.

TNF-Induced NFkB1 Signaling Pathway

NFkB is a family of transcription factors composed of five proteins: (i) NFkB1 (p50 and its precursor p105), (ii) NFkB2 (p52 and its precursor p100), (iii) RelA (p65), (iv) RelB, and (v) c-Rel. These proteins interact with each other to form different types of homo- and hetero-dimers and regulate important biological processes, including inflammation, immunity, and apoptosis. In an unstimulated cell, NFkB1 dimer is sequestered in the cytoplasm through physical association with NFkB1 inhibitory protein (IkB). The TNF, IL1, LPS, or various other external stimuli induce the canonical signal transduction pathway of NFkB1 (46). Activation of this pathway involves site-specific phosphorylation of IKKβ and formation of an active kinase complex IKK, which subsequently phosphorylates and degrades the downstream target IkB. This leads to the release and translocation of NFkB1 to the nucleus and activates the target gene transcripts involved in immune response, including *IL6* and *CXCL8* (**Figure 4B**). The VDR binds to IKKβ and prevents its phosphorylation and formation of IKK, and thus inhibit the NFkB1 activation (47). The 1,25 D binds with VDR and enhances the interaction between VDR-IKKβ; thus, a study concluded that 1,25 D blocks the TNF induced NFkB1 activation (47). Our analysis found that NFkB1 regulates the expression of *IL6* and *CXCL8* (*IL8*, with the lowest adjusted p-value), which are over-expressed in SARS-CoV-2 infected cells (**Figure 4B**). High levels of IL6 and CXCL8 are associated with severe cases of COVID-19 pathogenesis. Collectively, the data suggest that the active form of Vitamin D could prevent the translocation of NFkB1 to the nucleus and, consequently, inhibit the cytokine storm in COVID-19.

IFN-α–Induced Jak-STAT Signaling Pathway

The IFN has an essential role in the innate immune response against viruses. Viral infection in human activates type 1 IFNs (IFN-α, IFN-β, IFN-ϵ, IFN-κ, IFN-ω), type II IFN (IFN-γ), and type III IFN (IFN-λ) (45). IFN-α binds to the cell receptor and activates the JAK1 and Tyk2, which subsequently phosphorylate the downstream targets STAT1 and STAT2 (48). The phosphorylation causes the dimerization of STAT1 with STAT2, which associates with IRF9 to forms a major transcription factor ISGF3 complex. The activation and translocation of ISGF3 from the cytoplasm to the nucleus induces interferon-stimulated genes (ISGs) that provide the antiviral activity of a cell (49).

Prior studies showed that Vitamin D deficiency had a poor response of IFN-α based therapy on chronic hepatitis C virus (HCV) (50, 51). In Huh-7.5 cells, vitamin D inhibited the production of infectious HCV in a dose-dependent manner (50). A subsequent study revealed that a combination of 1,25 D with IFN-α increases the inhibitory effect on HCV replication (52). Furthermore, the study observed a constitutive inhibitory interaction between VDR with STAT1 in Huh-7.5 cells infected with HCV; however, this interaction decreased after cells were treated with IFN-α alone and completed abolished when cells were treated with both 1,25 D and IFN-α. Consequently, STAT1 dissociated from VDR and formed the ISGF3, which moved to the nucleus for inducing its target genes (52). Furthermore, the study demonstrated that the silencing of VDR expression in cells and then treated with IFN-α resulted in a significantly more potent induction of mRNA expression of ISGs (*IFI27L* and *IFI44L*) than the control cells. Thus the study concluded that VDR is an inhibitor of IFN-α induced signaling through the Jak–STAT pathway (52).

Our study indicates that the high expression of *IRF9* in the SARS-CoV-2 infected cells could be involved in the formation of transcription factor ISGF3, which subsequently increases the expression of ISGs. The genes *ILI44L*, *OAS1*, *OAS2*, and *OAS3*, whose expression are under the control of ISGF3, were over-expressed in SARS-CoV-2 infected cells compared to control (**Figure 4B**). Furthermore, this study showed over-expression of *TLR2* and *CYP27B1* in SARS-CoV-2 infected cells compared to

control. A prior study demonstrated that 1,25 D supplement enhances the TLR-mediated macrophage ability to fight against Mycobacterium tuberculosis (53). The *CYP27B1* gene encodes an enzyme 1α-hydroxylase that converts vitamin D to its active form, 1,25 D. A previous study showed that insufficient Vitamin D and polymorphism in promotor of *CYP27B1*-1260 have been associated with chronic HCV infection as well as a poor response to IFN-α based therapy (50, 51, 54). Furthermore, *IRF7* is up-regulated in both DEHGs and $DEHGs^{COVID\text{-}19}$ SARS-CoV-2 infected samples. A recent study identified the loss-of-function mutation at the loci of TLR3 and IRF7 in severe patients with influenza and COVID-19, resulting in preventing type 1 IFN production, thus emphasizing the significance of type 1 IFN in controlling virus production (55).

A study used transcription and serum profiling of COVID-19 patients and revealed that SARS-CoV-2 infection induces a high level of chemokines and pro-inflammatory cytokines such as IL6, while very low level of IFN-I or IFN-III resulting limited antiviral ISGs response (27). Accumulating finds suggested that an imbalance between a high level of pro-inflammatory cytokines production and a low IFN response could cause severe COVID-19 pathogenesis (27, 45). Based on our findings, we expect that a high level of vitamin D could have two consequences: (i) down-regulation of cytokine storm, and (ii) up-regulation of ISGs for a robust antiviral response. Analysis of differentially expressed host genes from high viral load compared to low virus load, $DEHGs^{COVID\text{-}19}$, demonstrated the crucial role of "Cytokines and Inflammatory Response" and "Non-genomic actions of 1,25 dihydroxyvitamin D3" pathways in COVID-19. It also revealed that *VDR* and pro-inflammatory genes are down-regulated, while *STAT1*, *STAT2*, and interferon response genes are up-regulated in the high viral load lung samples compared to the low viral lung samples of COVID-19 (**Table 4** and **Figures 6A, B**). Desai *et al*. reported that the COVID-19 patients classified as high viral load induced more interferon response pathway genes for antiviral defense programs, resulting in a significantly short duration of illness than patients with low viral load (37). A recent study showed that human epithelial cells infected with SARS-CoV-2 induces a high interferon response *via* Jak-STAT signaling pathway, which controls the viral replications and *de novo* virus production (56) Another study observed that type I IFNs suppress the SARS-CoV-2 activities in cultured cells, showing the potency of type I IFNs to treat COVID-19 (57).

Therefore, based upon accumulating evidence and gene expression data of SARS-CoV-2 infected samples, our finding revealed that an adequate level of Vitamin D binds with VDR that could results. (i) minimizes the expression of pro-inflammatory cytokines by blocking the TNF induced NFkB1 signaling pathway, and (ii) induces the expression of ISGs for antiviral defense through activating the IFN-α induced Jak-STAT signaling pathway for reducing the virus load.

The "Human Immune Response to Tuberculosis" Pathway

Only 10-20% of people infected with *Mycobacterium tuberculosis* have a lifetime risk of showing signs called "active" tuberculosis (TB) (58, 59). Pathogens responsible for both COVID-19 and TB show high similarity regarding its transmission mode and symptoms, including infecting the lungs, having fever, cough, and shortness of breath. A study observed that countries implemented mass vaccination programs for the Bacille Calmette-Guérin vaccine (BCG) against TB showed a significant reduction in COVID-19 mortality than those who never applied it (60). Thus, the authors suggested that BCG vaccination could protect people from COVID-19; however, the experimental evidence and underlying molecular mechanism are still lacking (60). IFN-γ is a crucial cytokine produced by $CD4^+$ T cells and activates macrophage to providing resistance to TB infection (61). Low plasma concentration of IFN-γ was associated with active TB infection (62). Moreover, studies identified that a single nucleotide polymorphism (+874T/A) at the first intron of *IFN-γ* increases the chance to develop active TB (62, 63). A study analyzed the blood transcriptome data and identified over-expression of IFN I and IFN II inducible genes, including *IFITM1*, *IRF1*, *IRF9*, *OAS1*, *MX1*, *STAT1*, and *STAT2* (**Figure 5B**), also identified up-regulated inflammatory genes in the neutrophils of active TB compared to healthy control (26). In contrast, the expression of T and B-cell specific genes were down-regulated in active tuberculosis (26, 58). In the current study, six genes (*IFITM1*, *IRF9*, *MX1*, *OAS1*, *STAT1*, and *STAT2*) of the "*host response signature network*" are significantly overlapping with the "human immune response to tuberculosis" pathway with p-value 1.57e-8 (**Table 3** and **Figure 5B**). Furthermore, 11 genes (*STAT1*, *STAT2, JAK2, PSMB8, TAP1, IFI35, IFIT1, IFITM1, IFIT3, OAS1*, and *MX1*) were up-regulated in the "human immune response to tuberculosis" pathways in the high viral load compared to the low viral load COVID-19 samples (**Table 4** and **Figure S8A**). Overlapping of activated genes and pathways between COVID-19 and active TB indicates both diseases are mechanistically related, which might be the reason that BCG vaccination could protect people from severe COVID-19 (60).

The "Lung Fibrosis" Pathway

The complication of COVID-19 includes the development of lung fibrosis, excessive deposition of collagen and extracellular matrix (EM), destroying normal lung architecture, resulting in difficulty breathing and lung failure (64). The ARDS is considered as one of the significant factors for the lung fibrosis (64). A prior study demonstrated that SARS-CoV-1 infection promotes lung fibrosis by enhancing the TGF-β signaling and reducing the ACE2 expression (65, 66). ACE2 receptor, responsible for SARS-CoV-1 and SARS-CoV-2 infection, is primarily involved in the degradation and clearance of ANG-II. Reducing the clearance of ANG-II induces extracellular matrix deposition and lung fibrosis (66).

Our analysis showed that eight genes (*CSF2*, *CSF3*, *CXCL2*, *CXCL8*, *IL1B*, *IL6*, *MMP9*, and *TNF*) are significantly overlapping with the lung fibrosis pathway with p-value 9.35e-11 (**Table 3** and **Figure 5B**). Furthermore, 15 genes (*IL1B*, *CCR3*, *CXCL8*, *IL12B*, *TNF*, *CYSLTR2*, *FGF1*, *IL6*, *BMP7*, *MMP9*, *MUC5B*, *SFTPC*, *MECP2*, *TGFA*, *HMOX1*) were up-regulated

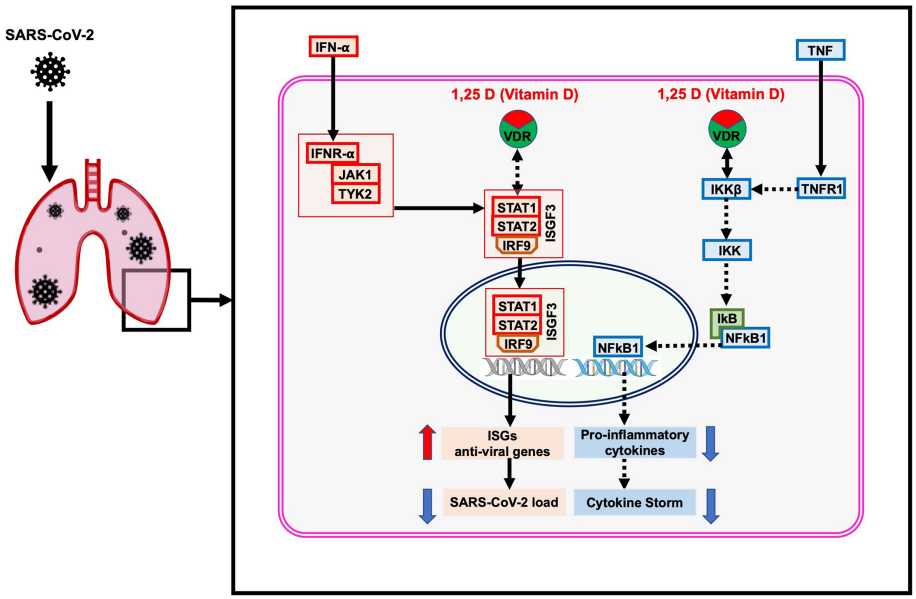


FIGURE 7 | The proposed model of the non-genomic actions of 1,25 dihydroxyvitamin D3 (1,25 D) in the lungs infected with SARS-CoV-2. The 1,25 D is a biologically active form of vitamin D that blocks the TNF induced NFkB1 activation. The 1,25 D binds with VDR and enhances the interaction between VDR-IKKβ, which prevents phosphorylation of IKKβ and formation of active IKK. Therefore, the degradation of IkB is blocked, resulting in preventing the translocation of NFkB1 to the nucleus. Consequently, the transcription and expression of NFkB1 target genes responsible for the cytokine storm are suppressed. In addition, 1,25 D enhances the IFN-α induced Jak-STAT signaling pathway. IFN-α activates the JAK1 and TYK2 signaling, which subsequently phosphorylate and activate the downstream targets STAT1 and STAT2. The 1,25 D binds with VDR and induces the dissociation between VDR-STAT1; thus, STAT1 is available for phosphorylation and formation of active TF complex ISGF3. The translocation of ISGF3 to the nucleus activates the transcription of interferon-stimulated genes (ISGs), which provide antiviral activity and reduce the SARS-CoV-2 load in cells. The figure was adapted from the WikiPathways (WP4341) www.wikipathways.org/instance/WP4341.

in the "lung fibrosis" pathways in the low viral load compared to the high viral load COVID-19 samples (**Table 4** and **Figure S8A**). Thus, our study indicates the relationship between SARS-CoV-2 infection and lung fibrosis. This study also supports the previous finding that high levels of NFkB, TNF, SFTPC, MUC5B, and other proteins could promote lung fibrosis (67). Diffuse alveolar damage and lung fibrosis are frequently observed in SARS-CoV-2 patients; however, the underlying mechanism is not clearly understood (37).

CONCLUSION

Our study uses bioinformatics and systems biology approaches and identified the SARS-CoV-2 induced altered host gene regulatory sub-network, *Cluster1*, responsible for cytokine storm.

Cluster 1 contains highly interconnected 31 genes under the regulation of STAT1, STAT2, STAT3, POU2F2, and NFkB1, making a "*host response signature network*". The association of "*host response signature network*" with "cytokines and inflammatory response", "non-genomic action of vitamin D", "the human immune response to tuberculosis", and "lung fibrosis" indicates that it plays an essential role in COVID-19 pathogenesis.

Our study revealed that Vitamin D could bind with its receptor and work through two pathways to suppress cytokine storm and reduce viral load (**Figure 7**). Our study has few limitations, including a small sample size of SARS-CoV-2 infected host cells and a lack of experimental validation supporting the identified mechanisms. Furthermore, this study did not include the influence of genetic polymorphism relevant to identified pathways, especially pro-inflammatory and type 1 IFN related signaling pathways on the severity of COVID-19. Therefore, this study proposed an urgent need to check the suitability of Vitamin D in combination with IFN-α to suppress the cytokine storm and reduce viral load in the SARS-CoV-2 infected experimental model. Our current study provides in-depth insight into a better understanding of the regulatory mechanism of cytokine storm and vitamin D; it might be helpful to develop a better approach for therapeutic intervention using vitamin D for severe COVID-19 patients.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE147507, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE150316.

AUTHOR CONTRIBUTIONS

FA conceptualized and designed the whole study, performed all the analyses, interpreted the results, wrote, and revised the manuscript.

ACKNOWLEDGMENTS

The author is grateful to Nazreen Fatma for critically reading the manuscript, improving language and presentation. The author would like to acknowledge the technical support provided by the Department of Biochemistry, and the University of Jeddah Center for Scientific and Medical Research (UJCSMR), University of Jeddah.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.590459/full#supplementary-material

REFERENCES

1. Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, Xu T, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. *Chin Med J (Engl)* (2020) 133:1015–24. doi: 10.1097/CM9.0000000000000722
2. Lu G, Wang Q, Gao GF. Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. *Trends Microbiol* (2015) 23(8):468–78. doi: 10.1016/j.tim.2015.06.003
3. Adams MJ, Lefkowitz EJ, King AM, Bamford DH, Breitbart M, Davison AJ, et al. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2015). *Arch Virol* (2015) 160 (7):1837–50. doi: 10.1007/s00705-015-2425-z
4. Choudhry H, Bakhrebah MA, Abdulaal WH, Zamzami MA, Baothman OA, Hassan MA, et al. Middle East respiratory syndrome: pathogenesis and therapeutic developments. *Future Virol* (2019) 14(4):237–46. doi: 10.2217/fvl-2018-0201
5. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* (2020) 181(2):271–80.e8. doi: 10.1016/j.cell.2020.02.052
6. Moore BJB, June CH. Cytokine release syndrome in severe COVID-19. *Science* (2020) 368:eabb8925. doi: 10.1126/science.abb8925
7. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* (2020) 8:475–81. doi: 10.1016/S2213-2600(20)30079-5
8. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* (2020) 395 (10223):497–506. doi: 10.1016/S0140-6736(20)30183-5
9. McKechnie JL, Blish CA. The Innate Immune System: Fighting on the Front Lines or Fanning the Flames of COVID-19? *Cell Host Microbe* (2020) 27 (6):863–9. doi: 10.1016/j.chom.2020.05.009
10. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. *Int J Antimicrob Agents* (2020) 55:105954. doi: 10.1016/j.ijantimicag.2020.105954
11. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* (2020) 8(4):420–2. doi: 10.1016/S2213-2600(20)30076-X
12. Hashemi R, Morshedi M, Asghari Jafarabadi M, Altafi D, Saeed Hosseini-Asl S, Rafie-Arefhosseini S. Anti-inflammatory effects of dietary vitamin D. *Neurol Genet* (2018) 4(6):e278. doi: 10.1212/NXG.0000000000000278
13. Hashemi R, Hosseini-Asl SS, Arefhosseini SR, Morshedi M. The impact of vitamin D3 intake on inflammatory markers in multiple sclerosis patients and their first-degree relatives. *PLoS One* (2020) 15(4):e0231145. doi: 10.1371/journal.pone.0231145
14. Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, et al. Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. *Nutrients* (2020) 12 (4):988. doi: 10.3390/nu12040988
15. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ* (2017) 356:i6583. doi: 10.1136/bmj.i6583
16. Hii CS, Ferrante A. The Non-Genomic Actions of Vitamin D. *Nutrients* (2016) 8(3):135. doi: 10.3390/nu8030135
17. Daneshkhah A, Agrawal V, Eshein A, Subramanian H, Roy HK, Backman V. The Possible Role of Vitamin D in Suppressing Cytokine Storm and Associated Mortality in COVID-19 Patients. *medRxiv* (2020). doi: 10.1101/2020.04.08.20058578
18. Ahmed F, Ansari JA, Ansari ZE, Alam Q, Gan SH, Kamal MA, et al. A molecular bridge: connecting type 2 diabetes and Alzheimer's disease. *CNS Neurol Disord Drug Targets* (2014) 13(2):312–21. doi: 10.2174/18715273113126660133
19. Ahmed F. Integrated Network Analysis Reveals FOXM1 and MYBL2 as Key Regulators of Cell Proliferation in Non-small Cell Lung Cancer. *Front Oncol* (2019) 9:1011. doi: 10.3389/fonc.2019.01011
20. Durmuş S, Çakır T, Özgür A. Guthke R. A review on computational systems biology of pathogen-host interactions. *Front Microbiol* (2015) 6:235. doi: 10.3389/fmicb.2015.00235
21. Ahmed F, Senthil-Kumar M, Lee S, Dai X, Mysore KS, Zhao PX. Comprehensive analysis of small RNA-seq data reveals that combination of miRNA with its isomiRs increase the accuracy of target prediction in Arabidopsis thaliana. *RNA Biol* (2014) 11(11):1414–29. doi: 10.1080/15476286.2014.996474
22. Ahmed F, Senthil-Kumar M, Dai X, Ramu VS, Lee S, Mysore KS, et al. pssRNAit-a web server for designing effective and specific plant siRNAs with genome-wide off-target assessment. *Plant Physiol* (2020) 184:65–81. doi: 10.1104/pp.20.00293
23. Alzahrani FA, Ahmed F, Sharma M, Rehan M, Mahfuz M, Baeshen MN, et al. Investigating the pathogenic SNPs in BLM helicase and their biological consequences by computational approach. *Sci Rep* (2020) 10(1):12377. doi: 10.1038/s41598-020-69033-8
24. Ramilo O, Mejías A. Shifting the paradigm: host gene signatures for diagnosis of infectious diseases. *Cell Host Microbe* (2009) 6(3):199–200. doi: 10.1016/j.chom.2009.08.007
25. Zaas AK, Chen M, Varkey J, Veldman T, Hero AO, Lucas J, et al. Gene expression signatures diagnose influenza and other symptomatic respiratory viral infections in humans. *Cell Host Microbe* (2009) 6(3):207–17. doi: 10.1016/j.chom.2009.07.006
26. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* (2010) 466(7309):973–7. doi: 10.1038/nature09247
27. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell* (2020) 181(5):1036–45.e9. doi: 10.1016/j.cell.2020.04.026
28. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* (2014) 15(12):550. doi: 10.1186/s13059-014-0550-8
29. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res* (2015) 43(Database issue):D447–52. doi: 10.1093/nar/gku1003
30. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* (2003) 13(11):2498–504. doi: 10.1101/gr.1239303
31. Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinf* (2003) 4:2. doi: 10.1186/1471-2105-4-2

32. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. *Bioinformatics* (2011) 27(12):1739–40. doi: 10.1093/bioinformatics/btr260
33. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* (2015) 1(6):417–25. doi: 10.1016/j.cels.2015.12.004
34. Janky R, Verfaillie A, Imrichova H, Van de Sande B, Standaert L, Christiaens V, et al. iRegulon: from a gene list to a gene regulatory network using large motif and track collections. *PLoS Comput Biol* (2014) 10(7):e1003731. doi: 10.1371/journal.pcbi.1003731
35. Pratt D, Chen J, Welker D, Rivas R, Pillich R, Rynkov V, et al. NDEx, the Network Data Exchange. *Cell Syst* (2015) 1(4):302–5. doi: 10.1016/j.cels.2015.10.001
36. Pratt D, Chen J, Pillich R, Rynkov V, Gary A, Demchak B, et al. NDEx 2.0: A Clearinghouse for Research on Cancer Pathways. *Cancer Res* (2017) 77(21):e58–61. doi: 10.1158/0008-5472.CAN-17-0606
37. Desai N, Neyaz A, Szabolcs A, Shih AR, Chen JH, Thapar V, et al. Temporal and Spatial Heterogeneity of Host Response to SARS-CoV-2 Pulmonary Infection. *medRxiv* (2020). doi: 10.1101/2020.07.30.20165241
38. Hamilton JA. GM-CSF-Dependent Inflammatory Pathways. *Front Immunol* (2019) 10:2055. doi: 10.3389/fimmu.2019.02055
39. Kitamura T, Tanaka N, Watanabe J, Uchida, Kanegasaki S, Yamada Y, et al. Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. *J Exp Med* (1999) 190(6):875–80. doi: 10.1084/jem.190.6.875
40. Stanley E, Lieschke GJ, Grail D, Metcalf D, Hodgson G, Gall JA, et al. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci USA* (1994) 91(12):5592–6. doi: 10.1073/pnas.91.12.5592
41. Wang H, FitzPatrick M, Wilson NJ, Anthony D, Reading PC, Satzke C, et al. CSF3R/CD114 mediates infection-dependent transition to severe asthma. *J Allergy Clin Immunol* (2019) 143(2):785–8.e6. doi: 10.1016/j.jaci.2018.10.001
42. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). *Front Immunol* (2020) 11:827. doi: 10.3389/fimmu.2020.00827
43. Krausgruber T, Fortelny N, Fife-Gernedl V, Senekowitsch M, Schuster LC, Lercher A, et al. Structural cells are key regulators of organ-specific immune responses. *Nature* (2020) 583(7815):296–302. doi: 10.1038/s41586-020-2424-4
44. Maghbooli Z, Sahraian MA, Ebrahimi M, Pazoki M, Kafan S, Tabriz HM, et al. Vitamin D sufficiency, a serum 25-hydroxyvitamin D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection. *PLoS One* (2020) 15(9):e0239799. doi: 10.1371/journal.pone.0239799
45. Park A, Iwasaki A, Type I. and Type III Interferons - Induction, Signaling, Evasion, and Application to Combat COVID-19. *Cell Host Microbe* (2020) 27(6):870–8. doi: 10.1016/j.chom.2020.05.008
46. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther* (2017) 2:e17023. doi: 10.1038/sigtrans.2017.23
47. Chen Y, Zhang J, Ge X, Du J, Deb DK, Li YC. Vitamin D receptor inhibits nuclear factor κB activation by interacting with IκB kinase β protein. *J Biol Chem* (2013) 288(27):19450–8. doi: 10.1074/jbc.M113.467670
48. Schindler C, Levy DE, Decker T. JAK-STAT signaling: from interferons to cytokines. *J Biol Chem* (2007) 282(28):20059–63. doi: 10.1074/jbc.R700016200
49. Schindler C, Shuai K, Prezioso VR, Darnell JE. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science* (1992) 257(5071):809–13. doi: 10.1126/science.1496401
50. Gal-Tanamy M, Bachmetov L, Ravid A, Koren R, Erman A, Tur-Kaspa R, et al. Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. *Hepatology* (2011) 54(5):1570–9. doi: 10.1002/hep.24575
51. Lange CM, Bojunga J, Ramos-Lopez E, von Wagner M, Hassler A, Vermehren J, et al. Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. *J Hepatol* (2011) 54(5):887–93. doi: 10.1016/j.jhep.2010.08.036
52. Lange CM, Gouttenoire J, Duong FH, Morikawa K, Heim MH, Moradpour D. Vitamin D receptor and Jak-STAT signaling crosstalk results in calcitriol-mediated increase of hepatocellular response to IFN-α. *J Immunol* (2014) 192(12):6037–44. doi: 10.4049/jimmunol.1302296
53. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* (2006) 311(5768):1770–3. doi: 10.1126/science.1123933
54. Lange CM, Bibert S, Kutalik Z, Burgisser P, Cerny A, Dufour JF, et al. A genetic validation study reveals a role of vitamin D metabolism in the response to interferon-alfa-based therapy of chronic hepatitis C. *PLoS One* (2012) 7(7):e40159. doi: 10.1371/journal.pone.0040159
55. Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science* (2020) 371:eabd4570. doi: 10.1126/science.abd4570
56. Stanifer ML, Kee C, Cortese M, Zumaran CM, Triana S, Mukenhirn M, et al. Critical Role of Type III Interferon in Controlling SARS-CoV-2 Infection in Human Intestinal Epithelial Cells. *Cell Rep* (2020) 32(1):107863. doi: 10.1016/j.celrep.2020.107863
57. Mantlo E, Bukreyeva N, Maruyama J, Paessler S, Huang C. Antiviral activities of type I interferons to SARS-CoV-2 infection. *Antiviral Res* (2020) 179:104811. doi: 10.1016/j.antiviral.2020.104811
58. Cliff JM, Kaufmann SH, McShane H, van Helden P, O'Garra A. The human immune response to tuberculosis and its treatment: a view from the blood. *Immunol Rev* (2015) 264(1):88–102. doi: 10.1111/imr.12269
59. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. *Annu Rev Immunol* (2013) 31:475–527. doi: 10.1146/annurev-immunol-032712-095939
60. Escobar LE, Molina-Cruz A, Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). *Proc Natl Acad Sci USA* (2020) 117:17720–6. doi: 10.1073/pnas.2008410117
61. Rossouw M, Nel HJ, Cooke GS, van Helden PD, Hoal EG. Association between tuberculosis and a polymorphic NFkappaB binding site in the interferon gamma gene. *Lancet* (2003) 361(9372):1871–2. doi: 10.1016/S0140-6736(03)13491-5
62. Vallinoto AC, Graça ES, Araújo MS, Azevedo VN, Cayres-Vallinoto I, Machado LF, et al. IFNG +874T/A polymorphism and cytokine plasma levels are associated with susceptibility to Mycobacterium tuberculosis infection and clinical manifestation of tuberculosis. *Hum Immunol* (2010) 71(7):692–6. doi: 10.1016/j.humimm.2010.03.008
63. de Albuquerque AC, Rocha LQ, de Morais Batista AH, Teixeira AB, Dos Santos DB, Nogueira NA. Association of polymorphism +874 A/T of interferon-γ and susceptibility to the development of tuberculosis: meta-analysis. *Eur J Clin Microbiol Infect Dis* (2012) 31(11):2887–95. doi: 10.1007/s10096-012-1660-4
64. Lechowicz K, Drożdżal S, Machaj F, Rosik J, Szostak B, Zegan-Barańska M, et al. COVID-19: The Potential Treatment of Pulmonary Fibrosis Associated with SARS-CoV-2 Infection. *J Clin Med* (2020) 9(6):1917. doi: 10.3390/jcm9061917
65. Zhao X, Nicholls JM, Chen YG. Severe acute respiratory syndrome-associated coronavirus nucleocapsid protein interacts with Smad3 and modulates transforming growth factor-beta signaling. *J Biol Chem* (2008) 283(6):3272–80. doi: 10.1074/jbc.M708033200
66. Zuo W, Zhao X, Chen Y-G. SARS Coronavirus and Lung Fibrosis. In: SK Lal, editor. *Molecular Biology of the SARS-Coronavirus*. Berlin, Heidelberg: Springer (2010). p. 247–58.
67. Hancock LA, Hennessy CE, Solomon GM, Dobrinskikh E, Estrella A, Hara N, et al. Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice. *Nat Commun* (2018) 9(1):5363. doi: 10.1038/s41467-018-07768-9

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Ahmed. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

REVIEW
published: 13 January 2021
doi: 10.3389/fnut.2020.570235

Ramadan Fasting During the COVID-19 Pandemic; Observance of Health, Nutrition and Exercise Criteria for Improving the Immune System

Majid Taati Moghadam [1,2], Behzad Taati [3], Seyed Mojtaba Paydar Ardakani [4] and Katsuhiko Suzuki [5]*

[1] Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran, [2] Student Research Committee, Iran University of Medical Sciences, Tehran, Iran, [3] Department of Exercise Physiology, Faculty of Sports Sciences, University of Guilan, Rasht, Iran, [4] Department of Sport Sciences, Faculty of Humanities and Social Sciences, Ardakan University, Yazd, Iran, [5] Faculty of Sport Sciences, Waseda University, Tokorozawa, Japan

Fasting is one of the religious rituals of Muslims worldwide who refrain from eating foods and liquids every year during Ramadan. This year (2020), Ramadan is very different from previous years due to the outbreak of a terrible microscopic giant called coronavirus disease 2019 (COVID-19). The pandemic COVID-19 has made Ramadan very important this year because the virus has infected millions of people around the world and killed thousands, especially people with immunodeficiency. In dealing with COVID-19, maintaining good hygiene and supporting the immune system are effective, preventive approaches. Moderate exercise training and proper nutrition are the most important factors to support immune function. Lack of facilities, poor health and many traditions that lead to public community gatherings have made many Islamic countries susceptible to this dangerous virus. In such an unprecedented situation, there are many Muslims who doubt whether they can fast or not. Therefore, the proposal of usable exercise programs and effective nutritional strategies is imperative. In this study, we will look at the proposed health effects of fasting and its impact on the immune system, the effects of Ramadan intermittent fasting on resting values and responses of immunological/antioxidant biomarkers in elite and recreational athletes, together with the important health, nutrition, and exercise advice that fasting people need to follow in the event of a COVID-19 outbreak.

Keywords: coronavirus disease 2019, exercise training, functional foods, inflammatory responses, muslim athletes, lifestyle modifications

OPEN ACCESS

Edited by:
Ioannis Zabetakis, University of Limerick, Ireland

Reviewed by:
Brian P. Carson, University of Limerick, Ireland
Markus Xie, Immunology Discovery, Genentech, United States

***Correspondence:**
Behzad Taati
taati.behzad@yahoo.com

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Nutrition

Received: *06 June 2020*
Accepted: *14 December 2020*
Published: *13 January 2021*

Citation:
Moghadam MT, Taati B, Paydar Ardakani SM and Suzuki K (2021) Ramadan Fasting During the COVID-19 Pandemic; Observance of Health, Nutrition and Exercise Criteria for Improving the Immune System. Front. Nutr. 7:570235. doi: 10.3389/fnut.2020.570235

INTRODUCTION

Muslims were estimated to make up about one-fourth (1.6 billion) of the world's population, and Islam is the second largest religion after Christianity (1). One of the religious rituals of healthy adult Muslims is fasting from sunrise to sunset in Ramadan (during 29 or 30 days). Fasting means avoiding drinking and eating over time periods that vary from 13 to 18 h a day, depending on

the season (2). Since human coronaviruses have been identified around the world, they have been identified as mild human pathogens. The most well-known coronaviruses are human pathogens, including Middle East Respiratory Syndrome (MERS-CoV) and Acute Respiratory Syndrome (SARS-CoV), which have killed large numbers of people in Asia and the Middle East (3). The MERS-CoV virus was first reported in 2012 in Saudi Arabia from a sputum sample of a patient with pneumonia (4). However, in 2019, coronavirus developed a more severe respiratory disease in lung cells, caused by a novel coronavirus called COVID-19. It has been several months since COVID-19 spread from China, but in such a short time a pandemic has occurred due to its very high spreading capability (5). Humans have failed to fight this deadly virus successfully, and about 6 million people worldwide have been infected with the virus, thousands of whom have died. Older people with underlying health problems, and/or immunodeficiency, are more likely to have a poor outcome when they are infected with the virus (5, 6). So far, the exact number of deaths from COVID-19 is not known, due to the actual number of patients not having been identified, but previous studies have reported that ∼2. 84% of patients die and at least 10% show symptoms (7).

Islamic countries have some of the most populous political and economic centers in the world with special religious and cultural practices, such as the annual Hajj of Mecca in Saudi Arabia, the pilgrimage of Imam Reza in Mashhad, and the consumption of unique animal foods such as camels (8, 9). About 2.5 million Muslims from Islamic countries travel to Mecca each year to perform the Umrah pilgrimage. Also, more than 20 million people visit Karbala in Iraq, another important Shia Muslim religious event, which goes on for 40 days. It has been reported that many pilgrims are hospitalized due to respiratory illness during these gatherings (10–12). One of the most important issues in recent research is that COVID-19 has a surface receptor on lung cells, esophageal epithelial cells, and ileum enterocytes: this is called the angiotensin converting enzyme 2 (ACE-2) (13, 14). It is noteworthy that the expression of this receptor is higher in people of Asian countries than in Europe and the United States, and also in men more than women (15, 16). In addition, many Islamic countries can be very vulnerable to COVID-19 due to their poor healthcare system and lack of facilities. The lack of facilities has probably led to many people with COVID-19 are not identified in Islamic countries, and the number of people infected with this dangerous virus is higher than reported. Currently, the World Health Organization (WHO) report has shown that, of all the Islamic countries infected with the COVID-19 virus, Iran has the highest incidence, with about 300,000 cases and 17,000 deaths (**Figure 1**) (17). The WHO website (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports) shows daily report of disease in all parts of the world.

With the spread of the deadly COVID-19 virus, there may be many questions such as whether fasting can be a predisposing factor to the COVID-19 virus? Does fasting impair the immune system? Does fasting reduce physical strength?

HEALTH-RELATED EFFECTS OF RAMADAN FASTING

Consumption of high-calorie foods leads to obesity and subsequent conditions of chronic inflammatory disease, which is known as low-grade inflammation (18, 19). Under these conditions, the observed concentration of inflammatory cytokines usually increases by 2 to 3 times. This leads to many autoimmune diseases such as rheumatoid arthritis and inflammatory diseases such as insulin resistance, atherosclerosis, tissue damage associated with different types of cancer, and cardiovascular disease (20). However, human and animal studies have shown that diets, especially those that mimic fasting, improve many health indicators, both in healthy people and in people with chronic diseases. Although the results can vary slightly depending on the type of fasting patterns and the species studied, all fasting-like diets can result in a fundamental metabolic change and may associate with some health outcomes (21, 22). It is interesting to note that, over the past few years, the beneficial effects of some types of fasting such as reduced meal frequency (diets with reduced meal frequencies such as every-other-day fasting), caloric restriction (typically involving a 15–40% reduction in daily energy intake with maintenance of nutrition), and alternate-day fasting (generally comprise 24-h periods of fasting alternating with 24 h periods of *ad libitum* feeding) have been shown to increase lifespan, improve insulin sensitivity, reduce oxidative stress and inflammation, as well as mortality of cancer and cardiovascular disease (23–27). However, major beneficial effects of Ramadan fasting on people's health (**Figure 2**) also will be discussed, together with the main mechanisms for improvement, in the following sentences (28):

1) It causes weight loss and maintenance among overweight and obese people (29).
2) Glucose homeostasis occurs amongst obese individuals with type 2 diabetes, together with reduction in percentage body fat and in HbA1c (29).
3) There is a greatly improved survival rate and recovery of heart function and modulate cardiovascular risks (30).
4) It may protect neurons against aging disorders (e.g., Alzheimer's disease and stroke) (31, 32).
5) Fasting reduces insulin resistance vs. continuous energy restriction amongst the overweight and, in obese, non-diabetic subjects, it may therefore have an important role in protecting against obesity-related cancers (33).
6) There are decreases in resting heart rate, insulin, circulating levels of glucose and homocysteine which are favorable with regards to the risk of cardiovascular disease (34, 35).
7) It reduces fat mass, total cholesterol, and LDL cholesterol (29).
8) There is equivalent reduction in blood pressure (35).
9) There is a reduction in serum triglycerides, markers of oxidative stress and inflammation (29).
10) Circulating ketone levels are also elevated on the fasting days (36).
11) Effects on a number of cancer risk biomarkers occur (e.g., insulin, cytokines, and the inflammation-related

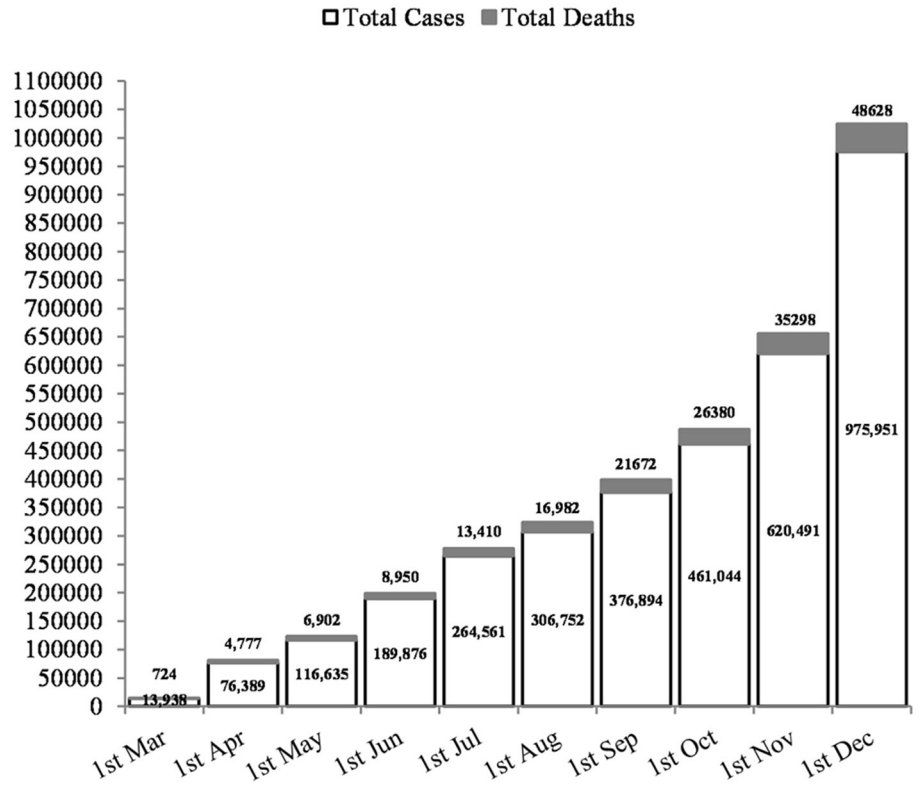


FIGURE 1 | Rates of COVID-19 cases and deaths in Iran, as an Islamic country with the highest incidence.

molecules leptin and adiponectin) which are thought to mediate the effects of adiposity and excessive energy intake on the development and growth of cancers in humans (35, 37).

12) A reduction in insulin-like growth factor 1 has been reported in normal and overweight subjects (38).

Overall, the abovementioned recent small trials of Ramadan fasting in multiple patients have been presented promising results that prepare the main reason for moving forward to perform larger clinical trials in patients with a wide range of disorders. However, this area of research is still in its infancy and requires the cooperation of various researchers and further research before reaching a definitive conclusion.

RAMADAN FASTING MAY AFFECT IMMUNE FUNCTION

The immune system in the human body is an organization consisting of cells and molecules that play a role in defending against infections. The two types of immune system include the innate immune response, which acts the same when exposed to an infectious agent multiple times, and the acquired immune response, which is enhanced by repeated exposure to the infectious agent (39). In the innate immune response, phagocytic cells (macrophages, neutrophils, and monocytes), cells that secrete inflammatory mediators (eosinophils, mast cells, and basophils), and natural killer cells play key roles. Also, cytokines, complement, and acute phase proteins are molecular components of the innate immune system. But the cells involved in the acquired immune response are antigen-specific B and T cells, which proliferate when their surface receptors bind to antigens. B cells release antibodies that target extracellular microorganisms. The role of T cells in acquired immunity is to help B cells to producing antibodies and also kill virus-infected cells and intracellular pathogens by activating macrophages (39).

There have been many studies on the effects of Ramadan fasting on the immune system, which have shown that fasting can restore the immune system (20, 40). Fasting for at least 3 days allows the body to start producing new white blood cells, which rejuvenates the immune system to fight infection. Although it has been shown in humans and animals that the number of white blood cells decreases with long-term fasting, blood cells return when they are re-fed (40). In this way, Ramadan fasting mimicking diets for 3 days (intermittent fasting during Ramadan, time-restricted feeding, and alternate day fasting) forces the body to consume glucose and fat stores, and a significant amount of white blood cells is broken down. As a result, changes in the body cause the stem cells to regenerate new cells in the immune system (20, 41). The promising results of studies have shown that inflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α), oxidative stress markers and C-reactive protein (CRP) might be reduced by Ramadan fasting (20, 42, 43).

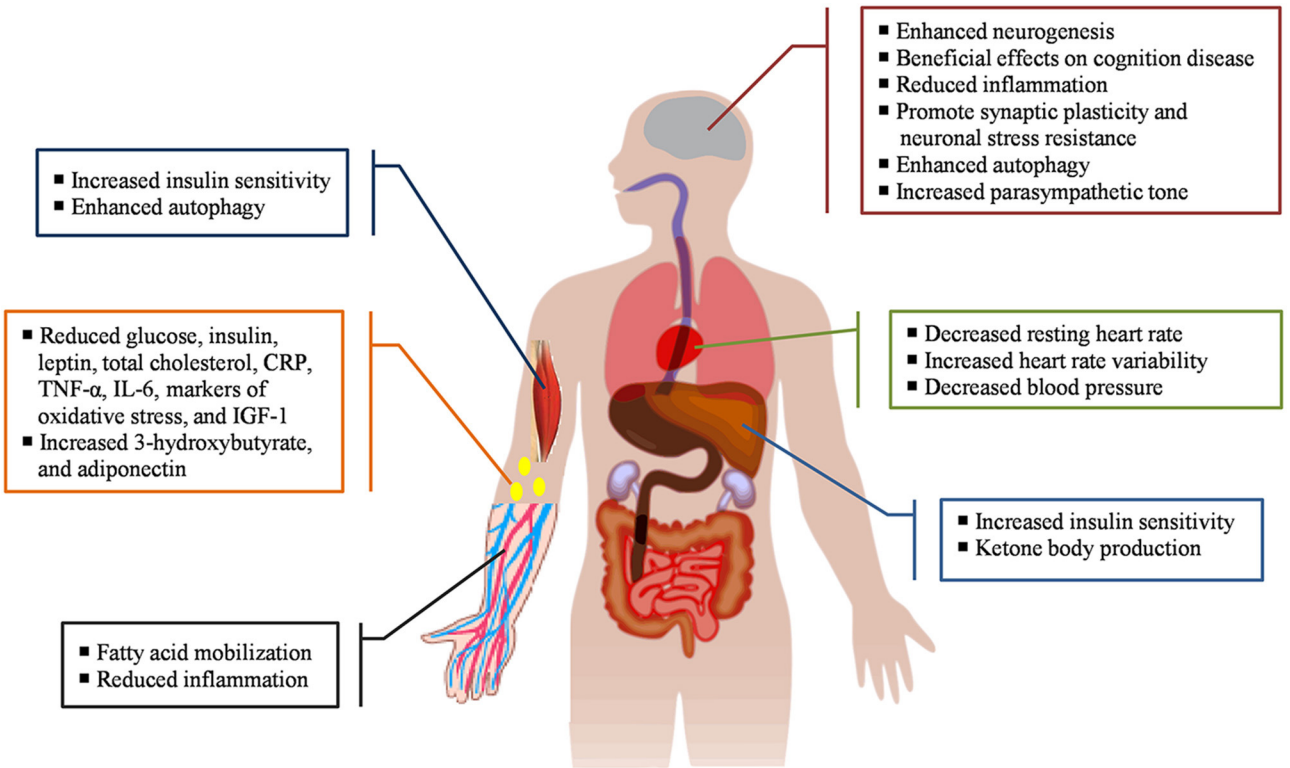


FIGURE 2 | A summary of the main beneficial effects of fasting in different parts of the body.

On the other hand, various studies have confirmed that cytokine storms are an essential mechanism of coronaviruses, which produces large amounts of inflammatory cytokines such as IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL- 18, IL-33, TNF-α, G-CSF, IP-10, MCP-1, MIP-1A, TGF-β, as well as chemokines including CXCL9 and CXCL10 against the virus infection (44–46). The cytokine storm triggers an uncontrollable systemic inflammatory response in the patient that causes the immune system to attack the body and cause diseases such as respiratory failure, acute respiratory distress syndrome (ARDS), multiple organ failure, shock, and death (severe cases) (47, 48). Accordingly, the main mechanism of the COVID-19 virus is the inflammatory cytokine storm. However, Ramadan fasting in healthy people has been concluded to have a small impact on the markers of oxidative stress (protein carbonyls, 8-isoprostane, nitrotyrosine, and 4-hydroxynonenal adducts) and inflammation (serum IL-1β, IL-6, TNF-α, and C-reactive protein) (42). In the case of the COVID-19 pandemic, scientists, jurisprudential scholars, and physicians are not sure whether fasting is safe or not. On the other hand, in some Islamic schools, the fear and danger of infection are not considered a good justification for not fasting. Therefore, the final decision to Ramadan fasting in these circumstances should be made by each person (based on the fatwa) based on the recommendations of the responsible doctors (32). Here are some preventative suggestions for people who are eager to fasting under the COVID-19 pandemic conditions:

- Adherence to the WHO recommendations: this includes frequent hand washing; maintaining a distance of at least one meter from others, and wearing a mask.
- Most religious authorities have always stated that, if a person has problems with ill-health, it is better to refrain from fasting.
- During fasting, avoid being in a crowded public space such as bus, subway, etc.
- Have more rest during fasting.
- Avoid going to religious places for prayer and supplication (49, 50).

It has been proven that supporting immune function and enhancing individual resistance are essential to fighting COVID-19, and the most important ways to strengthen and boost personal immunity are to avoiding overconsumption of calories and to undertake proper exercise program (28, 51–53).

EXERCISE TRAINING, RAMADAN FASTING AND IMMUNE FUNCTION

Based on available literature in the area of exercise immunology, it can be stated that, in general, regular moderate-intensity exercise training with a short-lasting duration (i.e., 45–60 min) has anti-inflammatory and antioxidant effects that improve immune function through enhancing the functional activity of tissue macrophages against pathogens, recirculation of neutrophils, anti-inflammatory cytokines, immature B cells,

cytotoxic T cells, natural killer cells, and immunoglobulins such as immunoglobulin A (54–59). In particular, each acute exercise bout can induce these immunoenhancing effects that add up over time to strengthen immune defense. For instance, a brief session of moderate-intensity exercise induced significant alterations in the pattern of circulatory cytokines related to increased cellular immune function (60). However, vigorous exercise training for prolonged periods of time (i.e., more than 2 h) may cause immunodepression through induction of oxidative stress, inflammatory cytokine cascade, and muscle damage or fatigue. This can lead to acute inflammation and increased susceptibility to infection and, for example, to upper respiratory tract illness (URTI) (54, 61–63).

Exercise training can be considered as a real challenge for Ramadan-fasted individuals, especially during COVID-19 outbreaks. The inability to eat and drink for many hours before and during exercise bouts contributes to the possibility of reduced levels of endogenous fuel in parallel with dehydration which continue until the end of the workout (64, 65). Moreover, daytime sleepiness, and feelings of increased malaise as well as lethargy are the factors that may negatively affect Muslim athletes toward undesirable mood swings (64, 65). Therefore, Ramadan-fasted individuals may experience relatively higher levels of fatigue and perceived effort in response to the same amount of work or exercise training during the month of Ramadan when compared to the non-Ramadan period (65–67). Although most athletes can observe Ramadan with a small loss of physical performance, Ramadan intermittent fast (RIF) can be dangerous for individuals with type I diabetes mellitus, and for participants in ultra-endurance events, particularly under hot conditions (68). However, avoiding exercise training during Ramadan (i.e., 29–30 consecutive days) results in a regression of some important exercise-induced changes such as cardiovascular and resistance adaptations. Thus, maintaining what you have performed in the previous month, without considerable progress in your exercise routine, would be imperative during Ramadan (69).

Much of the Ramadan fasting literature that has evaluated immunological or antioxidant biomarkers has primarily focused on the effect of RIF on resting measures in elite or recreational athletes maintaining their usual high training loads (70–74). In elite athletes who continue to train intensely during Ramadan, RIF may lead to small but significant increases in plasma IL-6 concentration (74), serum CRP, immunoglobulin A and G, non-enzymatic antioxidants such as vitamin A, haptoglobin, and α1-antitrypsin, whereas circulating leukocyte numbers, prealbumin, and homocysteine remained relatively unchanged, and serum vitamin E decreased (70). In another study, RIF had no effect on serum CRP, urea, apoprotein A1, apoprotein B, and cortisol in well-trained middle-distance runners who continued their usual competitive training in Ramadan (74). Moreover, RIF may affect the diurnal variations in immune parameters in trained athletes so that leukocyte counts measured in the afternoon were significantly lower than pre-Ramadan, while in the morning CRP levels were lower than pre-Ramadan and cortisol was higher than afternoon (73).

With regard to resistance exercise, maintaining a hypertrophic training program (4 sessions per week, 4–6 resistance exercises per session, 4 sets with a load of 10 RM, 2–3 min rest intervals between exercises and sets) throughout Ramadan has been shown to have no effect on CRP, plasma thiobarbituric acid reactive substance (TBARS), and mean circulating leukocyte, neutrophil, lymphocyte, and monocyte counts. However, it has increased uric acid values, and total activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) in trained bodybuilders (71, 72).

It seems that, in general, high-load exercise training programs completed by experienced athletes during Ramadan produce a myriad of small fluctuations in inflammatory and antioxidant responses, most of which are minor, and within the normal clinical ranges. Future studies, however, should be carried out

TABLE 1 | Exercise guidelines for the inactive or the less active fasting people during COVID-19 outbreaks in Ramadan.

Type	Intensity	Duration	Frequency
Warm-up	40–50% of HRmax	7–10 min	At least 3 and preferably 5 days per week
Aerobic training	50–75% of HRmax	20–30 min	
Stretching	Until the onset of pain in the muscle	6–10 s per limb/muscle	
Resistance training	50–70% of 1RM	20–30 min	
Cool-down	40–50% of HRmax	5 min	

HRmax, Maximal heart rate; 1RM, 1-repetition maximum.

TABLE 2 | Practical nutritional guidelines for adult athletes who are eager to fasting under the COVID-19 pandemic situation.

Nutrient	Description
Carbohydrate (82, 90)	• *Before exercise:* 1 g/kg of carbohydrate-rich foods such as pasta, rice and bread at the iftar meal (1 h prior to exercise) • *During exercise:* small amounts of carbohydrate-electrolyte drinks including mouth rinse • *After exercise:* 1.0–1.2 g/kg/h of carbohydrate sources such as potatoes and pasta
Protein (86, 90)	• *After exercise:* 20–25 g for young, and 40 g for elderly athletes
Fruits and vegetables (91)	• Frequent meals of citrus fruits, tree fruits, stone fruits, grapes, apples, most berries, strawberries, and bananas from the iftar to sahour meal • Onions, parsley, celery seed, oregano, soybeans, soy-based foods, and legumes can be used in the iftar and sahour meals
Vitamins (81, 92, 93)	• Vitamin C: 200 mg per day • Vitamin D: 2000 IU (50 μg) per day • Vitamin B_6: 1.3 mg per day • Vitamin B_9: 400 μg per day • Vitamin B_{12}: 2.4 μg per day
Others (81, 93, 94)	• Zinc: 8–11 mg per day • Selenium: 400 μg per day • Omega-3 fatty acids: 250 mg per day of EPA + DHA

EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

to support these findings and ascertain other immune-related aspects of RIF.

Another important aspect of research concerning exercise training during Ramadan is related to the effects of RIF on acute immune responses to an exercise bout (75–77), in which there is an apparent need for more studies and better understanding of the pattern of these responses. A brief maximal exercise (30-s Wingate test) in active young men has been shown to result in a significant increase in plasma concentration of IL-12, as a pro-inflammatory cytokine, during (the 1st and 4th weeks) and after Ramadan (3 weeks later). However, these changes during the 4th week were lower than that of the 1st week and after Ramadan (75). On the other hand, time-of-day affects antioxidant status and muscle inflammatory responses following exercise during Ramadan. In this context, one study examined the effects of RIF and time-of-day on biochemical responses of male athletes to an intermittent exercise test. The study reported that post-exercise levels of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the evening (5 P.M.) were higher than in the morning (7 A.M.) during the 2nd and 4th week of Ramadan, whereas total antioxidant status was better in the morning (76).

Despite the biochemical fluctuations mentioned which lead to changes in the hormonal, immune and antioxidant defense systems, there is no obvious scientific evidence to propose a significant increase in physiological stress or chronic systemic inflammation (70, 73, 76). Therefore, given the importance of exercise training to maintain proper immune function, especially during the COVID-19 pandemic, a modified training approach (e.g., home-based workouts) aiming to maintain physical fitness and body mass or avoiding detraining would seem worthwhile for the sedentary fasting people (**Table 1**). Nevertheless, for Muslim athletes, the same training load as that being undertaken immediately before Ramadan is proposed (69, 78). It should also be noted that it is best to do exercise sessions in the morning or after breaking the fast during the month of Ramadan to minimize performance degradation and exercise-induced inflammatory responses (76, 78, 79). We propose, however, that exercise session

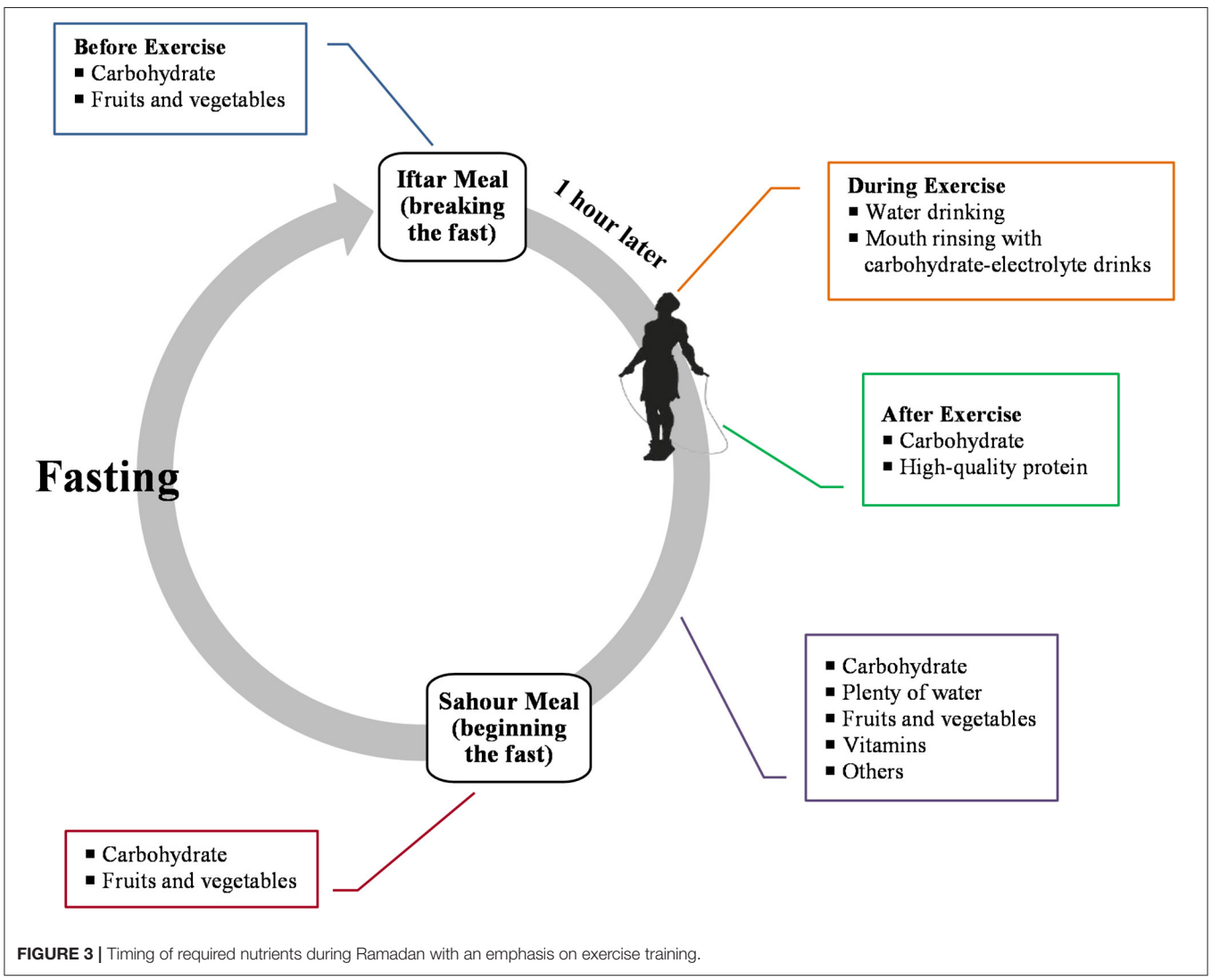


FIGURE 3 | Timing of required nutrients during Ramadan with an emphasis on exercise training.

can be performed about 1 h after iftar (meal eaten after sunset) ensuring that only a light meal is taken.

NUTRITIONAL STRATEGIES FOR RECOVERY FROM EXERCISE TRAINING IN RAMADAN

The homeostasis of nutritional metabolism has a vital role in maintaining cell survival and normal immunologic functions (80). It is suggested that along with exercise training, the nutritional approach should also be taken into account to provide acute nutritional needs, to optimize the body's recovery after each exercise bout and to help the stability of the immune system (54, 55, 78, 81). Indeed, finding a balance between exercise training load and nutrition is a challenging concern during Ramadan. Fasted individuals cannot ingest anything (e.g., carbohydrate, water, etc.) during and immediately following day-time exercise: this is likely to result in altered immunological responses to exercise bouts (70–73, 75, 76, 78). Nevertheless, night-time training can be a simple and logical way out of the problem because it allows athletes to stay relatively well-hydrated and to maintain blood glucose levels through the consumption of *ad libitum* drinking, so that recovery from exercise session can be effectively accomplished (78).

Sufficient intake of carbohydrates, protein substrates, polyphenols, minerals and vitamins may produce better outcomes regarding exercise recovery and efficient immune function (54, 55, 81). Therefore, in the situation of the COVID-19 pandemic, a well-balanced diet containing drinking water, carbohydrate-rich foods, grains, nuts, seeds, fruits, and vegetables is necessary to maintain viral protection and reduce exercise-induced inflammation (54, 55, 81). Fasted individuals should consume these nutritional items by a pattern of small frequent meals from the iftar to sahour (the last meal eaten before beginning the fast).

With regard to pre-exercise nutrition, it is proposed that a light meal containing at least 1 g of carbohydrate per kilogram of body mass (82) is suitable for the iftar meal to increase net carbohydrate availability during the subsequent exercise session and to reduce the risk of insulin increase and hypoglycemia after the onset of exercise. In addition, the exercise bout should be performed at least 1 h after the iftar meal.

Intended exercise duration discussed in the present article is about 60 min: this could enhance immune function (55) and is not associated with limitations of energy supply if pre-exercise carbohydrate ingestion has been given adequate attention (82). However, frequent rinsing of the mouth with a carbohydrate solution, such as carbohydrate-electrolyte sports drinks, appears to lead to better performance (83).

During early recovery from moderate-intensity exercise that is suggested in the paper cited below, the availability of dietary carbohydrate (e.g., moderate and high glycaemic index carbohydrate-rich foods and drinks) is imperative to provide high rates of muscle glycogen synthesis (82) and promote pro-inflammatory reactions within skeletal muscle (84). The 1st h after exercise session are the "golden times" for storing glycogen and it seems that body's capacity reaches maximum with a carbohydrate intake of about 1 g per kilogram of body mass per hour (85).

Beside carbohydrate, post-exercise protein ingestion has some benefits on immune responses to exercise and skeletal muscle recovery (82, 86). Fasted individuals can intake 20–25 g of high-quality protein soon after the exercise bout to provide adequate plasma essential amino acids which are important for many training adaptations through the synthesis of new protein structures (e.g., myofibrillar, sarcoplasmic and mitochondrial contents) during the recovery period (87, 88). Fast digesting proteins (e.g., whey component of milk) may be a good choice to increase the plasma leucine content after exercise (89). **Table 2** summarizes, in general, the potential nutritional suggestions that may result in better exercise-induced outcomes during COVID-19 outbreaks in the month of Ramadan.

CONCLUSIONS

COVID-19 is a serious respiratory disease that has become a deadly pandemic with a very rapid spread in countries around the world, especially in Islamic countries because of their poor healthcare system and lack of facilities. Whilst Ramadan fasting may seem to be a harmful challenge for Muslims in this situation, some health benefits have been proposed in humans (**Figure 2**). Nevertheless, improving immune function and increasing individual resistance are essential to help fight COVID-19 (28, 51, 52).

Exercise training has been known as a lifestyle factor that can maintain and even promote immune function and act like a vaccine against certain diseases/infections through producing physiological stress in the human body which lead to a series of adaptations occurring to overcome these stimuli (95). The overall health promotion effects of exercise training along with its disease prevention have resulted in the important statement of "Exercise is Medicine" (54, 96). These positive adaptations, however, may not be achieved without ingesting some functional foods (e.g., carbohydrate, protein, polyphenols, vitamins, and minerals) and fluids together with suitable timing (**Figure 3**) in order to help maintain exercise performance and immune function without causing harmful side effects on health (97).

AUTHOR CONTRIBUTIONS

MM and BT developed the idea, wrote, and drafted the paper. SP co-wrote and provided the figures and tables. KS co-wrote and developed the article. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

We wish to thank all physicians, nurses, midwives, psychologists, hospital medical staff, and laboratory technicians who are the guardians of people's health.

REFERENCES

1. Dabaja E, Dabaja K, Ismail M, Haidous M, Hamka A, Blackwood RA, et al. Pediatric muslim fasting practices in Southeast Michigan: a community survey. *J Community Health.* (2020) 45:732–8. doi: 10.1007/s10900-020-00788-x
2. Azizi F. Islamic fasting and health. *Ann Nutr Metab.* (2010) 56:273–82. doi: 10.1159/000295848
3. World Health Organization. *Laboratory Testing for Coronavirus Disease 2019 (COVID-19) in Suspected Human Cases: Interim Guidance*. World Health Organization (2020). Available online at: https://apps.who.int/iris/handle/10665/331329 (accessed March 2, 2020).
4. Yavarian J, Rezaei F, Shadab A, Soroush M, Gooya MM, Azad TM. Cluster of Middle East respiratory syndrome coronavirus infections in Iran, 2014. *Emerg Infect Dis.* (2015) 21:362. doi: 10.3201/eid2102.141405
5. Chen Z, Zhang W, Lu Y, Guo C, Guo Z, Liao C, et al. From sars-cov to wuhan 2019-ncov outbreak: similarity of early epidemic and prediction of future trends. *Cell Host Microbe*. (2020). doi: 10.1101/2020.01.24.919241
6. World Health Organization. *WHO Coronavirus Disease (COVID-19) Dashboard*. (2020). Available online at: https://covid19.who.int/?gclid=CjwKCAjwjqT5BRAPEiwAJlBuBeCQu4rosBrLlnT6WTYvcz6uT-ep5GcG4d25AEZhuoI3g2mDi2relxoCTtkQAvD_BwE (accessed 19 December, 2020).
7. Wang W, Tang J, Wei F. Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. *J Med Virol.* (2020) 92:441–7. doi: 10.1002/jmv.25689
8. Pinto PG. Pilgrimage, commodities, and religious objectification: the making of transnational Shiism between Iran and Syria. *Com Stud South Asia Africa Middle East.* (2007) 27:109–25. doi: 10.1215/1089201x-2006-047
9. Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen K-Y. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clin Microbiol Rev.* (2015) 28:465–522. doi: 10.1128/CMR.00102-14
10. Gautret P, Benkouiten S, Al-Tawfiq JA, Memish ZA. Hajj-associated viral respiratory infections: a systematic review. *Travel Med Infect Dis.* (2016) 14:92–109. doi: 10.1016/j.tmaid.2015.12.008
11. Yavarian J, Jandaghi NZS, Naseri M, Hemmati P, Dadras M, Gouya MM, et al. Influenza virus but not MERS coronavirus circulation in Iran, 2013–2016: comparison between pilgrims and general population. *Travel Med Infect Dis.* (2018) 21:51–5. doi: 10.1016/j.tmaid.2017.10.007
12. Al-Ghamdi SM, Akbar HO, Qari YA, Fathaldin OA, Al-Rashed RS. Pattern of admission to hospitals during muslim pilgrimage (Hajj). *Saudi Med J.* (2003) 24:1073–6.
13. Hoffmann M, Kleine-Weber H, Krüger N, Mueller MA, Drosten C, Pöhlmann S. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *BioRxiv*. (2020). doi: 10.1101/2020.01.31.929042
14. Zhang H, Kang Z, Gong H, Xu D, Wang J, Li Z, et al. The digestive system is a potential route of 2019-nCov infection: a bioinformatics analysis based on single-cell transcriptomes. *BioRxiv*. (2020). doi: 10.1101/2020.01.30.927806
15. Zhang Q, Cong M, Wang N, Li X, Zhang H, Zhang K, et al. Association of angiotensin-converting enzyme 2 gene polymorphism and enzymatic activity with essential hypertension in different gender: a case–control study. *Medicine.* (2018) 97:e12917. doi: 10.1097/MD.0000000000012917
16. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. *bioRxiv.* (2020). doi: 10.1101/2020.01.26.919985
17. World Health Organization. *Iran (Islamic Republic of) Situation: Worldometer*. (2020). Available online at: https://www.worldometers.info/coronavirus/country/iran/ (accessed 19 December, 2020).
18. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes.* (2007) 56:1010–3. doi: 10.2337/db06-1656
19. You T, Yang R, Lyles MF, Gong D, Nicklas BJ. Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. *Am J Physiol Endocrinol Metab.* (2005) 288:E741–7. doi: 10.1152/ajpendo.00419.2004
20. Faris MeA-IE, Kacimi S, Al-Kurd RaA, Fararjeh MA, Bustanji YK, Mohammad MK, et al. Intermittent fasting during Ramadan attenuates proinflammatory cytokines and immune cells in healthy subjects. *Nutr Res.* (2012) 32:947–55. doi: 10.1016/j.nutres.2012.06.021
21. Fond G, Macgregor A, Leboyer M, Michalsen A. Fasting in mood disorders: neurobiology and effectiveness a review of the literature. *Psychiatr Res.* (2013) 209:253–8. doi: 10.1016/j.psychres.2012.12.018
22. Wegman MP, Guo MH, Bennion DM, Shankar MN, Chrzanowski SM, Goldberg LA, et al. Practicality of intermittent fasting in humans and its effect on oxidative stress and genes related to aging and metabolism. *Rejuvenation Res.* (2015) 18:162–72. doi: 10.1089/rej.2014.1624
23. Mattson MP, Wan R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. *J Nutr Biochem.* (2005) 16:129–37. doi: 10.1016/j.jnutbio.2004.12.007
24. Varady KA, Roohk D, McEvoy-Hein B, Gaylinn B, Thorner M, Hellerstein M. Modified alternate-day fasting regimens reduce cell proliferation rates to a similar extent as daily calorie restriction in mice. *FASEB J.* (2008) 22:2090–6. doi: 10.1096/fj.07-098178
25. Wan R, Ahmet I, Brown M, Cheng A, Kamimura N, Talan M, et al. Cardioprotective effect of intermittent fasting is associated with an elevation of adiponectin levels in rats. *J Nutr Biochem.* (2010) 21:413–7. doi: 10.1016/j.jnutbio.2009.01.020
26. Lu J, E L, Wang W, Frontera J, Zhu H, Wang WT, et al. Alternate day fasting impacts the brain insulin-signaling pathway of young adult male C57BL/6 mice. *J Neurochem.* (2011) 117:154–63. doi: 10.1111/j.1471-4159.2011.07184.x
27. Castello L, Froio T, Maina M, Cavallini G, Biasi F, Leonarduzzi G, et al. Alternate-day fasting protects the rat heart against age-induced inflammation and fibrosis by inhibiting oxidative damage and NF-kB activation. *Free Radic Biol Med.* (2010) 48:47–54. doi: 10.1016/j.freeradbiomed.2009.10.003
28. Mattson MP, Longo VD, Harvie M. Impact of intermittent fasting on health and disease processes. *Ageing Res Rev.* (2017) 39:46–58. doi: 10.1016/j.arr.2016.10.005
29. Kul S, Savaş E, Öztürk ZA, Karadag G. Does Ramadan fasting alter body weight and blood lipids and fasting blood glucose in a healthy population? A meta-analysis. *J Relig Health.* (2014) 53:929–42. doi: 10.1007/s10943-013-9687-0
30. Yousefi B, Faghfoori Z, Samadi N, Karami H, Ahmadi Y, Badalzadeh R, et al. The effects of Ramadan fasting on endothelial function in patients with cardiovascular diseases. *Eur J Clin Nutr.* (2014) 68:835–9. doi: 10.1038/ejcn.2014.61
31. El-Mitwalli A, Zaher AA, El-Salammohamed M, Elmenshawi E. The effect of Ramadan fasting on cerebral stroke: a prospective hospital-based study. *Eur J Neurol.* (2009) 16:e80. doi: 10.1111/j.1468-1331.2009.02566.x
32. Tootee A, Larijani B. Ramadan fasting during Covid-19 pandemic. *J Diabetes Metab Disord.* (2020) 19:1–4. doi: 10.1007/s40200-020-00534-z
33. Shariatpanahi ZV, Shariatpanahi MV, Shahbazi S, Hossaini A, Abadi A. Effect of Ramadan fasting on some indices of insulin resistance and components of the metabolic syndrome in healthy male adults. *Br J Nutr.* (2008) 100:147–51. doi: 10.1017/S000711450787231X
34. Hammouda O, Chtourou H, Aloui A, Chahed H, Kallel C, Miled A, et al. Concomitant effects of Ramadan fasting and time-of-day on apolipoprotein AI, B, LP-a and homocysteine responses during aerobic exercise in Tunisian soccer players. *PLoS ONE.* (2013) 8:e79873. doi: 10.1371/journal.pone.0079873
35. Meo SA, Hassan A. Physiological changes during fasting in Ramadan. *J Pak Med Assoc.* (2015) 65:S6–14.
36. Lessan N, Ali T. Energy metabolism and intermittent fasting: the Ramadan perspective. *Nutrients.* (2019) 11:1192. doi: 10.3390/nu11051192
37. Feizollahzadeh S, Rasuli J, Kheirouri S, Alizadeh M. Augmented plasma adiponectin after prolonged fasting during ramadan in men. *Health Promot Perspect.* (2014) 4:77–81. doi: 10.5681/hpp.2014.010
38. Rahbar AR, Safavi E, Rooholamini M, Jaafari F, Darvishi S, Rahbar A. Effects of intermittent fasting during Ramadan on insulin-like growth factor-1, interleukin 2, and lipid profile in healthy Muslims. *Int J Prev Med.* (2019) 10:7. doi: 10.4103/ijpvm.IJPVM_252_17
39. Delves PJ, Roitt IM. The immune system. *N Engl J Med.* (2000) 343:37–49. doi: 10.1056/NEJM200007063430107

40. Javanmard SH, Otroj Z. Ramadan fasting and risk of covid-19. *Int J Prev Med.* (2020) 11:60. doi: 10.4103/ijpvm.IJPVM_236_20
41. Cheng C-W, Adams GB, Perin L, Wei M, Zhou X, Lam BS, et al. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. *Cell stem cell.* (2014) 14:810–23. doi: 10.1016/j.stem.2014.04.014
42. Faris MeA-IE, Jahrami HA, Obaideen AA, Madkour MI. Impact of diurnal intermittent fasting during Ramadan on inflammatory and oxidative stress markers in healthy people: systematic review and meta-analysis. *J Nutr Intermed Metab.* (2019) 15:18–26. doi: 10.1016/j.jnim.2018.11.005
43. Mushtaq R, Akram A, Mushtaq R, Khwaja S, Ahmed S. The role of inflammatory markers following Ramadan fasting. *Pak J Med Sci.* (2019) 35:77–81. doi: 10.12669/pjms.35.1.95
44. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
45. Williams AE, Chambers RC. The mercurial nature of neutrophils: still an enigma in ARDS? *Am J Physiol Lung Cell Mol Physiol.* (2014) 306:L217–30. doi: 10.1152/ajplung.00311.2013
46. Cameron MJ, Bermejo-Martin JF, Danesh A, Muller MP, Kelvin DJ. Human immunopathogenesis of severe acute respiratory syndrome (SARS). *Virus Res.* (2008) 133:13–9. doi: 10.1016/j.virusres.2007.02.014
47. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med.* (2020) 8:420–2. doi: 10.1016/S2213-2600(20)30076-X
48. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. *Asian Pac J Allergy Immunol.* (2020) 38:1–9. doi: 10.12932/AP-200220-0772
49. Abdi M. Coronavirus disease 2019 (COVID-19) outbreak in Iran: actions and problems. *Infect Control Hosp Epidemiol.* (2020) 41:754–5. doi: 10.1017/ice.2020.86
50. World Health Organization. *Coronavirus Disease (COVID-19) Advice for the Public.* (2020). Available online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public (accessed 19 December, 2020).
51. Yoshikawa TT, High KP. Nutritional strategies to boost immunity and prevent infection in elderly individuals. *Clin Infect Dis.* (2001) 33:1892–900. doi: 10.1086/324509
52. Rebaï H, Chtourou H, Zarrouk N, Harzallah A, Kanoun I, Dogui M, et al. Reducing resistance training volume during Ramadan improves muscle strength and power in football players. *Int J Sports Med.* (2014) 35:432–7. doi: 10.1055/s-0033-1353216
53. Dousari AS, Moghadam MT, Satarzadeh N. COVID-19 (Coronavirus disease 2019): a new coronavirus disease. *Infect Drug Resist.* (2020) 13:2819–28. doi: 10.2147/IDR.S259279
54. Suzuki K. Chronic inflammation as an immunological abnormality and effectiveness of exercise. *Biomolecules.* (2019) 9:223. doi: 10.3390/biom9060223
55. Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system. *J Sport Health Sci.* (2019) 8:201–17. doi: 10.1016/j.jshs.2018.09.009
56. Arazi H, Taati B, Suzuki K. A review of the effects of leucine metabolite (β-Hydroxy-β-methylbutyrate) supplementation and resistance training on inflammatory markers: a new approach to oxidative stress and cardiovascular risk factors. *Antioxidants.* (2018) 7:148. doi: 10.3390/antiox7100148
57. Khammassi M, Ouerghi N, Said M, Feki M, Khammassi Y, Pereira B, et al. Continuous moderate-intensity but not high-intensity interval training improves immune function biomarkers in healthy young men. *J Strength Cond Res.* (2020) 34:249–56. doi: 10.1519/JSC.0000000000002737
58. Abd El-Kader SM, Al-Shreef FM. Inflammatory cytokines and immune system modulation by aerobic versus resisted exercise training for elderly. *Afr Health Sci.* (2018) 18:120–31. doi: 10.4314/ahs.v18i1.16
59. Klentrou P, Cieslak T, MacNeil M, Vintinner A, Plyley M. Effect of moderate exercise on salivary immunoglobulin A and infection risk in humans. *Eur J Appl Physiol.* (2002) 87:153–8. doi: 10.1007/s00421-002-0609-1
60. de Souza DC, Matos VA, dos Santos VO, Medeiros IF, Marinho CS, Nascimento PR, et al. Effects of high-intensity interval and moderate-intensity continuous exercise on inflammatory, leptin, IgA, and lipid peroxidation responses in obese males. *Front Immunol.* (2018) 9:567. doi: 10.3389/fphys.2018.00567
61. Cerqueira É, Marinho DA, Neiva HP, Lourenço O. Inflammatory effects of high and moderate intensity exercise—a systematic review. *Front Physiol.* (2020) 10:1550. doi: 10.3389/fphys.2019.01550
62. Zwetsloot KA, John CS, Lawrence MM, Battista RA, Shanely RA. High-intensity interval training induces a modest systemic inflammatory response in active, young men. *J Inflamm Res.* (2014) 7:9–17. doi: 10.2147/JIR.S54721
63. Tharp GD, Barnes MW. Reduction of saliva immunoglobulin levels by swim training. *Eur J Appl Physiol.* (1990) 60:61–4. doi: 10.1007/BF00572187
64. Shephard RJ. The impact of Ramadan observance upon athletic performance. *Nutrients.* (2012) 4:491–505. doi: 10.3390/nu4060491
65. Damit NF, Lim VTW, Muhamed AMC, Chaouachi A, Chamari K, Singh R, et al. Exercise responses and training during daytime fasting in the month of Ramadan and its impact on training-induced adaptations. In: Chtourou H, editor. *Effects of Ramadan fasting on health and Athletic Performance: OMICS International*. Foster City, CA: OMICS Group eBooks (2014). p. 3–12.
66. Brisswalter J, Bouhlel E, Falola JM, Abbiss CR, Vallier JM, Hauswirth C. Effects of Ramadan intermittent fasting on middle-distance running performance in well-trained runners. *Clin J Sport Med.* (2011) 21:422–7. doi: 10.1097/JSM.0b013e3182293891
67. Aziz AR, Wahid MF, Png W, Jesuvadian CV. Effects of Ramadan fasting on 60 min of endurance running performance in moderately trained men. *Br J Sports Med.* (2010) 44:516–21. doi: 10.1136/bjsm.2009.070425
68. Shephard RJ. Sport participation and Ramadan observance: advice for the athlete. *J Nutr Fast Health.* (2015) 3:65–73. doi: 10.22038/jfh.2015.4670
69. Maughan R, Fallah J, Coyle EF. The effects of fasting on metabolism and performance. *Br J Sports Med.* (2010) 44:490–4. doi: 10.1136/bjsm.2010.072181
70. Chaouachi A, Coutts AJ, Wong DP, Roky R, Mbazaa A, Amri M, et al. Haematological, inflammatory, and immunological responses in elite judo athletes maintaining high training loads during Ramadan. *Appl Physiol Nutr Metab.* (2009) 34:907–15. doi: 10.1139/H09-095
71. Trabelsi K, Stannard SR, Maughan RJ, Jammoussi K, Zeghal K, Hakim A. Effect of resistance training during Ramadan on body composition and markers of renal function, metabolism, inflammation, and immunity in recreational bodybuilders. *Int J Sport Nutr Exerc Metab.* (2012) 22:267–75. doi: 10.1123/ijsnem.22.4.267
72. Elabed K, Trabelsi K, Ghlissi Z, Stannard SR, Leeuwenburgh C, Jamoussi K, et al. Effect of resistance training during the month of Ramadan on antioxidants and oxidative stress biomarkers in recreational bodybuilders. *Biol Rhythm Res.* (2015) 46:459–70. doi: 10.1080/09291016.2015.1015232
73. Maughan RJ, Leiper JB, Bartagi Z, Zrifi R, Zerguini Y, Dvorak J. Effect of Ramadan fasting on some biochemical and haematological parameters in Tunisian youth soccer players undertaking their usual training and competition schedule. *J Sports Sci.* (2008) 26:S39–46. doi: 10.1080/02640410802491368
74. Chennaoui M, Desgorces F, Drogou C, Boudjemaa B, Tomaszewski A, Depiesse F, et al. Effects of Ramadan fasting on physical performance and metabolic, hormonal, and inflammatory parameters in middle-distance runners. *Appl Physiol Nutr Metab.* (2009) 34:587–94. doi: 10.1139/H09-014
75. Abedelmalek S, Souissi N, Takayuki A, Hadouk S, Tabka Z. Effect of acute maximal exercise on circulating levels of interleukin-12 during Ramadan fasting. *Asian J Sports Med.* (2011) 2:154–60. doi: 10.5812/asjsm.34751
76. Hammouda O, Chtourou H, Aloui A, Mejri MA, Chahed H, Miled A, et al. Does Ramadan fasting affect the diurnal variations in metabolic responses and total antioxidant capacity during exercise in young soccer players? *Sport Sci Health.* (2014) 10:97–104. doi: 10.1007/s11332-014-0179-8
77. Bouzid MA, Abaidia AE, Bouchiba M, Ghattassi K, Daab W, Engel FA, et al. Effects of Ramadan fasting on recovery following a simulated soccer match in professional soccer players: a pilot study. *Front Physiol.* (2019) 10:1480. doi: 10.3389/fphys.2019.01480
78. Mujika I, Chaouachi A, Chamari K. Precompetition taper and nutritional strategies: special reference to training during Ramadan intermittent fast. *Br J Sports Med.* (2010) 44:495–501. doi: 10.1136/bjsm.2009.071274

79. Chtourou H, Hammouda O, Aloui A, Souissi N, Chaouachi A. The optimal time of day for training during Ramadan: a review study. *J Nutr Fast Health.* (2014) 2:46–52. doi: 10.22038/jfh.2014.2578
80. He L, Zhang J, Zhao J, Ma N, Kim SW, Qiao S, et al. Autophagy: the last defense against cellular nutritional stress. *Adv Nutr.* (2018) 9:493–504. doi: 10.1093/advances/nmy011
81. Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients.* (2020) 12:1181. doi: 10.3390/nu12041181
82. Burke LM, King C. Ramadan fasting and the goals of sports nutrition around exercise. *J Sports Sci.* (2012) 30:S21–31. doi: 10.1080/02640414.2012.680484
83. Jeukendrup AE, Chambers ES. Oral carbohydrate sensing and exercise performance. *Curr Opin Clin Nutr Metab Care.* (2010) 13:447–51. doi: 10.1097/MCO.0b013e328339de83
84. Ross ML, Halson SL, Suzuki K, Garnham A, Hawley JA, Cameron-Smith D, et al. Cytokine responses to carbohydrate ingestion during recovery from exercise-induced muscle injury. *J Interferon Cytokine Res.* (2010) 30:329–37. doi: 10.1089/jir.2009.0079
85. Jentjens R, Jeukendrup AE. Determinants of post-exercise glycogen synthesis during short-term recovery. *Sports Med.* (2003) 33:117–44. doi: 10.2165/00007256-200333020-00004
86. Peake JM, Neubauer O, Walsh NP, Simpson RJ. Recovery of the immune system after exercise. *J Appl Physiol.* (2017) 122:1077–87. doi: 10.1152/japplphysiol.00622.2016
87. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr.* (2009) 89:161–8. doi: 10.3945/ajcn.2008.26401
88. Hawley JA, Burke LM, Phillips SM, Spriet LL. Nutritional modulation of training-induced skeletal muscle adaptations. *J Appl Physiol.* (2011) 110:834–45. doi: 10.1152/japplphysiol.00949.2010
89. West DW, Burd NA, Coffey VG, Baker SK, Burke LM, Hawley JA, et al. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise–. *Am J Clin Nutr.* (2011) 94:795–803. doi: 10.3945/ajcn.111.013722
90. Naderi A, de Oliveira EP, Ziegenfuss TN, Willems ME. Timing, optimal dose and intake duration of dietary supplements with evidence-based use in sports nutrition. *J Exerc Nutrition Biochem.* (2016) 20:1–12. doi: 10.20463/jenb.2016.0031
91. Balentine DA, Dwyer JT, Erdman Jr JW, Ferruzzi MG, Gaine PC, Harnly JM, et al. Recommendations on reporting requirements for flavonoids in research. *Am J Clin Nutr.* (2015) 101:1113–25. doi: 10.3945/ajcn.113.071274
92. Aslam M, Majeed S, Aslam S, Irfan J. Vitamins: key role players in boosting up immune response—a mini review. *Vitam Miner.* (2017) 6:153. doi: 10.4172/2376-1318.1000153
93. Zhang L, Liu Y. Potential interventions for novel coronavirus in China: a systemic review. *J Med Virol.* (2020) 92:479–90. doi: 10.1002/jmv.25707
94. Hoffmann PR, Berry MJ. The influence of selenium on immune responses. *Mol Nutr Food Res.* (2008) 52:1273–80. doi: 10.1002/mnfr.200700330
95. Jee Y-S. Exercise is an antigen for vaccination: first series of scientific evidence. *J Exerc Rehabil.* (2019) 15:339–40. doi: 10.12965/jer.1938302.151
96. Roberts L, Suzuki K. Exercise and inflammation. *Antioxidants.* (2019) 8:155. doi: 10.3390/antiox8060155
97. Suzuki K, Tominaga T, Ruhee RT, Ma S. Characterization and modulation of systemic inflammatory response to exhaustive exercise in relation to oxidative stress. *Antioxidants.* (2020) 9:401. doi: 10.3390/antiox9050401

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Moghadam, Taati, Paydar Ardakani and Suzuki. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 21 January 2021
doi: 10.3389/fnut.2020.619850

The Burden of Malnutrition and Fatal COVID-19: A Global Burden of Disease Analysis

Elly Mertens and José L. Peñalvo*

Unit of Noncommunicable Diseases, Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium

Background: Although reasonable to assume, it is not yet clear whether malnourished countries are at higher risk for severe or fatal coronavirus disease 2019 (COVID-19). This study aims to identify the countries where prevalent malnutrition may be a driving factor for fatal disease after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Methods: Using estimates from the Global Burden of Disease 2019, country-level burden of malnutrition was quantified using four indicators: death rates for child growth failure (underweight, stunting, and/or wasting) and years lived with disability (YLD) attributed to iron and vitamin A deficiencies and high body mass index (BMI). Global mortality descriptors of the ongoing COVID-19 pandemic were extracted from the European Centre for Disease Prevention and Control, and case fatality ratios (CFRs) were calculated introducing a lag time of 10 weeks after the first death of a confirmed case. Bivariate analyses for 172 countries were carried out for malnutrition indicators and fatal COVID-19. Correlations between burden indicators were characterized by Spearman's rank correlation coefficients (ρ) and visually by scatterplots. Restricted cubic splines and underlying negative binomial regressions adjusted for countries' age-structure, prevalent chronic comorbidities related to COVID-19, population density, and income group were used to explore non-linear relationships.

Results: Stratified by the World Bank income group, a moderate positive association between YLD rates for iron deficiency and CFRs for COVID-19 was observed for low-income countries ($\rho = 0.60$, $p = 0.027$), whereas no clear indications for the association with child growth failure, vitamin A deficiency, or high BMI were found ($\rho < 0.30$). Countries ranking high on at least three malnutrition indicators and presenting also an elevated CFR for COVID-19 are sub-Saharan African countries, namely, Angola, Burkina Faso, Chad, Liberia, Mali, Niger, Sudan, and Tanzania, as well as Yemen and Guyana.

Conclusions: Population-level malnutrition appears to be related to increased rates of fatal COVID-19 in areas with an elevated burden of undernutrition, such as countries in the Sahel strip. COVID-19 response plans in malnourished countries, vulnerable to fatal COVID-19, should incorporate food security, nutrition, and social protection as a priority component in order to reduce COVID-19 fatality.

Keywords: malnutrition, undernutrition, overnutrition, BMI, global burden, COVID-19 mortality

OPEN ACCESS

Edited by:
Alexandros Tsoupras, University of Limerick, Ireland

Reviewed by:
Jose Paulo Andrade, Universidade do Porto, Portugal
Carla Ferreri, National Research Council (CNR), Italy

****Correspondence:***
Elly Mertens
ellymertens@itg.be

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *21 October 2020*
Accepted: *14 December 2020*
Published: *21 January 2021*

Citation:
Mertens E and Peñalvo JL (2021) The Burden of Malnutrition and Fatal COVID-19: A Global Burden of Disease Analysis. Front. Nutr. 7:619850. doi: 10.3389/fnut.2020.619850

INTRODUCTION

The ongoing pandemic of the coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a major cause of morbidity and mortality in 2020 across the globe, affecting more than 200 countries. Internationally, countries have reacted to COVID-19 by introducing key public health non-pharmaceutical practices, such as handwashing (1), physical distancing (2), and wearing facemasks (3) to hinder the spread of the virus and to protect vulnerable populations. Evidence, mainly from high-income countries (HIC), has emerged suggesting that the risk of suffering severe COVID-19 outcomes is higher in elderly populations and in individuals with pre-existing chronic comorbidities (4–6). Older age and multi-morbidity are also often associated with a higher risk and prevalence of disease-related malnutrition (7–9). It has been postulated that the nutritional status might also play a key role as a driver of SARS-CoV-2 virulence (10, 11). In this line, current patient care recommendations, as those published by the European Society for Clinical Nutrition and Metabolism (ESPEN) (12), among others (13, 14), include nutritional interventions for critical patients suffering from the SARS-CoV-2 infection.

Overall, malnutrition is the primary cause of immunodeficiency worldwide, affecting mostly infants, children, adolescents, and the elderly, increasing their vulnerability to infections (15). Nutritional status might be of particular concern in low- and lower–middle-income countries facing the so-called double burden of malnutrition, i.e. the coexistence of childhood undernutrition (commonly characterized by growth failure and/or micronutrient deficiencies) and overnutrition (overweight/obesity), affecting all levels of the population (countries, communities, families, and individuals) (16). Available evidence from HIC indicates that overweight and obesity as a form of malnutrition is a key predictor for severe COVID-19 at the individual level, and this also holds for young adults with no underlying conditions (17, 18). It is, however, reasonable to assume that also undernourished populations, including survivors of undernourishment, who often develop weaker immune systems, may be at greater risk of severe or fatal COVID-19 illness, since childhood malnutrition is associated with high morbidity and mortality globally, mainly due to infectious diseases (19).

To date, it is not yet clear whether population-level nutritional status plays a role in the population-level vulnerability to COVID-19. The aim of this study was therefore to identify the countries where a high burden of malnutrition coincides with higher rates of fatal disease after SARS-CoV-2 infection, indicating a potential relationship between these burdens.

MATERIALS AND METHODS

Data Sources

Country-level publicly available information for both exposure and outcome variables was used for these analyses. Indicators of countries' burden of malnutrition, defined as child growth failure, iron deficiency, vitamin A deficiency, and high body mass index (BMI), were obtained from the Global Burden of Disease (GBD) Study 2019 (20, 21). Child growth failure, including child underweight, child wasting, and child stunting, is a common indicator of malnutrition among children under 5 years old (22), whereas iron deficiency is the most widespread micronutrient deficiency worldwide affecting all layers of the population (23), followed by vitamin A deficiency, also especially burdensome in children under five (24). High BMI, defined as BMI $\geq$25 kg/m^2 for adults (aged 20+ years) and using thresholds from the International Obesity Task Force standards for children (aged <20 years), was used as a measure of countries' burden of malnutrition due to overnutrition. The GBD global health data exchange (GHDx) tool (25) was used to extract rates of deaths for child growth failure and rates of years lived with disability (YLD) for iron and vitamin A deficiencies and high BMI for both sexes and all ages in the year 2019, for each country. Rates of deaths for child growth failure (per 100,000) provide an understanding to the severity of underweight, wasting, or stunting to fatal outcomes at the country level. Rates of YLD (per 100,000) for iron and vitamin A deficiencies and high BMI are defined as the disability-adjusted prevalence of the conditions and reflect the non-fatal burden of malnutrition, and thus adding further insight to the severity of living with iron and/or vitamin A deficiency and/or high BMI at the country level.

To account for other factors related to both malnutrition and COVID-19, we adjusted our models with YLD rates for COVID-19-related vulnerable health conditions, as identified by WHO (26), as well as the percentage of the population aged 65 and older, population density, and income group. The YLD rate for COVID-19-related vulnerable health conditions was calculated for each country as the sum of YLD rates for chronic respiratory diseases, chronic heart diseases, chronic kidney diseases, chronic liver diseases, chronic neurological conditions, diabetes, problems with the spleen (e.g., sickle cell disorders, sickle cell trait), and cancers, as extracted from the GHDx tool for both sexes and all ages in the year 2019 (25). The percentage of the population aged 65 years and older was extracted from the World Bank (27), as well as the population density (28) and the countries' income classification, available for the year 2019 for 218 countries (29).

Outcome variables were obtained from Our World in Data (30), as reported by the European Centre for Disease Prevention and Control (ECDC), including total cases and total deaths (absolute and per million), available for 209 countries. From these data, we calculated weekly case fatality ratios (CFRs) for each country as the ratio between confirmed deaths and confirmed cases using the average of CFR for each day of that week starting from the day following the first death reported. Accounting for the potential biases introduced by calculating CFR during the early phases of the outbreak and to enable comparison between countries facing different stages of the pandemic, the present analyses included only countries with information on COVID-19 mortality at week 10 following the first death reported of a confirmed case and using CFR at that time point as a measure for countries' vulnerability to fatal COVID-19 (31).

Analyses

The present analyses included 172 countries for which data were available on both countries' burden of malnutrition and COVID-19 CFR. Each measure was divided in tertiles (low, mid, high) to facilitate visualization. Countries falling in the higher tertile of each measure were classified as highly vulnerable to either malnutrition or COVID-19 mortality. Bivariate groupings were established by considering the overlapping of the tertiles for measures of malnutrition and COVID-19. Relationships were visualized in scatterplots with the burden of malnutrition on the X-axis and CFRs on the Y-axis and using colors to depict income groups. Potential non-linear relationships at the global level were described using restricted cubic splines (RCS) with knots placed at the 5th, 35th, 65th, and 95th percentiles (32). The underlying negative binomial models for the RCS analyses used CFR for COVID-19 at week 10 following the first death reported as dependent variable and the measures of a countries' burden of malnutrition (child growth failure, iron deficiency, vitamin A deficiency, and high BMI) as independent variables. All models were adjusted as described above. Spearman's rank correlation coefficients (ρ) were used to describe the correlation between malnutrition measures and the CFR for COVID-19 at the global level and at the income group level (29), and p-values were adjusted for multiple testing according to Sidak. In addition, at the global level and stratified by income group, negative binomial models were used to compute adjusted prevalence ratios (PRs) and their 95% confidence intervals (CIs) for the relationship between a countries' burden of malnutrition and COVID-19 fatality, with countries classified into tertiles depending on their burden of malnutrition, using the lowest tertile (lowest burden) as reference.

Using the countries' position in the scatterplot, a heatmap of the world was created to visualize the countries where malnutrition is likely to be a driver for fatal COVID-19. Malnourished countries vulnerable to fatal COVID-19 were those countries with high rates (third tertile) for at least three malnutrition measures and a high (third tertile) CFR.

Sensitivity analyses included using the calculation of CFRs at week 15 following the first COVID-19 death reported, and the CFRs measured on the most recent day of the pandemic at the time of analyses (04/10/2020). As a measure of COVID-19 severity, the cumulative number of the intensive care unit (ICU) beds needed until week 10 following the first report of a confirmed COVID-19 death, as extracted from the GBD (25), was used in a secondary analysis to test the robustness of the results. A two-sided p-value below 0.05 was considered as statistically significant, and all analyses were carried out using STATA (Release 16/SE; StataCorp LP, College Station, TX, USA).

RESULTS

Countries' Burden of Malnutrition

Supplementary Table 1 shows for each country its vulnerability to fatal COVID-19 due to malnutrition, ordered by income group and CFR. Countries' burden of undernutrition decreased by income group, as measured by child growth failure and iron and vitamin A deficiencies. Median death rates for child growth failure per 100,000 assessed for both sexes and all ages were 45.5 (IQR 34.5, 85.2) in low-income countries (LIC), followed by 12.5 (IQR 5.6, 28.8) in lower–middle- and 1.86 (IQR 0.89, 5.81) in upper–middle-income countries, and were the lowest in HIC with a median rate of 0.62 (IQR 0.25, 1.76). Median YLD rates for iron deficiencies were 679 (IQR 504, 797) in LIC, followed by 435 (IQR 239, 595) in lower–middle- and 191 (IQR 137, 285) in upper–middle-income countries, and were the lowest in HIC with a median rate of 85 (IQR 33, 139). Median YLD rates for vitamin A deficiencies were 61.3 (IQR 40.1 86.6) in LIC, followed by 20.3 (IQR 9.54, 37.4) in lower–middle- and 5.03 (IQR 2.25, 8.71) in upper–middle-income countries, and were the lowest in HIC with a median rate of 5.03 (IQR 0.12, 1.16).

When countries' burden of overnutrition was assessed, the rates increased by income group. Median YLD rates for high BMI were 161 (IQR 132, 210) in LIC, followed by 351 (IQR 253, 509) in lower–middle- and 779 (IQR 622, 987) in upper–middle-income countries, and were the highest in HIC with a rate of 947 (IQR 736, 1,145).

Countries' Burden of COVID-19

Total COVID-19 confirmed cases at week 10 following the first death reported per 100,000 were the highest in HIC (1,516 cases; IQR 467, 3,138), followed by upper–middle- (318; IQR 133, 1,223) and lower–middle-income countries (161; IQR 64, 441), and were the lowest in LIC (72; IQR 29, 288). In addition, total COVID-19 deaths were the highest in HIC (32 deaths; IQR 15, 96), followed by upper–middle- (9.3; IQR 2.6, 37.8) and lower–middle-income countries (3.91; IQR 1.22, 8.05), and were the lowest in LIC (2.4; IQR 0.40, 5.50). Countries' vulnerability to fatal COVID-19, as measured by CFR, was the highest in HIC (3.9; IQR 1.7, 6.9), followed by lower–middle- (2.8; IQR 1.1, 3.9) and upper–middle-income countries (2.6; IQR 1.3, 4.8), and the lowest in LIC (1.8; IQR 0.9, 4.1).

Are Malnourished Countries More Vulnerable to Fatal COVID-19?

The potential relationship between prevalent malnutrition and COVID-19 fatality globally was assessed by each malnutrition indicator individually using models accounting for other recognized determinants of vulnerability to severe or fatal COVID-19 (**Supplementary Figure 1**), in an attempt to isolate the potential association of malnutrition with fatal COVID-19 (26); in particular for HIC only, a moderate positive correlation of CFR for COVID-19 with YLD rates for COVID-19 vulnerable health conditions ($\rho = 0.58$, $p < 0.001$) and with the percentage of individuals aged 65 or above ($\rho = 0.49$, $p = 0.006$) was observed.

Figure 1 shows the bivariate analyses stratified by income group and the non-linear shape of the adjusted association between rate of death for child growth failure and CFR for COVID-19, obtained by RCS analysis. No correlation was seen between the rate of death for child growth failure and CFR for COVID-19 at the global level, nor at the income group level ($\rho < 0.30$; **Table 1**). Countries' vulnerability to fatal COVID-19 seemed to be higher across increasing rates for child growth

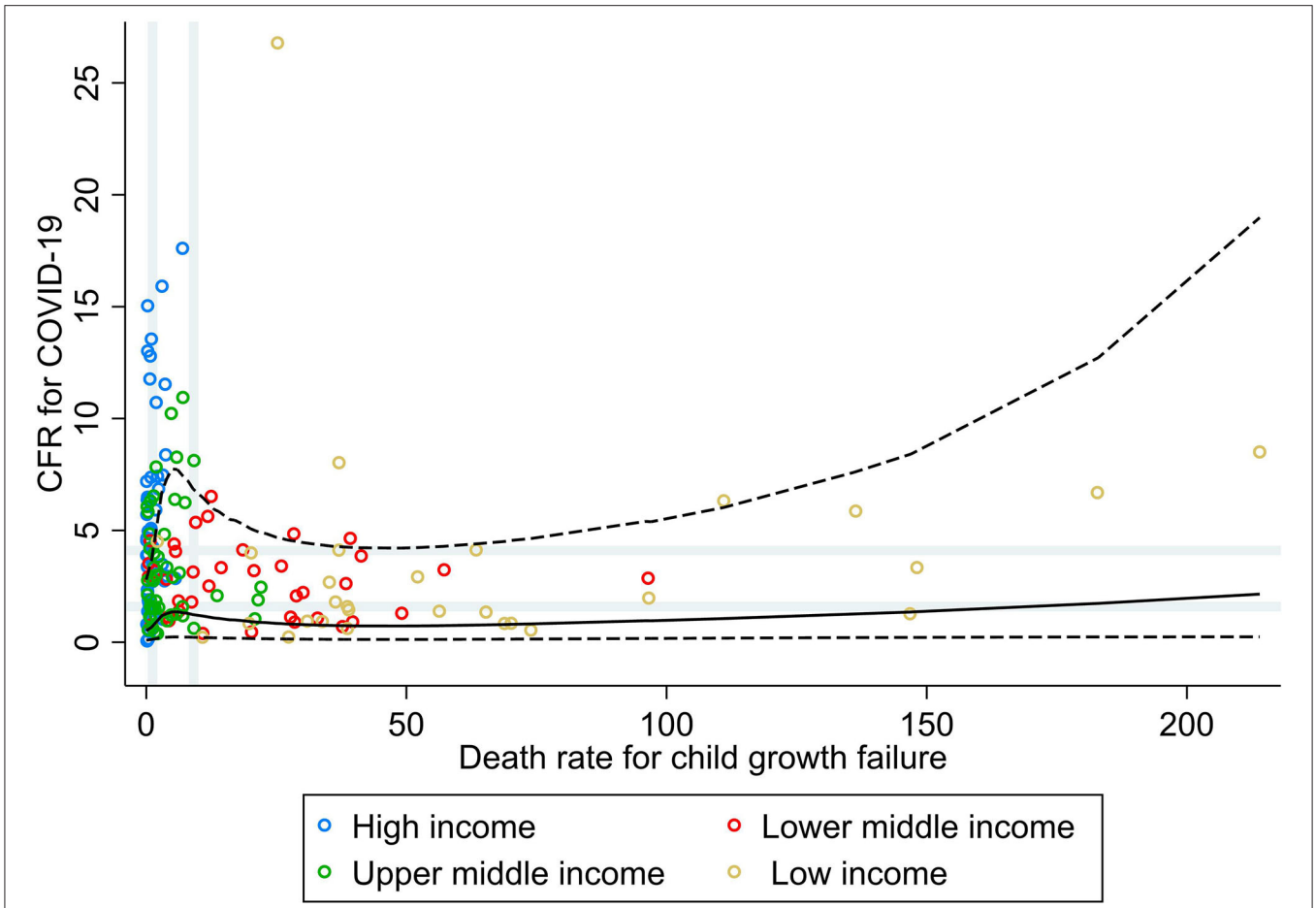


FIGURE 1 | Scatterplot of death rate for child growth failure (underweight, wasting, or stunting) against the average case fatality ratio for COVID-19 at week 10 following the first confirmed death reported, stratified by income group (29). All rates described are crude rates per 100,000 population. Gray vertical and horizontal gridlines indicate tertiles dividing lines for the measures: death rates for child growth failure (at 1.2 and 9.1) and case fatality ratio (CFR) for COVID-19 (at 1.6 and 4.1). Solid black line represents the restricted cubic spline, showing the shape of the adjusted association on a continuous scale with knots at the 5th, 35th, 65th, and 95th percentiles (corresponding to death rate for child growth failure of 0.2, 1.3, 7.4, and 74, respectively), with dashed black lines indicating the 95% confidence intervals.

failure (PR 1.76; 95% CI 0.92, 3.37), mainly driven by the association seen in upper–middle-income countries.

Figure 2 shows that countries with low and median rates of YLD for iron deficiencies did not necessarily have the higher CFR for COVID-19, whereas a slightly higher CFR for COVID-19 was seen for the countries with very high rates of YLD for iron deficiencies. Stratified by income group, a moderate positive correlation was observed in LIC ($\rho = 0.60$, $p = 0.027$; **Table 1**), whereas in the other income groups, a weak negative correlation was observed. In addition, when comparing countries in the highest tertile for the burden of iron deficiency with the lowest, increasing rates were associated with a higher vulnerability to fatal COVID-19 in LIC only (PR 3.61; 95% CI 1.45, 8.99), whereas in the other income groups, this association was inversed and significantly inversed in lower–middle- (PR 0.28; 95% CI 0.13, 0.63) and upper–middle-income countries (PR 0.45; 95% CI 0.25, 0.82).

Results from the RCS analysis and the bivariate analyses of YLD rate for vitamin A deficiency and CFR for COVID-19 (**Figure 3**) show that countries' vulnerability to fatal COVID-19 was slightly higher with increasing rates of vitamin A deficiency, with no further increases in CFR for COVID-19 for countries with very high rates of vitamin A deficiency. No correlations or associations were observed between the rate of YLD for vitamin A deficiencies and CFR for COVID-19 at the global level, nor at the income group level (**Table 1**).

Results from the RCS analysis and the bivariate analyses of YLD rate for high BMI and CFR for COVID-19 (**Figure 4**) show that countries' vulnerability to fatal COVID-19 was slightly higher with increasing rates of high BMI for countries with low and median rates of high BMI, with no further increases in CFR for COVID-19 for countries with high rates for high BMI. No correlations were seen between the rate of YLD for high BMI and CFR for COVID-19 at the global level, nor at the income group level ($\rho < 0.30$; **Table 1**). Increasing rates of high BMI were, however, associated with a higher vulnerability to fatal COVID-19 in LIC (PR 3.31; 95% CI 1.21, 9.05).

TABLE 1 | Spearman's rank correlation and prevalence ratio for countries' burden of malnutrition and fatal COVID-19, measured using CFR at week 10 following the first confirmed death reported, stratified by income group*.

	Death rate for child growth failure	YLD rate		
		Iron deficiency	Vitamin A deficiency	High BMI
Global ($n = 172$)				
Tertile 1, ref	<1.2	<139	<1.5	<441
Tertile 2	1.2–9.1	139–349	1.5–12	441–833
Tertile 3	≥9.1	≥349	≥12	≥833
ρ (p-value)	−0.09 (1.000)	−0.21 (0.186)	−0.16 (0.796)	0.26 (0.018)
PR, T3 vs. T1 (95% CI)	1.76 (0.92; 3.37)	0.87 (0.47; 1.61)	1.58 (0.70; 3.53)	1.77 (0.77; 4.09)
High-income countries ($n = 57$)				
Tertile 1, ref	<0.4	<43	<0.1	<781
Tertile 2	0.4–1.1	43–124	0.1–0.6	781–1,071
Tertile 3	≥1.1	≥124	≥0.6	≥1,071
ρ (p-value)	0.26 (0.815)	−0.15 (1.000)	−0.07 (1.000)	0.27 (0.775)
PR, T3 vs T1 (95% CI)	1.36 (0.76; 2.43)	0.74 (0.32; 1.71)	1.67 (0.82; 3.39)	0.79 (0.32; 1.98)
Upper–middle-income countries ($n = 48$)				
Tertile 1, ref	<1.4	<152	<3.3	<663
Tertile 2	1.4–4.8	152–247	3.3–7.7	663–913
Tertile 3	≥4.8	≥247	≥7.7	≥913
ρ (p-value)	0.03 (1.000)	−0.23 (0.969)	0.04 (1.000)	0.22 (0.985)
PR, T3 vs T1 (95% CI)	2.08 (1.01; 4.29)	0.45 (0.25; 0.82)	0.96 (0.43; 2.12)	0.84 (0.37; 1.84)
Lower–middle-income countries ($n = 38$)				
Tertile 1, ref	<8.7	<294	<12	<271
Tertile 2	8.7–28	294–539	12–31	271–440
Tertile 3	≥28	≥539	≥31	≥440
ρ (p-value)	−0.09 (1.000)	−0.44 (0.139)	−0.14 (1.000)	0.18 (1.000)
PR, T3 vs T1 (95% CI)	1.70 (0.58; 4.99)	0.28 (0.13; 0.63)	0.80 (0.29; 2.17)	0.45 (0.17; 1.16)
Low-income countries ($n = 29$)				
Tertile 1, ref	<36	<526	<51	<151
Tertile 2	36–69	526–712	51–84	151–196
Tertile 3	≥69	≥712	≥84	≥196
ρ (p-value)	0.30 (0.979)	0.60 (0.027)	0.47 (0.333)	−0.13 (1.000)
PR, T3 vs T1 (95% CI)	1.00 (0.32; 3.19)	3.61 (1.45; 8.99)	0.83 (0.25; 2.79)	3.32 (0.66; 16.8)

**According to World Bank 2019 (29). BMI, body mass index; CFR, case fatality ratio; CI, confidence interval; Death rate, rate of death per 100,000 for all ages; PR, prevalence ratio for COVID-19 fatality in the highest tertile of a countries' burden of malnutrition relative to the lowest tertile calculated in a negative binomial model; ρ, Spearman's rank correlation coefficient with p-value adjusted for multiple testing according to Sidak; YLD rate, rate of years lived with disability per 100,000 for all ages.*

Countries Where Malnutrition Is a Potential Driver for Fatal COVID-19

The heatmap for malnutrition shows that malnutrition could be considered as a driver for fatal COVID-19 in 13 countries (7.5%) (**Figure 5**). No country ranked consistently with high burden on all four malnutrition measures and fatal COVID-19. Countries with high burden of three malnutrition measures and fatal COVID-19 were: Angola, Burkina Faso, Chad, Liberia, Mali, Mauritania, Niger, Sierra Leone, Sudan, Tanzania, and Yemen for high burden of child growth failure and iron and vitamin A deficiencies; Guyana for high burden of child growth failure, iron deficiency, and high BMI; and Fiji for high burden of iron and vitamin A deficiencies and high BMI. When considering only one measure of malnutrition, child growth failure could be considered as a driver for fatal COVID-19 in 15 countries (8.7%), iron deficiencies in 14 countries (8.1%), vitamin A deficiencies in 14 countries (8.1%), and high BMI in 29 countries (16.9%).

Sensitivity Analyses

Using the CFR at week 15 or the most updated CFR as outcome variable did not alter conclusions of the RCS analyses, the Spearman's rank correlations, and the PRs (results not shown). Countries that were ranked consistently with high burden on at least two malnutrition measures and high burden on fatal COVID-19, as measured by CFR at weeks 10 and 15, and the most updated CFR were: Angola, Burkina Faso, Chad, Guyana, Liberia, Mali, Niger, Sudan, Tanzania, and Yemen. When using CFR at week 15, only Gambia was also additionally included, but Fiji, Mauritania, and Sierra Leone were excluded from the list (results not shown). When using the most updated CFR, the

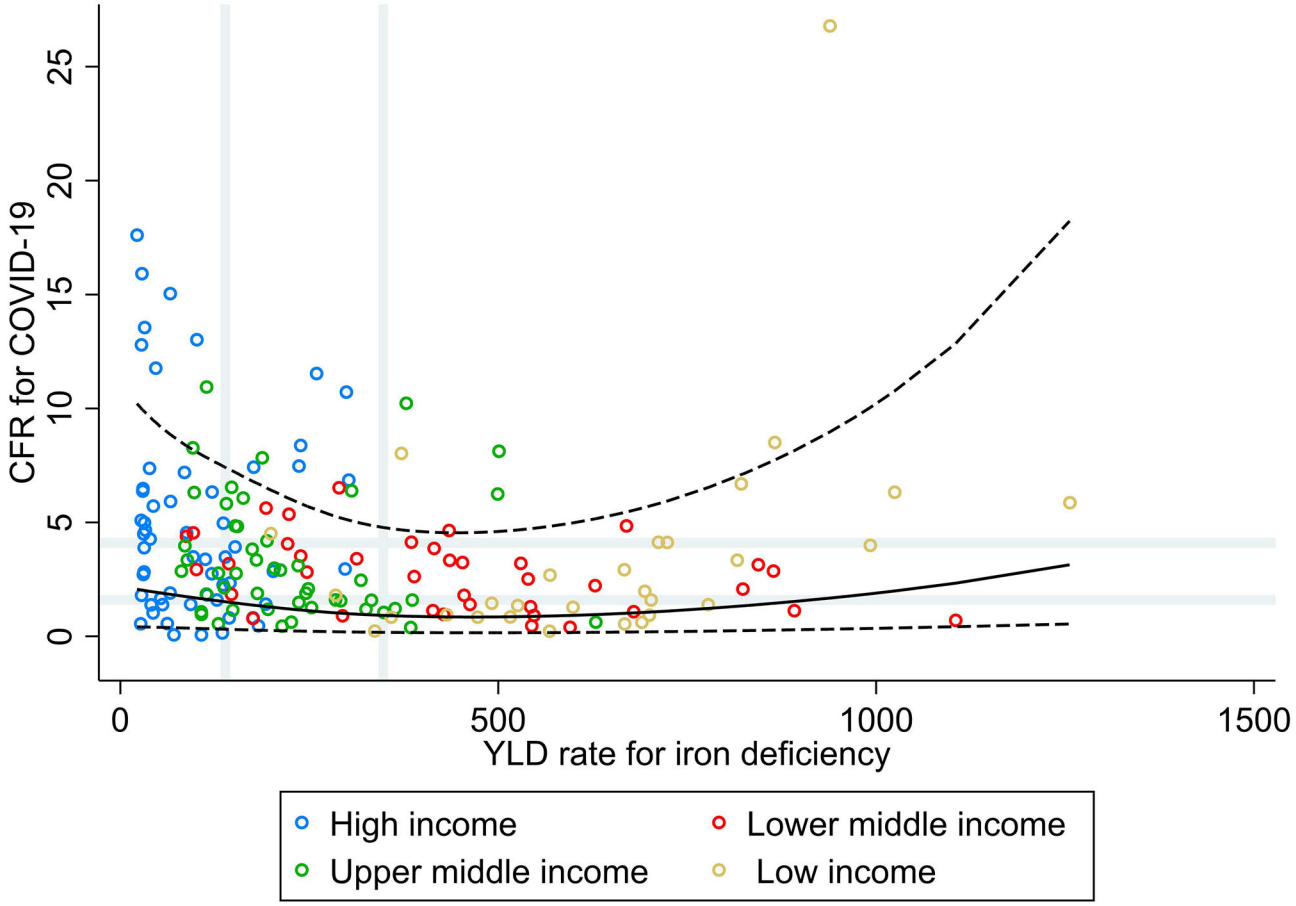


FIGURE 2 | Scatterplot of years lived with disability rate for iron deficiencies against the average case fatality ratio for COVID-19 at week 10 following the first confirmed death reported, stratified by income group (29). All rates described are crude rates per 100,000 population. Gray vertical and horizontal gridlines indicate tertiles dividing lines for the measures: years lived with disability (YLD) rates for iron deficiency (at 139 and 348) and case fatality ratio (CFR) for COVID-19 (at 1.6 and 4.1). Solid black line represents the restricted cubic spline, showing the shape of the adjusted association on a continuous scale with knots at the 5th, 35th, 65th, and 95th percentiles (corresponding to YLD rate for iron deficiency of 30, 144, 325, and 844, respectively), with black dashed lines indicating the 95% confidence intervals.

list of countries additionally included Bolivia, Gambia, Malawi, Somalia, Togo, and Zimbabwe.

Furthermore, when considering COVID-19 severity as measured by the daily needs of new ICU beds, countries that have the highest burden of high BMI are experiencing markedly worse COVID-19 severity rates, whereas countries with a high burden of undernutrition did not necessarily have a higher burden of severe COVID-19 cases, as likely related to the lower health care capacity in those countries (**Supplementary Figure 2**).

DISCUSSION

Using data on the country-specific burden of malnutrition and fatal COVID-19, the present study identified 10 countries where malnutrition might have played a key role in increasing the country's vulnerability to fatal COVID-19 as suggested by the coexistence of a high burden of malnutrition and elevated mortality among COVID-19 cases. The most affected countries are LIC in sub-Saharan Africa (SSA), such as Angola, Liberia, Tanzania, and particularly among those in the Sahel strip, such as Burkina Faso, Chad, Mali, Niger, and Sudan, as well as Yemen in the Middle East and North African region. Guyana in the Latin America and Caribbean region and Fiji in the East Asia and Pacific region were the only countries vulnerable to fatal COVID-19 through malnutrition, among the list of upper–middle income areas.

These countries that have the highest burden of undernutrition, and particularly high YLD rates for iron deficiency, are also experiencing markedly higher COVID-19 fatality rates. Iron deficiency is generally the consequence of inadequate dietary intake of iron (33), and in particular, an insufficient consumption of animal protein containing high amounts of bioavailable heme iron. A deficiency in iron intake is known to be a strong determinant of anemia, particularly in children and women of reproductive age (34). Although the relationship of anemia with an increased susceptibility to infectious diseases remains controversial and may depend on

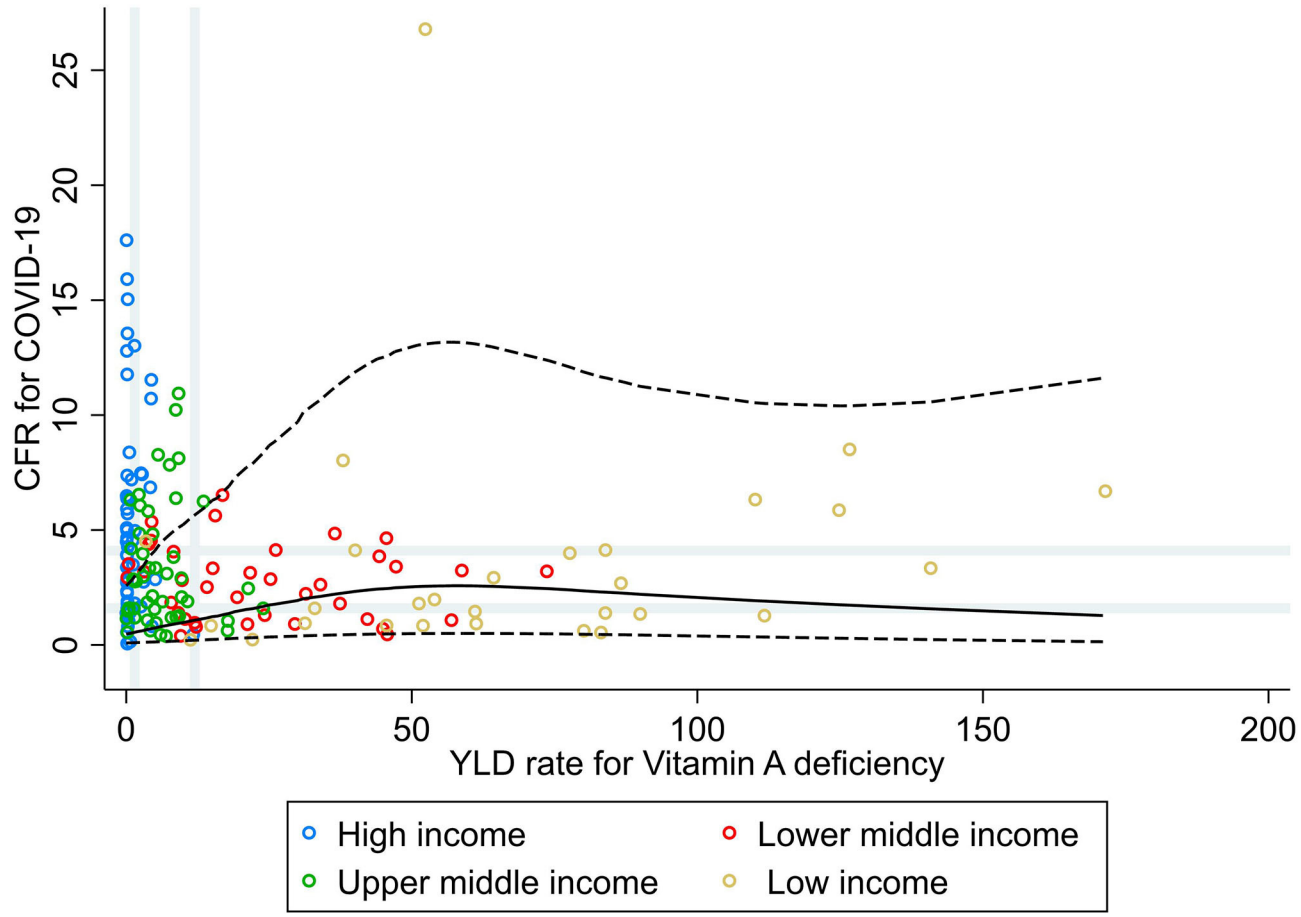


FIGURE 3 | Scatterplot of years lived with disability rate for vitamin A deficiencies against the average case fatality ratio for COVID-19 at week 10 following the first confirmed death reported, stratified by income group (27). All rates described are crude rates per 100,000 population. Gray vertical and horizontal gridlines indicate tertiles dividing lines for the measures: years lived with disability (YLD) rates for vitamin A deficiency (at 1.5 and 12) and case fatality ratio (CFR) for COVID-19 (at 1.6 and 4.1). Solid black line represents the restricted cubic spline, showing the shape of the adjusted association on a continuous scale with knots at the 5th, 35th, 65th, and 95th percentiles (corresponding YLD rate for vitamin A deficiency of 0.1, 2.3, 10.7, and 84, respectively), with black dashed lines indicating the 95% confidence intervals.

other immune factors of the community (35), consideration to food and nutrition security are especially needed for those countries lacking access to optimal diets (36). Indeed, albeit not a clear relationship between the burden of vitamin A deficiency and COVID-19 mortality was observed in this study, it is likely that this frequent condition adds to the influence of the burden of undernutrition on COVID-19 fatality, especially in SSA where the prevalence of vitamin A deficiency is the highest (24). Vitamin A deficiency is associated with an increased risk of mortality from measles and diarrhea in children, and although the numbers have decreased globally in the last two decades (37), it has not been the case for the SSA region where this deficiency accounts to 2% of child deaths (24).

Furthermore, considering a broader concept of malnutrition, other dietary risks should be taken into account when looking at the overall impact of suboptimal diets in the vulnerability to COVID-19. While chronic deficiency of essential vitamins and minerals (micronutrients) driven by hunger is a major concern, particularly in SSA (38), global nutrition transition and the spreading prevalence of suboptimal diets high in sodium and low in minimally processed plant-based foods, such as nuts and seeds, rich in omega-3 fatty acids have been consistently identified as major risk factors of the burden of chronic non-communicable diseases (NCDs) (39). This transition towards unhealthier diets is evidenced, for example, in the markedly different food intakes observed in southern SSA compared with other SSA regions in terms of lower consumption of fruits and legumes, increased intake of red meat and sugary drinks, and elevated ratio between omega-6/omega-3 fatty acids (39), similar to the levels observed in Western diets. An increased omega-6/omega-3 ratio has been associated with an elevated risk of cardiovascular, inflammatory, and autoimmune diseases (40).

Factors, such as international exposure, size of urban population, and strength of the health system, were initially identified as determinants of COVID-19 cases in Africa (41). In SSA countries, however, preventive measures, such

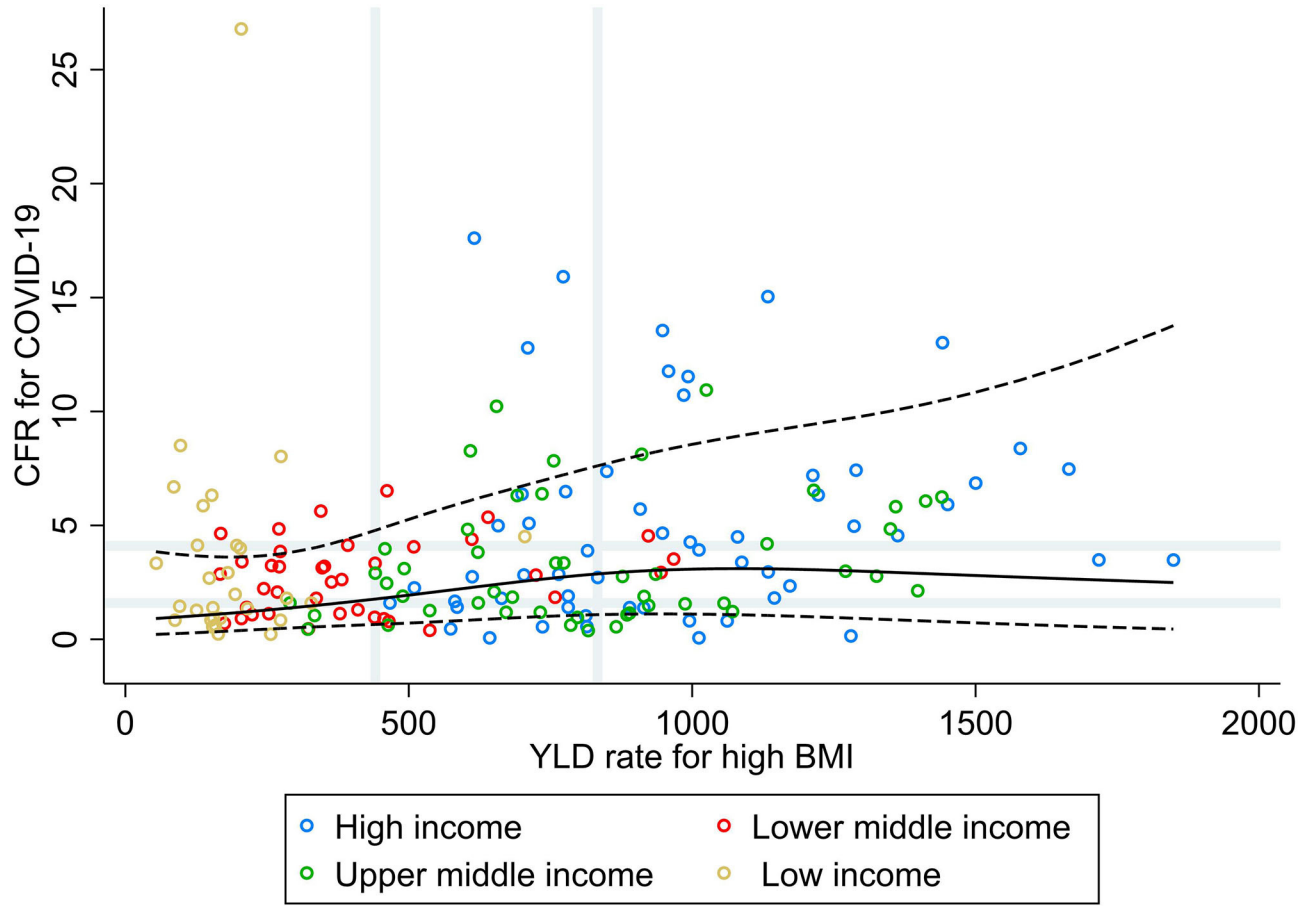


FIGURE 4 | Scatterplot of years lived with disability rate for high BMI against the average case fatality ratio for COVID-19 at week 10 following the first confirmed death reported, stratified by income group (29). All rates described are crude rates per 100,000 population. Gray vertical and horizontal gridlines indicate tertiles dividing lines for the measures: years lived with disability (YLD) rates for high BMI (at 441 and 833) and case fatality ratio for COVID-19 (at 1.6 and 4.1). Solid black line represents the restricted cubic spline, showing the shape of the adjusted association on a continuous scale with knots at the 5th, 35th, 65th, and 95th percentiles (corresponding to YLD rate for high BMI of 148, 461, 814, and 1,412, respectively), with black dashed lines indicating the 95% confidence intervals.

as self-isolation at home, particularly made COVID-19 to exacerbate food insecurity as a result of lower purchase power due to unemployment and pressure on the planting season due to a lack of inputs and labor (36) resulting in lower food production, as reported in Nigeria (42) and Bangladesh (43), and likely mirroring the situation across most parts of the African continent (44). In particular, the Sahel strip, one of the world's areas most affected by hunger before COVID-19, is now facing exceptional high levels of food insecurity with the number of food insecure people doubling or even tripling during the pandemic (45, 46). Early estimates also suggested that low- and middle-income countries should anticipate indirect large increases in childhood undernutrition and in maternal and child deaths resulting from the reduced access to food and the widespread disruption of the health systems due to COVID-19 (47, 48). Although the favorable clinical course of COVID-19 in children (49), the indirect effects of COVID-19 are likely to have a long-lasting impact on children in LIC, because of increasing poverty levels, disrupted schooling and lack of access to school feeding schemes, reduced access to health facilities, and interruptions in vaccination and other child health programs (50). This underlines the fact that malnutrition is the outcome of a disturbed interplay between the individual and household decision-making, the agri-food, environmental, and health systems at the community and/or national level (51), and has complicated relationships with infectious diseases (15), including COVID-19 (45–48). In future investigations during the coming months and years, it would be important to measure excess mortality for the countries' burden to fatal COVID-19 directly and indirectly, to reveal the real impact of COVID-19 on population health and inform public health measures, such as efforts to minimize disruption to health care and maximize food security, in particular to improve population maternal and child health (MCH).

Findings from the present study show that LIC and lower–middle-income countries are mostly dispersed above the highest tertile line of undernutrition and below the lowest tertile line of overnutrition, and that countries with higher burden of iron deficiencies presented higher CFR, most clearly for LIC.

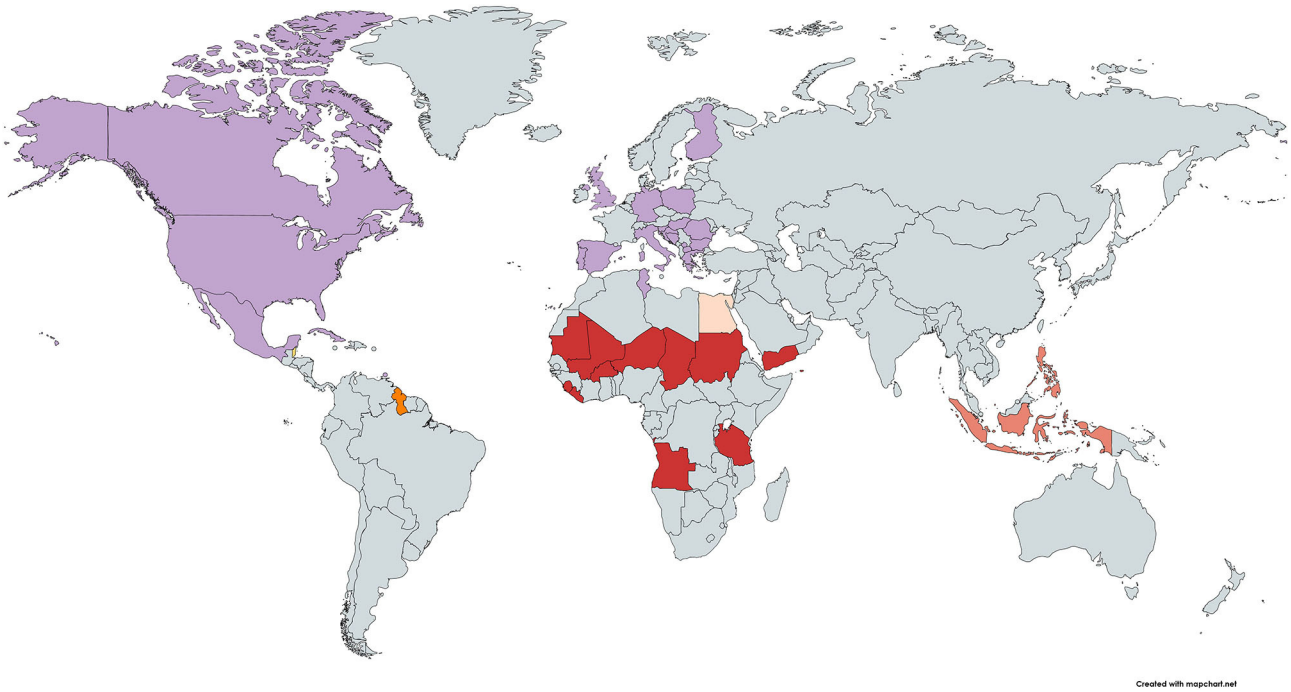


FIGURE 5 | Heatmap of countries where population-level malnutrition appears to be a driver of an increased risk for fatal COVID-19. High (third tertile) CFR at week 10 and ■ high rates for child growth failure, iron deficiency, and vitamin A deficiency, ■ high rates for child growth failure, iron deficiency, and high BMI; ■ high rates for child growth failure and vitamin A deficiency; ■ high rates for child growth failure only; ■ high rates for iron deficiency only; and ■ high rates for high BMI only. Note: Countries not on the map with high CFR and high rates for iron deficiencies, vitamin A deficiencies, and BMI were: Fiji; and high rates for high BMI only were: Antigua and Barbuda, Barbados, Bermuda, Northern Mariana Islands, and Virgin Islands (US). CFR, case fatality ratio; BMI, body mass index.

Contrastingly, our results indicate that HIC are mostly burdened by elevated rates of high BMI, whereas undernutrition, measured as either child growth failure or iron and vitamin A deficiencies, is much less prevalent in comparison with low- and middle-income countries. None of the measures, however, seem to be clearly related with greater vulnerability to COVID-19, neither at the global level nor at the income group level. At the individual level, however, evidence shows that obesity (high BMI) has been associated with severe COVID-19 (4, 5). In addition, health disparities in diet and obesity are believed to disproportionately affect the burden of infections, hospitalizations, and deaths from COVID-19 after infection, as seen, for example, in the U.S. where Native and African Americans have a four-to-five times higher hospitalization rates than White Americans (52). As mentioned, other factors, such as aging and health loss due to chronic conditions, also have a substantial impact on COVID-19 severity (26). A previous published modeling study estimated that one in five individuals worldwide has an underlying chronic cause of disease that could predispose them to severe COVID-19 if infected, with a higher share of the population at increased risk for Europe, Central Asia, and North America (53). Yet, a number of other important factors related to the management of the pandemic will also contribute to the extent of fatal COVID-19, including, but not limited to: (sub)national responses to the outbreak through public health non-pharmaceutical practices of handwashing, physical distancing, and the use of facemasks; health care access and capacities; and population density. Thus, a complete risk assessment of a countries' vulnerability to COVID-19 should take into account all elements that play a role in this pandemic.

Undernutrition early in life is a risk factor for overnutrition in adulthood, hence rising concerns for countries with a high prevalence of early life undernutrition being at higher risk for being affected by multiple burdens of malnutrition (54, 55). The present paper addresses double and triple burdens of malnutrition by including measures of malnutrition both for MCH and for NCDs. Malnutrition for MCH has been traditionally considered as insufficient caloric or nutrient intake, leading to child growth failure (i.e., insufficient physical growth, rapid weight loss, or failure to gain weight) and exacerbations of iron deficiency anemia, whereas malnutrition for NCDs as an excess consumption of calories, leading to high BMI. Countries' burden of malnutrition, as well as the burden of other conditions related to worse COVID-19 prognosis, was quantified using estimates from the GBD 2019 (20, 21), therefore using the most updated data, almost concurrent to the ongoing pandemic. Because of our aim to identify countries at high risk for fatal COVID-19 due to malnutrition, we opted for crude rates to more fairly represent countries' demographics, also considering that age and sex distributions influence the risk for fatal COVID-19, which is higher among older populations (53). Previous analysis have also acknowledged, by using crude rates of either

prevalence (53) or YLD (56) for COVID-19 vulnerable health conditions, the importance of identifying high-risk countries and high-risk groups within countries to mitigate the COVID-19 impact and inform public health strategies to shield those at the highest risk.

Countries' burden of COVID-19 fatality was quantified using CFR for COVID-19 at week 10 following the first confirmed death reported as a proxy for severity of the disease outcome, instead of infection fatality ratio (IFR), the more preferred measure to assess the severity of epidemics (57). As IFR estimates the proportion of deaths among all infected individuals, seroprevalence studies representative for the general population are needed to capture the undiagnosed asymptomatic and mildly symptomatic infections. The few available seroprevalence studies, mainly from HIC and China, point to a median global IFR of 0.27% with large variability across locations depending on population age-structure, and the case-mix of infected and deceased patients, as well as other local factors, such as population burden of underlying comorbidities, public health measures, and health care (58–60). There are indeed an elevated number of undetected cases for COVID-19 making CFR overestimating the true disease fatality. In any case, measures for disease fatality should be interpreted with caution because of its dependence on a particular context, time, and place related to the health care and testing capacity, the reporting practices of COVID-19 cases and deaths, the number of confirmed cases (for CFR), the number of infected individuals detected (for IFR), the number of confirmed deaths, the standard care response for suspected and confirmed COVID-19 patients, the COVID-19 patients' ability to recover, etc. In particular, with countries facing different stages of the pandemic at a particular time (61) and having different levels of testing efforts (30, 62), comparability of the fatality measures between countries might be limited. The present analysis used for each country the CFR measured at week 10 following the first death reported of a confirmed case in that country, as a way to avert the effect of the different stages of the pandemic on the calculation of CFR (31). Furthermore, sensitivity analyses also suggest robust findings, as using for each country CFR at week 15 and the most updated shows similar results. In secondary analyses, we used the cumulative number of ICU beds use from the first COVID-19 death reported up until 10 weeks later, as a proxy for COVID-19 severity at the country level. This measure is, however, not available for all countries (81%) and provides only a partial picture of the relationship between malnutrition and COVID-19's burdens. Countries with the greatest burden of high BMI are reporting markedly worse COVID-19 severity rates, thus confirming the relationship observed for CFR. However, countries where undernutrition is highly prevalent did not appear to be related with the higher use of ICU beds, likely reflecting a limited health system's capacity in those countries. The use of CFR is commonly regarded as a poor measure of mortality risk of COVID-19 throughout the early and middle stages of an ongoing outbreak, as it might under- or overestimate the true ratio between confirmed deaths and confirmed cases. However, due to the inconsistent reporting of other indicators, CFR represents the most comprehensive measures at the present time of the pandemic. With the accumulation of information about cases at the end of the pandemic, the estimate will be close to those finally observed once the epidemic recedes (63). Repeating the analyses at the end of the pandemic might further enlighten the role played by malnutrition on fatal COVID-19, and depending on the countries' reporting practices and availability of seroprevalence studies in the general population, the IFR could be used as a more accurate measure of disease fatality.

In conclusion, population-level malnutrition appears to be a driver of an increased risk for fatal COVID-19 in areas with marked burden of undernutrition as those in the Sahel strip. Using routine estimates of the risk factor burden allowed the rapid assessment for countries' vulnerability to COVID-19 due to malnutrition and identifying those countries where malnutrition is a virulence factor for severe COVID-19. Our findings are relevant to country-level, global interpretation, and cannot be extrapolated to high-risk individuals or populations within particular countries because of ecological fallacy. To mitigate the impact of severe COVID-19, preventive public health strategies of the response plans to COVID-19 should also pay attention to food, nutrition, and integrate sustainable food systems interventions to promote the production, distribution, and consumption of healthy diets, along with social protection, with the aim to improve the nutritional status of the population, especially those at higher risk, during and at the end of the pandemic (64).

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: http://ghdx.healthdata.org/gbd-results-tool; https://ourworldindata.org/coronavirus; https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups; https://population.un.org/wpp/Download/Standard/Population/.

AUTHOR CONTRIBUTIONS

JP and EM conceptualized and designed the study, identified the relevant data sources, retrieved the data, wrote the manuscript, and revised, read, and approved the submitted version. EM performed the statistical analyses. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.619850/full#supplementary-material

REFERENCES

1. WHO. *Water, Sanitation, Hygiene, and Waste Management for the COVID-19, Technical Brief*. Available online at: WHO/2019-nCoV/IPC_WASH/2020.3 (2020).
2. Ebrahim SH, Ahmed QA, Gozzer E, Schlagenhauf P, Memish ZA. Covid-19 and community mitigation strategies in a pandemic. *BMJ*. (2020) 368:m1066. doi: 10.1136/bmj.m1066
3. Schünemann HJ, Akl EA, Chou R, Chu DK, Loeb M, Lotfi T, et al. Use of facemasks during the COVID-19 pandemic. *Lancet Respir Med*. (2020) 8:954–5. doi: 10.1016/S2213-2600(20)30352-0
4. Matsushita K, Ding N, Kou M, Hu X, Chen M, Gao Y, et al. The relationship of COVID-19 severity with cardiovascular disease and its traditional risk factors: a systematic review and meta-analysis. *Global Heart*. (2020) 15:64. doi: 10.5334/gh.814
5. Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, et al. Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. *BMJ*. (2020) 369:m1985. doi: 10.1136/bmj.m1985
6. Banerjee A, Pasea L, Harris S, Gonzalez-Izquierdo A, Torralbo A, Shallcross L, et al. Estimating excess 1-year mortality associated with the COVID-19 pandemic according to underlying conditions and age: a population-based cohort study. *Lancet*. (2020) 395:1715–25. doi: 10.1016/S0140-6736(20)30854-0
7. Dent E, Visvanathan R, Piantadosi C, Chapman I. Nutritional screening tools as predictors of mortality, functional decline, and move to higher level care in older people: a systematic review. *J Nutr Gerontol Geriatr*. (2012) 31:97–145. doi: 10.1080/21551197.2012.678214
8. Burgos R, Joaquín C, Blay C, Vaqué C. Disease-related malnutrition in hospitalized chronic patients with complex needs. *Clin Nutr*. (2020) 39:1447–53. doi: 10.1016/j.clnu.2019.06.006
9. Chapman IM. Nutritional disorders in the elderly. *Med Clin N Am*. (2006) 90:887–907. doi: 10.1016/j.mcna.2006.05.010
10. Briguglio M, Pregliasco FE, Lombardi G, Perazzo P, Banfi G. The Malnutritional status of the host as a virulence factor for new coronavirus SARS-CoV-2. *Front Med*. (2020) 7:146. doi: 10.3389/fmed.2020.00146
11. Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients*. (2020) 12:1181. doi: 10.3390/nu12041181
12. Barazzoni R, Bischoff SC, Breda J, Wickramasinghe K, Krznaric Z, Nitzan D, et al. ESPEN expert statements and practical guidance for nutritional management of individuals with SARS-CoV-2 infection. *Clin Nutr*. (2020) 39:1631–8. doi: 10.1016/j.clnu.2020.03.022
13. Stachowska E, Folwarski M, Jamioł-Milc D, Maciejewska D, Skonieczna-Zydecka K. Nutritional support in coronavirus 2019 disease. *Medicina*. (2020) 56:289. doi: 10.3390/medicina56060289
14. ASPEN Clinical Practice Committee. *Resources for Clinicians Caring for Patients With Coronavirus* (2020).
15. Katona P, Katona-Apte J. The interaction between nutrition and infection. *Clin Infect Dis*. (2008) 46:1582–8. doi: 10.1086/587658
16. Popkin BM, Corvalan C, Grummer-Strawn LM. Dynamics of the double burden of malnutrition and the changing nutrition reality. *Lancet*. (2020) 395:65–74. doi: 10.1016/S0140-6736(19)32497-3
17. Kassir R. Risk of COVID-19 for patients with obesity. *Obesity Rev*. (2020) 21:e13034. doi: 10.1111/obr.13034
18. Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell LF, Chernyak Y, et al. Factors associated with hospitalization and critical illness among 4,103 patients with COVID-19 disease in New York City. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.04.08.20057794
19. Walson JL, Berkley JA. The impact of malnutrition on childhood infections. *Curr Opin Infect Dis*. (2018) 31:231–6. doi: 10.1097/QCO.0000000000000448
20. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. (2020) 396:1204–22. doi: 10.1016/S0140-6736(20)30925-9
21. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. (2020) 396:1223–49. doi: 10.1016/S0140-6736(20)30752-2
22. WHO & UNICEF. *WHO Child Growth Standards and the Identification of Severe Acute Malnutrition in Infants and Children: A Joint Statement* (2009).
23. Stoltzfus RJ. Defining iron-deficiency anemia in public health terms: a time for reflection. *J Nutr*. (2001) 131:565–7S. doi: 10.1093/jn/131.2.565S
24. Stevens GA, Bennett JE, Hennocq Q, Lu Y, De-Regil LM, Rogers L, et al. Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. *Lancet Global Health*. (2015) 3:e528–36. doi: 10.1016/S2214-109X(15)00039-X
25. Institute for Health Metrics and Evalution. *GBD Results Tool*. University of Washington, Seattle, WA (2018).
26. WHO. *COVID-19 and NCDs, Information Note* (2020).
27. The World Bank. *World Development Indicators, Based on Age/Sex Distributions of United Nations Population Division's World Population Prospects: 2017 Revision*. The World Bank Group (2017).
28. The World Bank. *Population Density (people per sq. km of land area)*. The World Bank Group (2019).
29. The World Bank. *Country Classification: World Bank Country and Lending Groups*. The World Bank Group (2019).
30. Roser M, Ritchie H, Ortiz-Ospina EHJ. *Coronavirus Pandemic (COVID-19)*. Available online at: OurWorldInData.org (2020).
31. Ghayda RA, Lee KH, Han YJ, Ryu S, Hong SH, Yoon S, et al. Estimation of global case fatality rate of coronavirus disease 2019 (COVID-19) using meta-analyses: comparison between calendar date and days since the outbreak of the first confirmed case. *Int J Infect Dis*. (2020) 100:302–8. doi: 10.1016/j.ijid.2020.08.065
32. Stone CJ, Koo C-Y. Additive splines in statistics. *Proc Stat Comput Sect ASA*. (1985) 27:45–8.
33. Mwangi MN, Phiri KS, Abkari A, Gbané M, Bourdet-Sicard R, Braesco VA, et al. Iron for Africa-Report of an expert workshop. *Nutrients*. (2017) 9:576. doi: 10.3390/nu9060576
34. Petry N, Olofin I, Hurrell RF, Boy E, Wirth JP, Moursi M, et al. The Proportion of anemia associated with iron deficiency in low, medium, and high human development index countries: a systematic analysis of national surveys. *Nutrients*. (2016) 8:693. doi: 10.3390/nu8110693
35. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. *J Nutr*. (2001) 131:616–35S. doi: 10.1093/jn/131.2.616S
36. Wageningen University & Research. *The Effects of COVID-19 on Food Systems: Rapid Assessments* (2020).
37. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet*. (2015) 385:430–40. doi: 10.1016/S0140-6736(14)61698-6
38. Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE. The global hidden hunger indices and maps: an advocacy tool for action. *PloS ONE*. (2013) 8:e67860. doi: 10.1371/journal.pone.0067860
39. GBD 2017 Diet Collaborators. Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. (2019) 393:1958–72. doi: 10.1016/S0140-6736(19)30041-8
40. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother*. (2002) 56:365–79. doi: 10.1016/S0753-3322(02)00253-6
41. Africa Center for Strategic Studies. *Mapping Risk Factors for the Spread of COVID-19 in Africa* (2020).
42. Kalu B. COVID-19 in Nigeria: a disease of hunger. *Lancet Respir Med*. (2020) 8:556–7. doi: 10.1016/S2213-2600(20)30220-4
43. Rashid SF, Theobald S, Ozano K. Towards a socially just model: balancing hunger and response to the COVID-19 pandemic in Bangladesh. *BMJ Glob Health*. (2020) 5:e002715. doi: 10.1136/bmjgh-2020-002715
44. Unicef. *West and Central Africa: More than 15 Million Cases of Acute Malnutrition Expected in 2020, Dakar* (2020).
45. OCHA United Nations Office for the Coordination of Humanitarian Affairs. *Humanitarian Needs Rise in the Sahel Amid COVID-19. OCHA United Nations Office for the Coordination of Humanitarian Affairs* (2020).

46. Refugees International. *Issue Brief Mounting Hunger in the Sahel: The Unintended Impact of COVID-19 Prevention, Refugees International*. Washington, DC (2020).
47. Roberton T, Carter ED, Chou VB, Stegmuller AR, Jackson BD, Tam Y, et al. Early estimates of the indirect effects of the COVID-19 pandemic on maternal and child mortality in low-income and middle-income countries: a modelling study. *Lancet Global Health.* (2020) 8:e901–8. doi: 10.1016/S2214-109X(20)30229-1
48. Headey D, Heidkamp R, Osendarp S, Ruel M, Scott N, Black R, et al. Impacts of COVID-19 on childhood malnutrition and nutrition-related mortality. *Lancet.* (2020) 396:519–21. doi: 10.1016/S0140-6736(20)31647-0
49. de Souza TH, Nadal JA, Nogueira RJN, Pereira RM, Brandão MB. Clinical manifestations of children with COVID-19: a systematic review. *Pediatr Pulmonol.* (2020) 55:1892–9. doi: 10.1002/ppul.24885
50. Zar HJ, Dawa J, Fischer GB, Castro-Rodriguez JA. Challenges of COVID-19 in children in low- and middle-income countries. *Paediatr Respir Rev*. (2020) 35:70–4. doi: 10.1016/j.prrv.2020.06.016
51. Gillespie S, van den Bold M. Agriculture, food systems, and nutrition: meeting the challenge. *Global Challenges.* (2017) 1:1600002. doi: 10.1002/gch2.201600002
52. Belanger MJ, Hill MA, Angelidi AM, Dalamaga M, Sowers JR, Mantzoros CS. Covid-19 and disparities in nutrition and obesity. *N Engl J Med*. (2020) 383:e69. doi: 10.1056/NEJMp2021264
53. Clark A, Jit M, Warren-Gash C, Guthrie B, Wang HH, Mercer SW, et al. How many are at increased risk of severe COVID-19 disease? Rapid global, regional and national estimates for 2020. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.04.18.20064774
54. Shrimpton R, Rokx C. *The Double Burden of Malnutrition: A Review of Global Evidence*. World Bank's Human Development Network, Washington, DC (2012).
55. Romieu I, Dossus L, Barquera S, Blottière HM, Franks PW, Gunter M, et al. Energy balance and obesity: what are the main drivers? *Cancer Causes Control.* (2017) 28:247–58. doi: 10.1007/s10552-017-0869-z
56. Wyper GMA, Assunção R, Cuschieri S, Devleeschauwer B, Fletcher E, Haagsma JA, et al. Population vulnerability to COVID-19 in Europe: a burden of disease analysis. *Arch Public Health.* (2020) 78:47. doi: 10.1186/s13690-020-00437-8
57. WHO. *Estimating mortality from COVID-19, Scientific Brief* (2020).
58. Meyerowitz-Katz G, Merone L. A systematic review and meta-analysis of published research data on COVID-19 infection-fatality rates. *Int J Infect Dis*. (2020) 101:38–48. doi: 10.1016/j.ijid.2020.09.1464
59. Levin AT, Hanage WP, Owusu-Boaitey N, Cochran KB, Walsh SP, Meyerowitz-Katz G. Assessing the age specificity of infection fatality rates for COVID-19: a systematic review, meta-analysis, and public policy implications. *Eur. J. Epidemiol.* (2020) 35:1123–38. doi: 10.3386/w27597
60. Ioannidis J. The infection fatality rate of COVID-19 inferred from seroprevalence data. *medRxiv [Preprint]*. (2020) doi: 10.1101/2020.05.13.20101253
61. European Centre for Disease Prevention and Control (ECDC). *COVID-19 Situation Update Worldwide, as of 13 October 2020*. An Agency of the European Union (2020).
62. Our World in Data (owid). *Coronavirus (COVID-19) Testing* (2020).
63. Ghani AC, Donnelly CA, Cox DR, Griffin JT, Fraser C, Lam TH, et al. Methods for estimating the case fatality ratio for a novel, emerging infectious disease. *Am J Epidemiol.* (2005) 162:479–86. doi: 10.1093/aje/kwi230
64. Development Initiatives. *Global Nutrition Report: Action on Equity to End Malnutrition*. Development Initiatives, Bristol (2020).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Mertens and Peñalvo. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

REVIEW
published: 04 March 2021
doi: 10.3389/fnut.2021.626432

The Impact of Lockdown During the COVID-19 Outbreak on Dietary Habits in Various Population Groups: A Scoping Review

Grace Bennett[1], *Elysia Young*[1], *Isabel Butler*[1] *and Shelly Coe*[1,2]*

[1] *Centre for Nutrition and Health, Department of Sport, Health Sciences and Social Work, Faculty of Health & Life Sciences, Oxford Brookes University, Oxford, United Kingdom,* [2] *Centre for Movement Occupational and Rehabilitation Sciences, Department of Sport, Health Sciences and Social Work, Faculty of Health & Life Sciences, Oxford Brookes University, Oxford, United Kingdom*

Background: Since the beginning of the COVID-19 pandemic, access to fresh food has been restricted, and people are spending more time inside and have limited their physical activity. However, more time at home may have resulted in some positive habits including an increase in cooking. The aim of this review was to assess dietary changes during the first lockdown. Themes and patterns were considered and associations with other lifestyle factors were assessed.

Methods: Between June and July 2020, the PubMed, Google Scholar, and Science Direct databases were searched, and results were screened for eligibility based on title, abstract, and full text. The inclusion criteria of this search included: papers published (or in pre-print) in the year 2020; studies that investigated the impact of COVID-19 lockdown on diet; papers published in English. Exclusion criteria were as follows: papers examining dietary changes in those following a structured diet based on diagnosed conditions or dietetic advice; literature, systematic, or narrative studies reviewing previous research. Researchers agreed on the study characteristics for extraction from final papers.

Results: Four thousand three hundred and twenty-two studies were originally considered with 23 final full-text papers included. Four themes were identified: dietary patterns, dietary habits (favorable), dietary habits (unfavorable), and other (includes physical activity levels, weight gain). A total of 10 studies reported an increase in the number of snacks consumed, while six studies found that participants increased their meal number and frequency during quarantine. Eleven studies reported favorable changes in dietary habits with an increase in fresh produce and home cooking and reductions in comfort food and alcohol consumption. However, nine studies found a reduction in fresh produce, with a further six reporting an increase in comfort foods including sweets, fried food, snack foods, and processed foods. Two studies reported an increase in alcohol consumption. In eight studies participants reported weight gain with seven studies reporting a reduction in physical exercise.

Conclusion: The effect of COVID-19 lockdown both negatively and positively impacted dietary practices throughout Europe and globally, and negative diet habits were

OPEN ACCESS

Edited by:
Christophe Matthys, KU Leuven, Belgium

Reviewed by:
José María Huerta, Instituto de Salud Carlos III (ISCIII), Spain
Leila Lujan-Barroso, Catalan Institute of Oncology (ICO). Bellvitge Biomedical Reserach Institute-IDIBELL, Spain
Luis Moreno, University of Zaragoza, Spain

***Correspondence:**
Shelly Coe
scoe@brookes.ac.uk

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *05 November 2020*
Accepted: *03 February 2021*
Published: *04 March 2021*

Citation:
Bennett G, Young E, Butler I and Coe S (2021) The Impact of Lockdown During the COVID-19 Outbreak on Dietary Habits in Various Population Groups: A Scoping Review. Front. Nutr. 8:626432. doi: 10.3389/fnut.2021.626432

associated with other poor lifestyle outcomes including weight gain, mental health issues, and limited physical activity. Both in the short term and if sustained in the long term, these changes may have significant impacts on the health of the population.

Keywords: diet, LockDown, COVID-19, lifestyle, fruit and vegetables

INTRODUCTION

In March 2020, the World Health Organization (WHO) declared the coronavirus outbreak as a global pandemic (1). Currently, little is known of the transmission, mechanism, and treatment of COVID-19. It is believed that COVID-19 is spread via person-person contact, through respiratory droplets and because of this social distancing, hand washing, and wearing face coverings is crucial to reduce the spread of the virus (2).

As of July 2020, there have been over 570,000 global deaths related to COVID-19 (3). With no vaccine to prevent infection and no guaranteed treatment once infected, many countries have restricted public access to supermarkets, shops, and recreation facilities. The seriousness of restrictions varies among countries and is dependent on the incidence rates of infection. Despite lockdown measures taken by governments worldwide, over 13 million cases have been reported in over 200 countries (3).

Although such restrictions are to limit interpersonal contact and therefore transmission, many experts have voiced their concern for the long-lasting affects that lockdown may pose on individuals' mental and physical health (4). Lockdown has restricted the number of hours permitted for outdoor physical activity, people's access to fresh food, and previous research has shown that increased stress, which is likely during a global pandemic, can have a severe impact on an individual's lifestyle habits (5). Stress and anxiety have been shown to result in an increased intake of alcohol and sugary foods, and energy imbalance is also likely as energy expenditure during lockdown is reduced (5). However, positive lifestyle habits may also have emerged from the pandemic, including more time for cooking and reduced fast-food consumption.

Although there has been increasing and significant interest on the impact of the pandemic on various health outcomes, no review has addressed the association between COVID-19 and diet patterns globally. The aim of this review is to assess the literature on the impact of lockdown on dietary changes in various population groups. The objectives will be to assess diet patterns and themes in order to understand the dietary and associated lifestyle changes during the initial lockdown. It will be concluded how such changes may affect health and wellbeing in the short-term during the initial COVID-19 lockdown period and this will then inform research into the longer term effects of the pandemic on diet changes and health outcomes.

Abbreviations: COVID-19, Coronavirus disease; FSM, free school meals; MedDiet, Mediterranean diet; PRISMA-ScR, preferred reporting items for systematic reviews and meta-analyses extension for scoping reviews; WHO, World Health Organization.

METHODS

This review followed the preferred reporting items for systematic reviews and meta-analyses extension for scoping reviews (PRISMA-ScR) and was in accordance with the JBI Manual for Evidence Synthesis: updated methodological guidance for the conduct of scoping reviews (6).

Literature Search

A systematic literature search of the PubMed, Google Scholar, and Science Direct databases was conducted for studies published in 2020. Initially, the following search terms were used in the three databases to obtain a general understanding of the current research on the topic area: (COVID-19) AND (nutrition OR diet) AND (lockdown); (Food Habits) AND (Lockdown); (Dietary change) AND (COVID-19) AND (Lockdown). Following this search, alternative phrasing was noted among relevant studies and guidance on the search strategy was received. The search terms were then finalized and the following search terms were used in the systematic search: (Covid-19 OR Coronavirus) AND (Diet* OR Food OR Nutrition OR Eat*) AND (Lockdown OR Confinement OR Containment OR Quarantine OR Isolation). This search was undertaken between June 2020 and July 2020. No restrictors and filters were used in the database search so as to not inadvertently exclude any papers of interest.

Selection Process

Results were screened for eligibility based on title, abstract, and finally full text. Two researchers independently screened the articles for eligibility (GB and EY) alongside the inclusion criteria. The inclusion criteria of this literature search were as follows:

1. Limit to papers published in the year 2020 (including pre-prints);
2. Studies that investigated the association of COVID-19 lockdown and diet;
3. Only research papers in English.

For this review, the exclusion criteria were as follows:

1. Papers examining dietary changes in those following a structured diet (e.g., diabetics/coeliacs) based on diagnosed conditions or dietetic advice;
2. Literature, systematic, or narrative studies reviewing previous research.

The search was kept broad in order to identify any relevant studies that would fit within the aims of the review, and as lockdown occurred during 2020, only papers within this year were searched. No authors were further contacted.

Data Charting and Synthesis

Researchers GB and EY discussed search terms and databases prior to the initial search. All searches were documented in a shared excel spreadsheet accessible to all four researchers. After discussion among all four researchers, GB and EY performed a second and final search with the altered search terms in the same databases. Once eligible studies were identified based on the inclusion/ exclusion criteria, they were extracted and screened. An initial extraction template was developed and study characteristics were exported. The following characteristics from each study were extracted: first author, year, title, journal, type of study, participant number, age, location, findings, and conclusion. After consideration, researchers revised the extraction template to only focus on what they believed to be the important and relevant data from each study in regards to the study aims. A final extraction template of study characteristics for final full-text studies was agreed upon amongst all four reviewers. Results are shown in **Table 1** and are composed of: study type/ design, assessment methods, location, gender, age, dietary change, and significant findings/findings that showed a change. Themes were agreed upon by all researchers and data from each paper for the relevant themes were gathered and grouped together for analysis.

RESULTS

Out of a total of 4,322 papers initially identified, 23 full papers were deemed eligible for the current review (**Figure 1**). Most studies included in this review are based on self-reported data among adult populations in the developed world. Seventeen studies were cross-sectional in nature (7–10, 13, 15–17, 19, 21–27, 29), and three cohort (12, 14, 18), one observational (20), one correlational (28), and one panel study (11) were included. Locations included global (9, 25), the US (10, 18, 28), Asia including Palestine (7), India (8, 15, 17), and China (29), Europe including Italy (11, 13, 20, 22, 26), France (12), Spain (23, 24), Poland (27), and the UK (19), Australia (14, 21), and Zimbabwe (16). The age range and total number of participants were not presented as these demographics were not stated in all the studies included in the review. A full overview of papers is included in **Table 1**, in which only results that showed a change were included. After reviewing all eligible papers, four research themes were derived from each paper for analysis and discussion purposes (**Table 2**). These included: dietary patterns (meal patterns and snacking), dietary habits (favorable), dietary habits (unfavorable), and other (includes physical activity levels, weight gain). The following discussion is separated into four parts, to support the research themes.

DISCUSSION OF RESULTS

Dietary Patterns

Snacking

A significant change in snacking frequency and behaviors was reported by a total of nine studies that were reviewed (8, 9, 12, 14, 15, 20, 26–28, 30). ALMughanis et al. (8) reported an increase in snacking at night in 44% of participants. This trend was supported by three other studies that found that increased snacking behaviors were typically seen at night or after the last meal of the day (9, 15, 28). Late night food intake is viewed by many as an unfavorable dietary pattern as research has shown an association between late-night eating and the development of metabolic syndrome (31). Snacks mostly consisted of energy-dense and non-nutrient-rich foods. Scarmozzino et al. (26) reported that in Italy over 40% of participants increased consumption of "comfort foods" such as chocolate, ice cream, and desserts during lockdown. Romeo-Arroyo et al. (24) reported a 50% increase in sweets consumed. Three papers (14, 20, 28) found that snacking was used as a mechanism to help cope with increased anxiety levels during self-quarantine. Following this finding, promotion of other avenues of stress relief, including physical activity and meditation, may be influential when advising population groups how to maintain sustainable and healthy lifestyle habits.

Meal Number

Six studies reported that participants saw an increase in their meal number and frequency during lockdown (7, 15, 17, 22, 26, 27, 30). In Mumbai, India, Mehta et al. (17) completed a 15-question survey with 50 participants and found that over half of participants increased their meal consumption, with a large portion choosing foods that had a low preparation time and meals based around ready-to-eat foods and animal produce. In a study by Deschasaux-Tanguy et al. (12), 37,252 adults from France completed a lockdown-specific questionnaire on nutritional behaviors before and after lockdown, between April and May 2020. This research found that although food intake increased, diet diversity and fresh produce, such as fruit and vegetables, intake reduced. Three of the studies investigated did not report an increase in food consumption (13, 21, 23). Phillipou et al. (21) found that almost 30% of participants had restricted their eating habits during confinement, primarily due to weight gain concerns. Husain et al. (15) found that in Kuwait, almost 42% of their cohort skipped breakfast during quarantine, which increased slightly from 39% before lockdown.

Change in Dietary Habits: Favorable

Increase in Fresh Produce

A total of 11 papers reported favorable changes in dietary habits during the coronavirus pandemic lockdown (7, 9–13, 15, 22, 23, 25, 26, 32). Pietrobelli et al. (22) identified an increase in fruit consumption in 41 children and adolescents in Italy ($p = 0.055$). An increase in fruit intake is supported by four other studies (7, 10, 23, 25). Ruiz-Roso et al. (25) found an increase in both fruit and vegetable intake in 820 adolescents during confinement. An explanation for this could be the increase in home cooking and the promotion from the WHO of the importance of fruit and vegetables during confinement. In 7,154 Spanish adults, an increase in fruit and vegetable intake during confinement was observed in a higher proportion for males than females compared to pre-confinement (23). In contrast, Bhutani et al. (10) reported a higher proportion of females in the United States (US) increasing fruit and vegetable consumption in confinement compared to US males. The difference in results could be due

TABLE 1 | Overview of papers included in this review.

N(ref)[1]	First author	Study type	Assessment method	Location	Female *N* (%)	Age (mean) in years	Dietary change	Findings that report a change and/or show significance
(7)	Allabadi et al.	Cross-sectional study	Demographic, diet, and lifestyle survey (telephone)	Asia (Palestine)	300 (50%)	10–19 (14.1)	Comfort food, fruit, and vegetable increase	Food intake increased during lockdown; weight gain seen ($p < 0.001$). Increased food intake higher in females than males ($p = 0.013$).
(8)	ALMughamis et al.	Cross-sectional study	Demographic, diet, and lifestyle survey (WhatsApp)	Asia (India)	380 (72.8%)	(41.8)	Snacking, food intake increase	Change in dietary habits. Increased snacking, particularly after dinner. Weight gain seen in lockdown ($p < 0.001$). Increase in sedentary levels.
(9)	Ammar et al.	Cross-sectional study	Mental and physical health and lifestyle behavior survey (online)	Global (40% Africa, 36% Asia, 21% Europe)	563 (53.8%)	18+	Meal number, comfort food, and snacking increase	Increase in number of meals consumed ($p < 0.001$). Binge eating habits and snacking increased during confinement ($p < 0.001$). Consumption of unhealthy food increased ($p < 0.001$). Binge alcohol drinking decreased ($p < 0.001$).
(10)	Bhutani et al.	Cross-sectional study	Demographic, diet, and lifestyle survey (online)	Americas (U.S.[4])	1,007 (56.6%)	18–75	Snacking, fruit, and vegetable increase	Increased intake of fruit and vegetables ($p > 0.05$). Increase in processed food intake and snacking ($p > 0.001$).
(11)	Bracale et al.	Panel study	Analysis of consumer trends	Europe (Italy)			Decrease in fresh food intake	Increase in long-life products: pasta, flour, eggs, homemade bread and pizza, and red or processed meat. Decrease in fresh produce.
(12)	Deschasau-Tanguy et al.	Cohort study	Demographic survey and 24-h recall (online)	Europe (France)	19,483 (52.3%)	15+ (52.1)	Snacking, comfort food increase, fresh food decrease	Change in dietary habits. Reported weight gain, particularly among young women. Increase in home cooking, snacking, comfort food, and alcohol intake. Reduction in fresh fruit, vegetable, meat, and fish.
(13)	Di Renzo et al.	Cross-sectional study	Demographic, diet, and lifestyle survey (online)	Europe (Italy)	2,486 (71%)	12–86	No change in meal number	Increase in homemade foods. Decrease in fresh fish, baked goods, and alcohol intake ($p = 0.002$).
(14)	Gallo et al.	Cohort study	Self-administered 24-h recall	Oceania (Australia)	295 (57.9%)	19–27	Snacking and food intake increase	Snacking and energy intake increase in females ($p < 0.05$). Increase in number of meals consumed at home ($p < 0.0001$). Reduction in walking ($p < 0.05$).
(15)	Husain et al.	Cross-sectional study	Demographic, diet, and lifestyle survey (online)	Asia (India)	285 (68.7%)	18–73 (38.5)	Snacking increase	Change in dietary habits, increase in number of meals ($p = 0.000$). Increase in snacking, particularly at night ($p = 0.000$). Increase in freshly made foods/home cooking. Decrease in red meat and fast food consumption ($p = 0.000$).
(16)	Matsungo and Chopera	Cross-sectional study	Demographic, diet, and lifestyle survey (online)	Africa (Zimbabwe)	318 (63%)	18+	Decrease in fruit and vegetables	Decrease in fruit, vegetables, nuts, seeds, cereals, breads, and tubers intake ($p < 0.006$). Increase in perceived body size seen in lockdown ($p < 0.001$).
(17)	Mehta	Cross-sectional study	Demographic, diet, and lifestyle survey	Asia (India)	25 (50%)	20–50 (37)	Snacking, meal numbers increase, fruit, and vegetable decrease	Change in dietary habits. Intake during meal times and snacking increased. Fruit and vegetable intake decreased during confinement.
(18)	Mitchell et al.	Retrospective cohort study	Dietary and lifestyle record (mobile app)	Americas (U.S.)	318,224 (83.4%)	18+ (47.8)	Decrease in fruit and vegetables	Decrease in fruit and vegetable intake. Increased in red and processed meat intake, particularly among men.
(19)	Parnham et al.	Cross-sectional study	COVID-19 related questionnaire	Europe (UK[5])		8+	Reduction in school meals	Half of children did not receive free school meals during lockdown ($p < 0.01$).
(20)	Pellegrini et al.	Retrospective observational study	Demographic, diet, and lifestyle survey (telephone)	Europe (Italy)		18–75 (47.9)	Snacking increase	Increase in BMI and self-reported weight gain ($p < 0.001$). Lower educational levels, higher anxiety/depression levels, and poor dietary habits associated with weight gain ($p < 0.001$). Increased consumption of snacks, sweets, and cereals ($p < 0.001$).

(Continued)

TABLE 1 | Continued

N(ref)[1]	First author	Study type	Assessment method	Location	Female *N* (%)	Age (mean) in years	Dietary change	Findings that report a change and/or show significance
(21)	Phillipou et al.	Cross-sectional study	Demographic, diet, and mood survey (online)	Oceania (Australia)	4,231 (80%)	18+	Food intake	Higher levels of binge eating (~35%) ($p < 0.003$), higher levels of restrictive eating (~28%) ($p < 0.001$).
(22)	Pietrobelli et al.	Cross-sectional study	Lifestyle questionnaire (in-person and telephone)	Europe (Italy)	19 (46%)	6–18 (14)	Meal number, comfort food increase	Increase in number of meals consumed (p <0.001). Increase in fruit consumption ($p = 0.055$). Intake of crisps sweets and sugary drinks increased ($p = 0.005$ – <0.001).
(23)	Rodriguez-Perez et al.	Cross-sectional study	Demographic and dietary intake survey (online)	Europe (Spain)	5,305 (70.6%)	18+	No increase in meal intake	No increase in eating habits. Decreased alcohol intake and physical activity levels. Difficulty finding fresh fish and vegetables. Males had significantly different intake levels of fruit, vegetables, and olive oil ($p < 0.006$).
(24)	Romeo-Arroyo et al.	Cross-sectional study	Diet and lifestyle survey (online)	Europe (Spain)	301 (50.1%)	18–68 (42.6)	Comfort food increase	Increase intake of sweets. Decrease in fresh fish intake ($p < 0.05$).
(25)	Ruiz-Roso et al.	Cross-sectional study	Demographic, diet, and lifestyle survey (online)	Global (EU[2] and South America)	495 (61.1%)	<14 to >17 (16)	Comfort food, fruit, and vegetable increase	Increase in fruit and vegetable ($p < 0.0001$) and legumes ($p < 0.05$) intake, higher compliance with recommendations. Fast food intake decreased ($p < 0.0001$). Sweet ($p < 0.0001$) and fried food ($p < 0.001$) intake increased.
(26)	Scarmozzino et al.	Cross-sectional study	Demographic and dietary intake survey (online)	Europe (Italy)		Anonymous	Comfort food, food intake increase	Increased eating during confinement. Increase in comfort food intake: chocolate, ice cream, and desserts. Increased snacking. Decreased alcohol intake.
(27)	Sidor et al.	Cross-sectional study	Demographic, diet, and lifestyle survey (online)	Europe (Poland)	1,042 (95%)	18+ (27.7)	Snacking and food intake increase	Increased eating and snacking during confinement in those with a higher BMI[3] ($p < 0.01$). Highest BMIs had lowest fruit and vegetable intake, highest dairy, red meat, and fast food intakes ($p < 0.05$).
(28)	Zachery et al.	Correlational study	Demographic, lifestyle, and social survey (online)	Americas (U.S.)	96 (55.5%)	(26)	Snacking increase	Increased snacking after dinner ($p < 0.001$ and increased eating in response to stress or boredom ($p = 0.041$) in those who gained weight during lockdown.
(29)	Zhao et al.	Cross-sectional study	Demographic, dietary, and household food diversity survey	Asia (China)	1,273 (65.7%)	18+	No increase in meal intake	Increased intake of nutritional supplements. Younger age group had a lower diet diversity score.

[1]*N(ref), reference number;* [2]*EU, European Union;* [3]*BMI, body mass index;* [4]*U.S., United States;* [5]*UK, United Kingdom; N, number.*

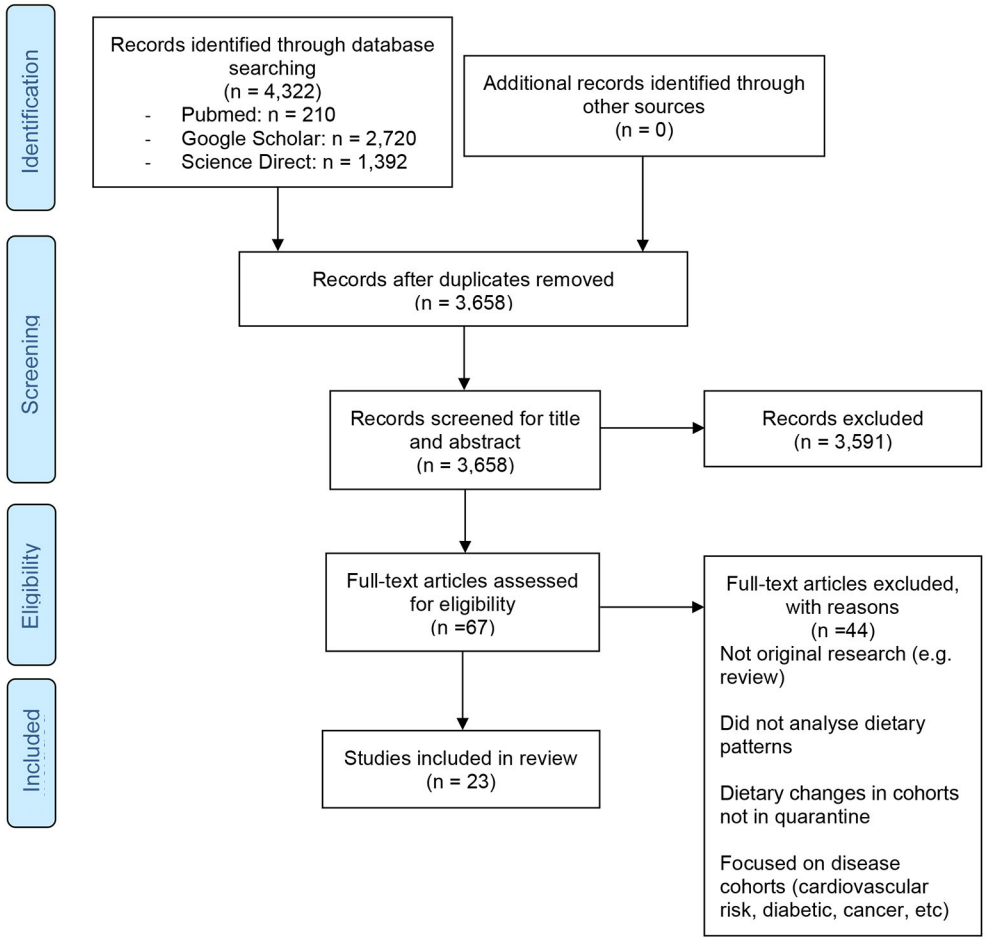


FIGURE 1 | PRISMA 2009 flow diagram displaying the selection process of the 23 final papers.

to Spanish female participants having a higher adherence to the Mediterranean diet (MedDiet), which is high in fruit and vegetables, prior to lockdown, and therefore any increase would be more noticeable in a US female cohort.

Increase in Home Cooking

Based on consumption trends, there was an increase in the amount of home cooking with a significant increase in foods such as homemade pizza and bread (11). This is supported by Deschasaux-Tanguy et al. (12) and Di Renzo et al. (13) who both reported an increase in home cooking. The increase in home cooking could be attributable to various reasons including reduced availability of eating out and/or fast food options, increase in family time, safety concerns, and finally it could be used as a method to overcome boredom.

Reduction in Comfort Foods

A decrease in total fast food consumption during lockdown has been reported in participants in Kuwait, Spain, Italy, Brazil, Colombia, and Chile (15, 25). In a cohort of 415 participants living in Kuwait, 82.2% did not consume any fast food during lockdown and this dietary habit reportedly continued post lockdown with 13.7% consuming fast food 1–2 times per week compared to 49.4% pre-lockdown. Consideration needs to be taken for the fact that many fast food outlets would have been closed during lockdown which would have significantly affected fast food intake.

Reduction in Alcohol Consumption

Ammar et al. (9) found via a short dietary behavior questionnaire that the largest dietary habit change during lockdown was a reduction in binge drinking. It is thought that the younger participants in the study would have limited access to alcohol and less social interaction with friends which may have impacted the results. Social incentives have been found to be more of an influence over coping motives for increased alcohol consumption in the younger population and therefore this may have caused a significant reduction on drinking in this group during the pandemic (33). A 36.8% reduction in alcohol consumption in Italy was reported, however this is thought to have been overexaggerated due to an underreporting of alcohol intake which is often associated with self-reporting questionnaires (26).

TABLE 2 | Twenty-three papers in the review divided by theme and sub theme.

Theme for discussion	Sub-sections	Paper reference numbers
Dietary patterns		(7–9, 12–15, 17, 20–24, 26–28)
	Snacking	(8, 9, 12, 14, 15, 20, 24, 26–28)
	Meal number	(7, 12, 13, 15, 17, 21–23, 26, 27)
Dietary habits: favorable		(7, 9–13, 15, 22, 23, 25, 26)
	Increase in fresh produce	(7, 10, 22, 23, 25)
	Increase in home cooking	(11–13)
	Reduction in comfort foods	(15, 25)
	Reduction in alcohol consumption	(9, 23, 26)
Dietary habits: unfavorable		(7, 9–13, 16–18, 20, 22–26, 29)
	Reduction in fresh produce	(7, 10–13, 16–18, 23, 24)
	Increase in comfort foods	(9, 12, 20, 22, 25, 26)
	Increase in alcohol consumption	(12, 29)
Other		(7, 8, 12–14, 16, 19–21, 23, 26–29)
	Reduction in physical activity	(7, 8, 12, 14, 16, 21, 23)
	Increase in supplementation	(29)
	Weight gain	(7, 8, 12, 13, 20, 26–28)
	School meals	(19)

However, Rodríguez-Pérez et al. (23) identified a lower intake of alcohol during lockdown in Spain and reported that these dietary changes continued with a higher adherence to the MedDiet post lockdown.

Change in Dietary Habits: Unfavorable

Reduction in Fresh Produce

Nine papers assessed the reduction in fresh produce (7, 10–13, 16–18, 24). Overall the papers showed a reduction in purchasing and consumption of fresh produce. Bracle et al. (11) assessed consumer purchase trends from February to March 2020 in an Italian population and found a dramatic reduction in fresh goods including fruit and vegetables. Similarly, Deschasaux-Tanguy et al. (12) found that 27.4% of participants reported buying less fresh products due to poorer access to usual food stores and/or products. Rodriguez-Perez et al. (23) reported just over a quarter (27%) had issues with buying certain foods which included meat (23.83%) and vegetables (13.86%).

Research by Matsungo and Chopera (16), using a cross-sectional online survey for 507 people living in Zimbabwe, found that 94.8% of people reported an increase in food price with decreased availability (64%) and a decrease in quality (43.9%), during a 4-week lockdown in Zimbabwe, showing the massive effect COVID-19 has had on the food chain supply system. Therefore, price or financial status may also play a role in reduced purchase as Mehta (17) found that 27% of participants reduced fruit and vegetable intake and although unavailability was a key reason, 32% reported that the increase in price of these consumables influenced their lack of purchase and/or consumption. Allabadi et al. (7) performed telephone interviews with 600 adolescents in Palestine. In total, 36.7% increased fried food intake and 46.5% increased sweets and sugar-added food intake, and this was associated with weight gain and worsening financial status during lockdown.

As mentioned above, stress and anxiety may have significant influence over food habits during lockdown as Mitchell et al. (18) found through a self-reported food intake questionnaire in 381,564 participants pre and during COVID-19 lockdown. Fruit (men −4.2%, women −5%), salad (men −5.9%, women −6.5%), vegetables (men −4.7%, women −6.3%), and lean protein (men −3.6%, women −3.9%) intake decreased. These results were compared to other studies in which stress during "natural disasters" has been shown to correlate with reduced fresh produce consumption (30). Matsungo and Chopera (16) found food prices increased and availability decreased. Over the 4-week lockdown period, 57.8% said they reduced vitamin A rich fruit and vegetable intake, 48.5% decreased other vegetable intake, and 64.9% decreased other fruit intake, with a reduction in fresh produce associated with higher anxiety.

Increase in Comfort Foods

Pietrobelli et al. (22) found that the consumption of unhealthy foods including potato chips, red meat, and sugary drinks significantly increased 3 weeks into lockdown in a sample of 41 young people in Italy who were classified as obese ($p < 0.005$–0.001). Ruiz Roso et al. (25) used an online questionnaire in a multi-country cohort of adolescents and found sweet food and fried food consumption increased during COVID-19 confinement compared to before COVID-19. There were significant country differences and those who watched TV during mealtimes had higher sweet food and fried food consumption. In another study, an increased consumption of sweets and chocolate (22%) and biscuits and cakes (20%) was found, which was associated with other poor diet habit changes (12). Therefore, multiple factors may have increased the intake of unhealthy or comfort foods during COVID-19 lockdown, including already being overweight or obese, more sedentary time at home, and an overall change in dietary habits/patterns.

In an international sample of 1,047 participants, unhealthy food consumption had increased during confinement, which was due to changes in mood including lack of motivation and anxiety and/or boredom (9). Scarmozzino et al. (26) found that, similar to Ammar et al. (9), the increase in negative eating behaviors was thought to be due to increased anxiety in this population during lockdown (42.7%). Pellegrini et al. (20) used a questionnaire in 150 obese participants in Northern Italy and found that unhealthy food consumption, specifically sweets intake increased by 50%. This increase was associated with weight gain during lockdown which may have been related to increased anxiety.

Increase in Alcohol Consumption

Two studies looked at increased alcohol consumption during lockdown (12, 29). Zhao et al. (29) used a cross-sectional

questionnaire-based survey in 1,938 Chinese participants to assess what lifestyle changes people used to "cope" with COVID-19 and found that 10.6% of participants increased their alcohol consumption intentionally. In another study, when considering food groups, alcoholic beverage consumption increased in 15% of the participants and decreased in 12%. The increase in alcohol intake was associated with other unhealthy changes in diet patterns, with higher anxiety and depression scores and with working from home (12).

Other

Reduction in Physical Activity

During a 4-week lockdown in Zimbabwe, Matsungo et al. (16) reported that 62.5% of people surveyed reduced their physical activity. This may be explained by an increase in screen time in 89.1% of respondents. Similarly, Rodriguez-Perez et al. (23) showed that while people's dietary pattern did not dramatically deviate from the MedDiet during lockdown, over 59.6% decreased their levels of physical activity. Three papers (7, 8, 12) showed that at least 50% of people surveyed had decreased levels of exercise in Kuwait, Palestine, and France due to confinement. An Australian study by Phillipou et al. (21) reported that in the general population group the majority of people (43.4%) decreased their exercise levels, which may be related to working from home and restrictions on sporting activities and gym closures during the peak of the pandemic. Gallo et al. (14) carried out a survey on US students comparing data from 2018/2019 to data collected during the 2020 lockdown. Although vigorous exercise levels remained the same, students reported a decrease in walking during 2020, which may be related to no longer commuting to campus or walking between classes. Examining a subsection of biomedical students, 80% were classed as "sufficiently active," which dropped to 62% for men and 55% for women in 2020, showing the negative effect lockdown has on physical activity levels among young adults.

Increase in Supplementation

Zhao et al. (29) showed that 37.7% of people surveyed intentionally consumed certain foods, supplements, or Chinese herbs as they thought it would help against COVID-19. These people had a higher Household Dietary Diversity Score which the paper concluded could mean they were more "in tune" with their health. People mainly consumed vitamin C (18.2%), however despite government advice, some people consumed alcohol (10.6%) or vinegar (16%) under the false assumption that consumption would kill or help fight the virus.

Weight Gain

Three Italian surveys noted increased weight gain among participants related to the consumption of certain foods (13, 20, 26). In Northern Italy, Pellegrini et al. (20) reported a significant increase of 1.51 kg on average among participants. Those of lower education levels, disinterest in the "healthiness" of foods, and who self-reported anxiety and depression were more likely to report weight gain. Di Renzo et al. (13) reported 48.6% of people saw an increase in weight, which may be due to an increased appetite during lockdown. Scarmozzino et al. (26) showed the lowest reported weight gain (19.5%). In the US, Zachary et al. (28) reported a lower number of people (22%) reporting weight gain but a larger increase in weight, ∼5–10 pounds (2.2–4.5 kg). Weight gain was linked to increased eating due to stress (52%), boredom (73%), seeing or smelling food (65%), snacking after dinner (65%), being around friends and family (65%), and cravings (52%). Four other papers reported an increase in weight due to changes in the type of food consumed (7, 8, 12, 27). ALMughanis et al. (8) reported in Kuwait that there was a significant change in weight (1.13 kg), which is unsurprising as 74.1% claimed they changed their dietary habits. Deschasaux-Tanguy et al. (12) showed that participants (35%) gained weight (1.8 kg) due to reduced physical activity and an increase in calories by over 10%. Energy intake vs. energy expenditure will have shifted during the initial lockdown and the resulting weight gain following these patterns is therefore not surprising. However, this new "normal" of confinement at home may necessitate the need to find alternative ways of managing weight, diet, and exercise, especially if continued (32).

School Meals

Parnham et al. (19) surveyed children eligible for free school meals (FSM) in the UK and found that children on FSM were more likely to receive vouchers during the 1st month of lockdown in the UK than those through the universal scheme. Secondary school children were more likely to receive the voucher than children from junior schools, but this could be due to them being classified by means testing for FSM not through the universal scheme. There was also a correlation between receiving FSM and the household visiting a food bank.

Dietary Changes by Country

Country specific changes in diet and lifestyle habits were also assessed in the current review to determine if any patterns were apparent. In Australia, Gallo et al. (14) and Phillipou et al. (21) both reported a reduction in physical activity during the initial lockdown. In the US, Bhutani et al. (10) and Mitchell et al. (18) assessed the reduction in the consumption of fresh produce, with only Mitchell et al. showing this trend. ALMughanis et al. (8) and Husain et al. (15) showed an increase in snacking behavior in the Indian population. In Italy, Bracale et al. (11) and Di Renzo et al. (13) showed evidence for an increase in home cooking yet a reduction of fresh produce, while Pellegrini et al. (20), Pietrobelli et al. (22), and Scarmozzino et al. (26) reported an increase in comfort foods. Di Renzo et al. (13), Pietrobelli et al. (22), and Scarmozzino et al. (26) asked participants if there were changes in the number of meals consumed with only Pietrobelli et al. and Scarmozzino et al. reporting an increase in the numbers of meals, while Di Renzo et al. found no change. Also, Di Renzo et al. (13), Pellegrini et al. (20), and Scarmozzino et al. (26) showed that participants reported weight gain. In Spain, Rodriguez-Perez et al. (23) and Romeo-Arroyo et al. (24) both reported a reduction in consuming fresh produce. Finally, Ammar et al. (9) and Ruiz-Roso et al. (25), which both covered multiple continents, both showed an increase in consumption of comfort foods. Therefore, although it is difficult to conclude anything definite, there seems to be agreement between studies within the same country and

this could potentially be due to the different lockdown timelines and policies implemented in these locations. For example, over half of the countries in this review entered either a full or partial lockdown by the end of March 2020, with the government restricting movement and social interaction, which may have had a sudden and significant impact on the frequency of accessing supermarkets to buy fresh produce, the lack of food-associated social occasions, and limiting exercise.

Strengths and Limitations

Previous research has highlighted the negative effects that lockdown has on health, however, this study is focused on the thematic analysis of dietary change and provides a unique insight into how the COVID-19 pandemic has impacted dietary behavior globally. The evidence included in this review is from a short time period in 2020 and therefore the studies are based on acute changes to diet and associated lifestyle factors. Even though this review is a starting point for considering long term effects of COVID-19 on health, so far this can only be predicted from the short-term studies. Only studies with full-text publications and published in English were considered for this review, which may lead to selection bias. As with most nutritional research, dietary intake was assessed through self-reported data, where misreporting, most commonly underreporting, is possible. Studies in this review were predominately cross-sectional in design and therefore the risk of bias and the quality of each study was difficult to assess due to the nature of the review and the studies included. It was not possible to assess quality compared to longer term cohort/ cause-effect research. Only findings that showed a change were reported in the results, as anything that did not significantly change did not differ compared to prior to lockdown. This review had limitations in its classification of a scoping review due to the short-term nature of the studies included and the limited literature in which to discuss the findings, however it was felt that this review type was the most appropriate for the current topic. Pre-print research papers were included in this review (8, 10, 12, 17, 18) and therefore despite having a DOI, these papers had not undergone peer-review prior to being included in this review. The search terms were not fact checked by a librarian and therefore the search strategy was constructed based on the expertise of the four authors of this review, who between them have an extensive background in the nutrition research field and in writing systematic and scoping reviews.

CONCLUSION

Although some studies noted a decrease in food consumption and healthier diet practices during lockdown, many studies found either an increase in snacking and meal numbers, or an increase in unfavorable food choices and dietary habits. Therefore, COVID-19 lockdown resulted in both favorable and unfavorable changes in eating practices, and this may have both short- and long-term consequences on health. Although over half of the studies included European populations, there is evidence to suggest that action is required globally to encourage people to re-adopt healthy lifestyle habits during and post lockdown.

The positive diet practices that resulted from lockdown included an increase in the consumption of fresh produce, mostly fruit and vegetables, and an increase in home cooking during lockdown. However, poor food habits were seen in the majority of studies including increased snacking and meal frequency, reduced fresh produce, an increase in comfort foods, and alcohol intake. Reasons for negative changes in food behavior predominately included limited availability and increased price, and there were associations with poor food choices and mental health conditions including depression and anxiety, and sedentary time and weight gain. Those with a higher BMI were more likely to develop unhealthy dietary habits during quarantine (27). The increase in negative food choice such as alcohol intake was associated with other unhealthy changes in diet patterns, with higher anxiety and depression scores and with working from home (12). Conversely, those who showed a healthier overall diet routine throughout lockdown were shown to exhibit other healthy behaviors such as a reduced alcohol intake and increased supplement use. Reduced physical activity and weight gain were the main concerns found in some studies, and alongside poorer eating habits these factors may overall cause significant public health worry.

If negative dietary patterns are sustained post lockdown, they may contribute to health issues among population groups in the future, as being overweight and obese leaves individuals more susceptible to chronic health conditions and disease. Indeed, sedentary behavior including increased screen time and television viewing is associated with unhealthy eating patterns, and therefore this is a pattern that is likely seen during confinement (34). It is also a possibility that future lockdowns may occur, and therefore changes in eating habits may continue. Although positive changes in dietary practices were seen during the first lockdown, the impact of COVID-19 on lifestyle behaviors including diet was detrimental across populations, and therefore future research should aim to help the recovery and maintenance of healthy lifestyle habits to prevent the long-term health effects of the pandemic.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.626432/full#supplementary-material

REFERENCES

1. *Listings of WHO's Response to COVID-19*. Available online at: https://www.who.int/news-room/detail/29-06-2020-covidtimeline (cited September 25, 2020).
2. *Social distancing: what you need to do - Coronavirus (COVID-19)*. NHS. Available online at: https://www.nhs.uk/conditions/coronavirus-covid-19/social-distancing/what-you-need-to-do/ (cited September 25, 2020).
3. WHO/Europe. *Coronavirus Disease (COVID-19) Outbreak*. Available online at: https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19 (cited September 28, 2020).
4. Cheval B, Sivaramakrishnan H, Maltagliati S, Fessler L, Forestier C, Sarrazin P, et al. Relationships between changes in self-reported physical activity and sedentary behaviours and health during the coronavirus (COVID-19) pandemic in france and switzerland. *J Sports Sci*. (2020). doi: 10.31236/osf.io/ydv84. [Epub ahead of print].
5. Mattioli AV, Sciomer S, Cocchi C, Maffei S, Gallina S. Quarantine during COVID-19 outbreak: changes in diet and physical activity increase the risk of cardiovascular disease. *Nutr Metab Cardiovasc Dis*. (2020) 30:1409–17. doi: 10.1016/j.numecd.2020.05.020
6. Peters MDJ, Marnie C, Tricco AC, Pollock D, Munn Z, Alexander L, et al. Updated methodological guidance for the conduct of scoping reviews. *JBI Evid Synthesis*. (2020) 18:2119–26. doi: 10.11124/JBIES-20-00167
7. Allabadi H, Dabis J, Aghabekian V, Khader A, Khammash U. Impact of COVID-19 lockdown on dietary and lifestyle behaviours among adolescents in Palestine. *Dyn Hum Health*. (2020) 2020:7.
8. ALMughamis N, AlAsfour S, Mehmood S. *Poor Eating Habits and Predictors of Weight Gain During the COVID-19 Quarantine Measures in Kuwait: A Cross Sectional Study*. Research Square (2020) (cited September 24, 2020).
9. Ammar A, Brach M, Trabelsi K, Chtourou H, Boukhris O, Masmoudi L, et al. Effects of COVID-19 home confinement on eating behaviour and physical activity: results of the ECLB-COVID19 international online survey. *Nutrients*. (2020) 12:1583. doi: 10.3390/nu12061583
10. Bhutani S, Cooper JA, Vandellen MR. Self-reported changes in energy balance behaviors during COVID-19 related home confinement: a cross-sectional study. *medRxiv*. (2020). doi: 10.1101/2020.06.10.20127753
11. Bracale R, Vaccaro CM. Changes in food choice following restrictive measures due to Covid-19. *Nutr Metab Cardiovasc Dis*. (2020) 30:1423–6. doi: 10.1016/j.numecd.2020.05.027
12. Deschasaux-Tanguy M, Druesne-Pecollo N, Esseddik Y, Szabo de Edelenyi F, Alles B, Andreeva VA, et al. Diet and physical activity during the COVID-19 lockdown period (March-May 2020): results from the French NutriNet-Sante cohort study. *medRxiv*. (2020). doi: 10.1101/2020.06.04.20121855
13. Di Renzo L, Gualtieri P, Pivari F, Soldati L, Attinà A, Cinelli G, et al. Eating habits and lifestyle changes during COVID-19 lockdown: an Italian survey. *J Transl Med*. (2020) 18:229. doi: 10.1186/s12967-020-02399-5
14. Gallo LA, Gallo TF, Young SL, Moritz KM, Akison LK. The impact of isolation measures due to covid-19 on energy intake and physical activity levels in australian University students. *Nutrients*. (2020) 12:1–14. doi: 10.1101/2020.05.10.20076414
15. Husain W, Ashkanani F. Does COVID-19 change dietary habits and lifestyle behaviours in Kuwait? *Environ Health Prev Med*. (2020) 25:1–13. doi: 10.20944/preprints202006.0154.v1
16. Matsungo TM, Chopera P. The effect of the COVID-19 induced lockdown on nutrition, health and lifestyle patterns among adults in Zimbabwe. *BMJ, Nutrition, Prevention and Health*. (2020) 3:205–12. doi: 10.1101/2020.06.16.20130278
17. Mehta V. *The Impact of COVID-19 on the Dietary Habits of Middle-Class Population in Mulund, Mumbai, India*. AIJR Preprints. AIJR Publisher (2020). Available online at: https://preprints.aijr.org/index.php/ap/preprint/view/82 doi: 10.21467/preprints.82 (cited September 24, 2020).
18. Mitchell SE, Yang Q, Behr H, Deluca L, Schaffer P, Mitchell S. Self-reported food choices before and during COVID-19 lockdown. *medRxiv*. (2020). doi: 10.1101/2020.06.15.20131888
19. Parnham JC, Laverty AA, Majeed A, Vamos EP. Half of children entitled to free school meals do not have access to the scheme during the COVID-19 lockdown in the UK. *medRxiv*. (2020) 87:161–64. doi: 10.1101/2020.06.19.20135392
20. Pellegrini M, Ponzo V, Rosato R, Scumaci E, Goitre I, Benso A, et al. Changes in weight and nutritional habits in adults with obesity during the "lockdown" period caused by the COVID-19 virus emergency. *Nutrients*. (2020) 12:1–11. doi: 10.3390/nu12072016
21. Phillipou A, Meyer D, Neill E, Tan EJ, Toh WL, Van Rheenen TE, et al. Eating and exercise behaviors in eating disorders and the general population during the COVID-19 pandemic in Australia: initial results from the COLLATEproject. *Int J Eat Disord*. (2020) 53:1158–65. doi: 10.1002/eat.23317
22. Pietrobelli A, Pecoraro L, Ferruzzi A, Heo M, Faith M, Zoller T, et al. Effects of COVID-19 lockdown on lifestyle behaviors in children with obesity living in Verona, Italy: a longitudinal study. *Obesity*. (2020) 28:1382–5. doi: 10.1002/oby.22861
23. Rodríguez-Pérez C, Molina-Montes E, Verardo V, Artacho R, García-Villanova B, Guerra-Hernández EJ, et al. Changes in dietary behaviours during the COVID-19 outbreak confinement in the Spanish COVIDiet study. *Nutrients*. (2020) 12:1–19. doi: 10.3390/nu12061730
24. Romeo-Arroyo E, Mora M, Vázquez-Araújo L. Consumer behavior in confinement times: food choice and cooking attitudes in Spain. *Int J Gastron Food Sci*. (2020) 21:100226. doi: 10.1016/j.ijgfs.2020.100226
25. Ruiz-Roso MB, Padilha P de C, Mantilla-Escalante DC, Ulloa N, Brun P, Acevedo-Correa D, et al. Covid-19 confinement and changes of adolescent's dietary trends in Italy, Spain, Chile, Colombia and Brazil. *Nutrients*. (2020) 12:1–18. doi: 10.3390/nu12061807
26. Scarmozzino F, Visioli F. Covid-19 and the subsequent lockdown modified dietary habits of almost half the population in an Italian sample. *Foods*. (2020) 9:675. doi: 10.3390/foods9050675
27. Sidor A, Rzymski P. Dietary choices and habits during COVID-19 lockdown: experience from Poland. *Nutrients*. (2020) 12:1657. doi: 10.3390/nu12061657
28. Zachary Z, Brianna F, Brianna L, Garrett P, Jade W, Alyssa D, et al. Self-quarantine and weight gain related risk factors during the COVID-19 pandemic. *Obes Res Clin Pract*. (2020) 14:210–6. doi: 10.1016/j.orcp.2020.05.004
29. Zhao A, Li Z, Ke Y, Huo S, Ma Y, Zhang Y, et al. Dietary diversity among chinese residents during the COVID-19 outbreak and its associated factors. *Nutrients*. (2020) 12:1–13. doi: 10.3390/nu12061699
30. Schelleman-Offermans K, Kuntsche E, Knibbe RA. Associations between drinking motives and changes in adolescents' alcohol consumption: a full cross-lagged panel study. *Addiction*. (2011) 106:1882. doi: 10.1111/j.1360-0443.2011.03423.x
31. Yoshida J, Eguchi E, Nagaoka K, Ito T, Ogino K. Association of night eating habits with metabolic syndrome and its components: a longitudinal study. *BMC Public Health*. (2018) 18:1366. doi: 10.1186/s12889-018-6262-3
32. Bhutani S, Cooper JA. COVID-19-related home confinement in adults: weight gain risks and opportunities. *Obesity*. (2020) 28:1576–7. doi: 10.1002/oby.22904
33. Uemura M, Ohira T, Yasumura S, Otsuru A, Maeda M, Harigane M, et al. Association between psychological distress and dietary intake among evacuees after the great East japan earthquake in a cross-sectional study: the Fukushima Health Management Survey. *BMJ Open*. (2016) 6:11534. doi: 10.1136/bmjopen-2016-011534
34. Pearson N. Biddle SJH. Sedentary behavior and dietary intake in children, adolescents, and adults: a systematic review. *Am J Prev Med*. (2011) 41:178–88. doi: 10.1016/j.amepre.2011.05.002

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Bennett, Young, Butler and Coe. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

PERSPECTIVE
published: 05 March 2021
doi: 10.3389/fnut.2021.591964

Negative Association Between Mediterranean Diet Adherence and COVID-19 Cases and Related Deaths in Spain and 23 OECD Countries: An Ecological Study

Michael W. Greene[1,2]*, *Alexis P. Roberts*[1] *and Andrew D. Frugé*[1,2]

[1] *Department of Nutrition, Auburn University, Auburn, AL, United States,* [2] *Boshell Diabetes and Metabolic Disease Research Program, Auburn University, Auburn, AL, United States*

In December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-Cov2) emerged in Wuhan, China, sparking the Coronavirus disease 2019 (COVID-19) pandemic. The high prevalence of nutrition-related COVID-19 risk factors including obesity, type 2 diabetes, and hypertension, suggests that healthy dietary approaches may mitigate COVID-19 related outcomes and possibly SARS-CoV-2 infection. Based on the fundamental role of nutrition in immune function and the well-documented association between Mediterranean diet consumption and risk reduction for chronic diseases that are comorbidities in COVID-19 patients, we hypothesized that there would be a relationship between Mediterranean diet adherence and COVID-19 cases and related deaths. In this perspective, we examined the association between regional adherence to a Mediterranean diet and COVID-19 cases and deaths using an ecological study design. We observed that Mediterranean diet adherence was negatively associated with both COVID-19 cases and related deaths across 17 regions in Spain and that the relationship remained when adjusted for factors of well-being. We also observed a negative association between Mediterranean diet adherence and COVID-19 related deaths across 23 countries when adjusted for factors of well-being and physical inactivity. The anti-inflammatory properties of the Mediterranean diet - likely due to the polyphenol content of the diet - may be a biological basis to explain our findings. However, there are confounding factors unrelated to dietary factors driving COVID-19 cases and related deaths across the regions in Spain and the 23 countries examined in our analysis. Our findings will need to be confirmed and further explored in cohort studies.

Keywords: COVID-19, mediterranean diet, coronavirus, chronic disease, healthy diet, pandemic

OPEN ACCESS

Edited by:
Alexandros Tsoupras, University of Limerick, Ireland

Reviewed by:
Jose Paulo Andrade, Universidade do Porto, Portugal
Rosa Casas Rodriguez, Institut de Recerca Biomèdica August Pi i Sunyer (IDIBAPS), Spain

***Correspondence:**
Michael W. Greene
mwg0006@auburn.edu

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *05 August 2020*
Accepted: *10 February 2021*
Published: *05 March 2021*

Citation:
Greene MW, Roberts AP and Frugé AD (2021) Negative Association Between Mediterranean Diet Adherence and COVID-19 Cases and Related Deaths in Spain and 23 OECD Countries: An Ecological Study. Front. Nutr. 8:591964. doi: 10.3389/fnut.2021.591964

INTRODUCTION

Epidemics and pandemics have shaped the historys of humanity. Deaths from pandemics have ranged from up to half of the world's population in the Post-classical period to very few deaths in the Late Modern period (1). The Coronavirus disease 2019 (COVID-19) pandemic is just the most recent of a series of epidemics and pandemics that are arising (1) likely due to environmental

pressure from the expansion of human populations (2). Increasing population density and economic development, or westernization, has not only been associated with infectious diseases, but non-communicable diseases such as obesity, cardiovascular disease, and type 2 diabetes.

Consumption of a Western diet which includes high consumption of energy dense foods, such as simple sugars and fats, contributes to the development of obesity and pathophysiological chronic inflammation (3, 4) which are risk factors for the development of chronic diseases including cardiovascular disease, type 2 diabetes, and certain forms of cancer (5). In addition, obesity is associated with immunomodulatory effects related to infectious, gastrointestinal, and respiratory diseases (6). In contrast, essential micronutrients and polyphenolic compounds which are enriched in healthy diets are anti-inflammatory and may have benefits for risk reduction of communicable and non-communicable diseases (7–9). Adherence to a traditional Mediterranean diet, a plant-based diet rich in fresh fruits and vegetables, whole grains, nuts, fish and extra virgin olive oil, can reduce the risk of cardiovascular disease, type 2 diabetes, cognitive disorders, muscle atrophy and other signs of frailty (10–12). A Mediterranean diet is also rich in fiber which modulates nutrient absorption and satiety and contributes to maintaining a healthy weight (13).

Common comorbidities in COVID-19 patients include obesity, hypertension, cardiovascular disease, type 2 diabetes, chronic obstructive pulmonary disease, chronic kidney disease, cerebrovascular disease and cancer (14–16). Increased risk of COVID-19 related death - reported in a meta-analysis of studies from China and the USA - has been observed for patients with chronic renal disease (OR, 9.4; 95% CI, 3.2–27.4), cardiovascular disease (OR, 3.8; 95% CI, 2.1–6.9), hypertension (OR, 2.5; 95% CI, 2.1–3.1), type 2 diabetes (OR, 2.0; 95% CI, 1.7–2.3) (17), and in a separate analysis obesity (OR 1.7; 95% CI, 1.1–2.8) from a cohort in the USA (18). Obesity related comorbidities that increase the severity of COVID-19 include respiratory dysfunction resulting in lower lung volume, impaired gas exchange, and increased airway resistance (19). A greater association with comorbidities and in-hospital mortality has been observed in patients in America and Europe compared to Asia (20).

Because the Mediterranean diet is a dietary approach associated with overall well-being, and Mediterranean diet consumption is associated with risk reduction for the common comorbidities observed in COVID-19 patients, we hypothesized that Mediterranean diet adherence would be negatively associated with COVID-19 cases and related deaths. The absence of dietary data in COVID-19 patients precluded a direct examination of the relationship between Mediterranean diet adherence and COVID-19 cases and related deaths. Therefore, we undertook an ecological study to examine the association between regional adherence to a Mediterranean diet and COVID-19 cases and deaths.

ECOLOGICAL ANALYSIS

To determine whether there is relationship between Mediterranean diet adherence and COVID-19 cases and deaths we first chose to examine regional data from a country with a universal healthcare system to reduce confounding factors associated other healthcare models which may lack standardization of the COVID-19 response. We also sought to examine a country for which (1) regional differences in MD adherence have been reported, (2) regional COVID-19 cases and deaths, and (3) risk-modifying factors have been reported. Based on our criteria, we examined the relationship across 17 autonomous communities (regions) of Spain between Mediterranean Adequacy Index score (21) and COVID-19 cases and deaths as of June 9, 2020 (22). In an unadjusted linear model, we observed a significant negative association between Mediterranean Adequacy Index score and COVID-19 cases ($p = 0.023$) and COVID-19 related deaths ($p = 0.032$) (**Table 1**). We used a multivariable linear model to adjust for social determinants of health (factors of well-being) which can influence health outcomes (23–26). The factors of well-being (income, education, housing, environment, and life satisfaction) were assessed using the Organization for Economic Co-operation and Development (OECD) Well-Being Database in the 17 regions of Spain (27). As shown in **Table 1**, a stronger significant negative association between Mediterranean Adequacy Index score and COVID-19 cases ($r^2 = 0.663$) and COVID-19 related deaths ($r^2 = 0.729$) was observed. The well-being factors of income, education, and life satisfaction added significantly ($p < 0.05$) to the relationship between Mediterranean Adequacy Index score and both COVID-19 cases and COVID-19 related deaths.

To further explore the relationship between Mediterranean diet adherence and COVID-19 cases and deaths, we examined Mediterranean adherence and COVID-19 cases and deaths across 23 countries in the OECD Well-Being Database for which Mediterranean Adequacy Index score (from highest to lowest: Turkey, Greece, Italy, Japan, Chile, Spain, Portugal, Israel, Finland, Norway, Iceland, United Kingdom, Ireland, France, Denmark, Sweden, Canada, Hungary, Germany, Austria, Australia, Switzerland, and United States) (28) and COVID-19 cases and deaths per million people (from highest to lowest: United Kingdom, Spain, Italy, France, Ireland, United States, Canada, Switzerland, Portugal, Chile, Germany, Denmark, Sweden, Austria, Turkey, Finland, Hungary, Norway, Israel, Iceland, Greece, Japan, and Australia) (29). As shown in **Table 2**, we did not observe an association between Mediterranean Adequacy Index score and COVID-19 cases or COVID-19 related deaths in an unadjusted linear model. There was also not an association between Mediterranean Adequacy Index score and COVID-19 cases when adjusted for well-being factors in the OECD Well-Being Database. However, a significant negative association between Mediterranean Adequacy Index score and COVID-19 related deaths ($r^2 = 0.768$, $p = 0.004$) was observed when adjusted for well-being factors (income, education, housing, environment, and life satisfaction) in the OECD Well-Being Database and physical inactivity from the World Health Organization Global Health Observatory data repository (30). The well-being factors of education, housing, environment, and life satisfaction added significantly ($p < 0.05$) to the relationship between Mediterranean Adequacy Index score and COVID-19 related deaths. A sensitivity analysis was

TABLE 1 | Multivariable linear regression analysis assessing Mediterranean diet adherence and COVID-19 cases and deaths between regions in Spain‡ adjusted for well-being factors.

	Cases†				Deaths†			
	β	SE	*p*-value	Adj. R^2	β	SE	p-value	Adj. R^2
Univariate Model			0.023*	0.255			0.032*	0.224
MAI[a]	−0.037	0.014	0.023*		−0.004	0.002	0.032*	
Multivariate Model			0.006	0.663			0.002*	0.729
MAI[a]	−0.032	0.014	0.045*		−0.003	0.001	0.043*	
Income[b]	0.008	0.002	0.007*		0.001	2.3e-04	0.001*	
Education[c]	−0.003	0.001	0.046*		−3.6e-04	1.1e-04	0.008*	
Housing[d]	−0.002	0.001	0.161		−3.0e-04	1.2e-04	0.034*	
Environment[e]	0.001	0.002	0.504		2.0e-04	1.4e-04	0.175	
Life satisfaction[f]	−0.001	5.2e-04	0.046*		−1.3e-04	4.9e-05	0.022*	

‡*Regions of Spain; Andalusia, Aragon, Asturias, Balearic Islands, Basque Country, Canary Islands, Cantabria, Castile and Leon, Castile-La Mancha, Catalonia, Ceuta, Extremadura, Galicia, La Roja, Madrid, Melilla, Murcia, Navarra, Valencia.*

†*Cases and deaths were normalized to the population in the regions.*

**Significant results ($P < 0.05$).*

[a]*MAI; Mediterranean Adherence Index Scores.*

[b]*Income; Household disposable income per capita (in real USD PPP).*

[c]*Education; Share of labor force with at least secondary education (%).*

[d]*Housing; Number of rooms per person (ratio).*

[e]*Environment; Estimated average exposure to air pollution in PM2.5 ($\mu g/m^3$), based on satellite imagery data.* [f]*Life satisfaction; Average self-evaluation of life satisfaction on a scale from 0 to 10.*

TABLE 2 | Multivariable linear regression analysis assessing Mediterranean diet adherence and COVID-19 cases and deaths between Countries‡ adjusted for well-being factors.

	Cases†				Deaths†			
	β	SE	*p*-Value	Adj. R^2	β	SE	*p*-Value	Adj. R^2
Univariate Model			0.738	−0.042			0.817	−0.047
MAI[a]	−0.358	1.057	0.738		−0.024	0.102	0.817	
Multivariate Model			0.121	0.343			0.004*	0.768
MAI[a]	−1.487	2.685	0.593		−0.597	0.176	0.010*	
Income[b]	8.3e-05	1.3e-04	0.526		2.6e-06	7.8e-06	0.745	
Education[c]	−0.013	0.006	0.073		−0.014	0.003	0.003*	
Housing[d]	0.001	0.273	0.958		0.065	0.018	0.009*	
Environment[e]	−0.161	0.010	0.126		−0.037	0.005	0.001*	
Life satisfaction[f]	2.576	1.573	0.136		0.298	0.097	0.016*	
Physical inactivity[g]	0.066	0.076	0.406		5.2e-03	4.8e-03	0.318	

‡*Countries; Australia, Austria, Canada, Chile, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Norway, Portugal, Spain, Sweden, Switzerland, Turkey, United Kingdom, United States.*

†*Cases and deaths were normalized to the population in the regions.*

**Significant results ($P < 0.05$).*

[a]*MAI, Mediterranean Adherence Index Scores.*

[b]*Income; Household disposable income per capita (in real USD PPP).*

[c]*Education; Share of labor force with at least secondary education (%).*

[d]*Housing; Number of rooms per person (ratio).*

[e]*Environment; Estimated average exposure to air pollution in PM2.5 ($\mu g/m^3$), based on satellite imagery data.*

[f]*Life satisfaction; Average self-evaluation of life satisfaction on a scale from 0 to 10.*

[g]*Physical inactivity: Prevalence of insufficient physical activity among adults aged 18+ years (age-standardized estimate).*

performed by removing the Mediterranean Adequacy Index score from the model. We found that the absence of the Mediterranean Adequacy Index score significantly ($p < 0.01$) weakened the adjusted square of the coefficient of multiple correlation from 0.768 to 0.500 (data not shown).

In a sub-analysis of only European countries, we observed a similar significant negative association between Mediterranean Adequacy Index score and COVID-19 related deaths ($r^2 = 0.771$, $p = 0.030$) when adjusted for well-being factors and physical inactivity (data not shown). We next examined whether

the association between Mediterranean Adequacy Index score and COVID-19 related deaths was modified by adding country wide obesity prevalence (31) to the model adjusted for well-being factors and physical inactivity. We still observed a significant negative association ($r^2 = 0.751$, $p = 0.012$) between Mediterranean Adequacy Index score and COVID-19 related deaths; however, obesity prevalence did not significantly add to the model (data not shown).

DISCUSSION

In the current ecological study, we observed that Mediterranean diet adherence was negatively associated with both COVID-19 cases and related deaths across 17 regions in Spain. The relationship between Mediterranean diet adherence and COVID-19 cases and related deaths remained when adjusted for factors of well-being. We then examined the relationship between Mediterranean diet adherence and COVID-19 cases and related deaths across 23 countries for which data was available for Mediterranean diet adherence, COVID-19 cases and deaths, and factors of well-being. In the models adjusted for factors of well-being and physical inactivity we observed a negative association between Mediterranean diet adherence and COVID-19 related deaths. Our findings that the socioeconomic factors of income and education added significantly to our models is consistent with prior work that income and education are significant factors in viral infection related hospitalization (32–35) and Mediterranean diet adherence (23, 36–40). The finding that life satisfaction added significantly to the adjusted models is consistent with epidemiological evidence linking life satisfaction to risk of chronic disease and mortality (41–43). It is important to note that our findings with COVID-19 cases and deaths predated use and approval of medical treatments known to reduce mortality such as dexamethasone and remdesivir (44).

In the analysis of the 23 OECD countries we observed that the United States and the United Kingdom had the 1st and 11th lowest Mediterranean diet adherence, respectively, and the 6th and 1st highest COVID-19 related deaths per million, respectively. In contrast, Italy and Spain were outliers in the association between Mediterranean diet adherence and COVID-19 related deaths in the 25 OECD countries. Italy and Spain had the 3rd and 6th highest in Mediterranean Adequacy Index score, respectively, yet high COVID-19 mortality (3rd and 2nd highest in deaths per million people, respectively). Italy and Spain along with China, Iran, Germany, France, South Korea, and Japan were the eight earliest majorly effected countries in the COVID-19 pandemic. The doubling time of COVID-19 cases in Italy during the first and second 3-week interval was greatest among the eight countries while the growth rate of COVID-19 cases from the first to second 3-week interval in Spain was greatest among the eight countries (45). Whether poor early containment was a factor in the high COVID-19 death rate is not known. Factors ranging from socio-cultural to health systems likely have contributed to the high COVID-19 death rate in Italy and Spain (46–48). Interestingly, Lombardy, Italy, a region in northern Italy which has low Mediterranean diet adherence compared to southern Italy (38, 49, 50), was the epicenter of COVID-19 cases in Europe.

The current study design does not allow for causative conclusions to be reached on our findings. However, there is a biological basis for our findings. Severe complications of COVID-19 include acute respiratory distress syndrome and death. In the subset of patients with severe complications, a hyper inflammation (a.k.a. the cytokine storm) state is observed (51) possibility due to a dysregulated macrophage response (52). It has been proposed that comorbidities including obesity, type 2 diabetes, cardiovascular disease, and hypertension contribute to the inflammation response leading to severe complications of COVID-19 (53).

Adherence to plant-based dietary approaches, including the Mediterranean diet, are associated with a reduction in the risk of developing major chronic diseases including the COVID-19 comorbidities of obesity, type 2 diabetes, cardiovascular disease, and hypertension (54–59). Low-grade chronic inflammation underlies the COVID-19 comorbidities (60). Importantly, consumption of a Mediterranean diet is associated with a reduction in the inflammation, coagulation, and endothelial dysfunction markers including c-reactive protein (CRP), interleukin 6 (IL-6), fibrinogen, homocysteine, and E-selectin (61–63) which is consistent with findings of a reduction in inflammation markers across plant-based diet consumption (64). An assessment of overall inflammatory potential of a diet, the dietary inflammatory index (DII) (65, 66) has been shown to link dietary inflammation to communicable disease and inflammation-related disease. A high DII has been reported to be associated with higher risk for stomach and intestinal illness (67) and with increased systemic inflammation and lower lung function (68). Importantly, a high DII is associated with low grade inflammation and low Mediterranean diet score (69). It has been proposed that the anti-inflammatory properties of the Mediterranean diet are due to the high polyphenol content (70–72) from fruits, vegetables and extra virgin olive oil (73). In the SUN cohort, participants with the highest Mediterranean diet adherence and polyphenol intake of flavonoids had a significantly lower incidence of cardiovascular disease events compared to participants with the lowest Mediterranean diet adherence and polyphenol intake of flavonoids (74). Consistent with these findings, it was observed in high cardiovascular disease risk patients in the PREDIMED study that the greatest reduction in plasma levels of inflammatory markers occurred in participants with the highest urinary total polyphenols (75). It should be noted that the PREDIMED study did not have full randomization to the Mediterranean diet treatment groups (76).

There are limitations to the current study. Importantly, the ecological design of the current study does not allow for direct examination of the relationship between Mediterranean diet adherence and COVID-19 cases and related deaths. The observational methodology used may be a source of errors that could lead to wrong conclusions on the association between Mediterranean diet adherence and COVID-19 cases and related deaths. Thus, causation cannot be inferred from these findings. Additionally, the possible age effect of each group (the habits and behaviors of each stage of life are different) and the period effect

(events that occur over time that affect all age groups and can alter the association) are limitations. Further, the Mediterranean diet adherence data may not reflect current dietary patterns in the regions of Spain and the OECD countries examined. Even though Mediterranean diet adherence has declined in Spain and other OECD countries in the Mediterranean Sea basin compared to historical data from the 1960s, it has stabilized in the past decades (21, 28). In addition, Mediterranean diet adherence assessed at two time points in the past two decades highly correlates across countries ($r^2 = 0.969$, $p < 0.001$) (data not shown). We acknowledge that there are confounding factors related to societal norms, cultural factors, and the governmental response to the COVID-19 pandemic and differences in health care systems across countries that may influence COVID-19 cases and deaths in Spain and the countries examined in the current study. As the COVID-19 pandemic continues our findings represent a snapshot in time of the pandemic.

Even though our findings suggest a negative relationship between Mediterranean diet adherence and COVID-19 cases and related deaths, further studies are required to examine whether Mediterranean diet consumption reduces the risk of COVID-19 and/or chronic disease risk reduction associated with Mediterranean diet consumption reduces the risk of COVID-19 death. In conclusion, the Mediterranean diet and other dietary approaches that reduce inflammation and risk for chronic disease might reduce the risk for severe COVID-19 pathology and mortality.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be obtained from the corresponding author (mwgreene@auburn.edu).

AUTHOR CONTRIBUTIONS

MG conceived of the work, performed data and statistical analysis, contributed to the writing, and offered critical comments. AR performed data analysis, wrote the first draft of the manuscript, and provided critical revisions to the content. AF provided critical revisions to the content. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Alabama Agricultural Experiment Station and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture (Project number ALA044-1-18037) (MG).

REFERENCES

1. Morens DM, Daszak P, Markel H, Taubenberger JK. Pandemic COVID-19 joins history's pandemic legion. *Mbio.* (2020) 11:e00812–20. doi: 10.1128/mBio.00812-20
2. Di Marco M, Baker ML, Daszak P, De Barro P, Eskew EA, Godde CM, et al. Opinion: sustainable development must account for pandemic risk. *Proc Natl Acad Sci USA.* (2020) 117:3888–92. doi: 10.1073/pnas.2001655117
3. de Heredia FP, Gómez-Martínez S, Marcos A. Obesity, inflammation and the immune system. *Proc Nutr Soc.* (2012) 71:332–8. doi: 10.1017/S0029665112000092
4. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology.* (2007) 132:2169–80. doi: 10.1053/j.gastro.2007.03.059
5. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC public health.* (2009) 9:88. doi: 10.1186/1471-2458-9-88
6. Myles IA. Fast food fever: reviewing the impacts of the Western diet on immunity. *Nutr J.* (2014) 13:1–17. doi: 10.1186/1475-2891-13-61
7. Farhadi S, Ovchinnikov RS. The relationship between nutrition and infectious diseases: a review. *Biomed Biotechnol Res J.* (2018) 2:168. doi: 10.4103/bbrj.bbrj_69_18
8. Gombart AF, Pierre A, Maggini S. A review of micronutrients and the immune System–Working in harmony to reduce the risk of infection. *Nutrients.* (2020) 12:236. doi: 10.3390/nu12010236
9. González-Gallego J, García-Mediavilla MV, Sánchez-Campos S, Tuñón MJ. Fruit polyphenols, immunity and inflammation. *Br J Nutr.* (2010) 104:S15–27. doi: 10.1017/S0007114510003910
10. Romagnolo DF, Selmin OI. Mediterranean diet and prevention of chronic diseases. *Nutr Today.* (2017) 52:208. doi: 10.1097/NT.0000000000000228
11. Sánchez-Sánchez ML, García-Vigara A, Hidalgo-Mora JJ, García-Pérez M, Tarín J, Cano A. Mediterranean diet and health: a systematic review of epidemiological studies and intervention trials. *Maturitas.* (2020) 136:25–37. doi: 10.1016/j.maturitas.2020.03.008
12. Tosti V, Bertozzi B, Fontana L. Health benefits of the Mediterranean diet: metabolic and molecular mechanisms. *J Gerontol A Biol Sci Med Sci.* (2018) 73:318–26. doi: 10.1093/gerona/glx227
13. Chen J-P, Chen G-C, Wang X-P, Qin L, Bai Y. Dietary fiber and metabolic syndrome: a meta-analysis and review of related mechanisms. *Nutrients.* (2018) 10:24. doi: 10.3390/nu10010024
14. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. *JAMA.* (2020) 323:2052–9. doi: 10.1001/jama.2020.6775
15. Singh AK, Gillies CL, Singh R, Singh A, Chudasama Y, Coles B, et al. Prevalence of co-morbidities and their association with mortality in patients with COVID-19: a systematic review and meta-analysis. *Diabetes Obes Metab.* (2020) 22:1915–24. doi: 10.1111/dom.14124
16. Onder G, Rezza G, Brusaferro S. Case-Fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. *JAMA.* (2020) 323:1775–6. doi: 10.1001/jama.2020.4683
17. Tian W, Jiang W, Yao J, Nicholson CJ, Li RH, Sigurslid HH, et al. Predictors of mortality in hospitalized COVID-19 patients: a systematic review and meta-analysis. *J Med Virol.* (2020) 92:1875–83. doi: 10.1002/jmv.26050
18. Pettit NN, MacKenzie EL, Ridgway J, Pursell K, Ash D, Patel B, et al. Obesity is associated with increased risk for mortality among hospitalized patients with COVID-19. *Obesity.* (2020) 28:1806–10. doi: 10.1002/oby.22941
19. Stefan N, Birkenfeld AL, Schulze MB, Ludwig DS. Obesity and impaired metabolic health in patients with COVID-19. *Nat Rev Endocrinol.* (2020) 16:341–2. doi: 10.1038/s41574-020-0364-6
20. Goel S, Jain T, Hooda A, Malhotra R, Johal G, Masoomi R, et al. Clinical characteristics and in-hospital mortality for COVID-19 across the globe. *Cardiol Therapy.* (2020) 9:553–9. doi: 10.1007/s40119-020-00189-0
21. Bach-Faig A, Fuentes-Bol C, Ramos D, Carrasco JL, Roman B, Bertomeu IF, et al. The Mediterranean diet in spain: adherence trends during the past two decades using the mediterranean adequacy index. *Public Health Nutr.* (2011) 14:622–8. doi: 10.1017/S1368980010002752
22. Spain MdSMoHo. *Actualización n° 131. Enfermedad por el coronavirus (COVID-19).* Madrid (2020).

23. Lowcock EC, Rosella LC, Foisy J, McGeer A, Crowcroft N. The social determinants of health and pandemic H1N1 2009 influenza severity. *Am J Public Health.* (2012) 102:e51–8. doi: 10.2105/AJPH.2012.300814
24. Wong CM, Yang L, Thach TQ, Chau PYK, Chan KP, Thomas GN, et al. Modification by influenza on health effects of air pollution in Hong Kong. *Environ Health Perspect.* (2009) 117:248–53. doi: 10.1289/ehp.11605
25. Cordoba E, Aiello AE. Social determinants of influenza illness and outbreaks in the United States. *North Carolina Med J.* (2016) 77:341–5. doi: 10.18043/ncm.77.5.341
26. Marmot M. Social determinants of health inequalities. *Lancet.* (2005) 365:1099–04. doi: 10.1016/S0140-6736(05)71146-6
27. Regional well-being (Edition 2016) (2016). Available from: https://www.oecd-ilibrary.org/content/data/d147c81c-en (accessed January 13, 2021).
28. Vilarnau C, Stracker DM, Funtikov A, da Silva R, Estruch R, Bach-Faig A. Worldwide adherence to mediterranean diet between 1960 and 2011. *Eur J Clin Nutr.* (2019) 72:83–91. doi: 10.1038/s41430-018-0313-9
29. World Health Organization. *WHO Coronavirus Disease (COVID-19) Dashboard*. Geneva: World Health Organization (2020).
30. *Global Health Observatory Data Repository*. World Health Organization (2021). Available from: https://apps.who.int/gho/data/node.main.A893?lang=en (accessed January 16, 2021).
31. Abarca-Gómez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C, et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128· 9 million children, adolescents, and adults. *Lancet.* (2017) 390:2627–42. doi: 10.1016/S0140-6736(17)32129-3
32. Chandrasekhar R, Sloan C, Mitchel E, Ndi D, Alden N, Thomas A, et al. Social determinants of influenza hospitalization in the United States. *Influenza Other Respir Viruses.* (2017) 11:479–88. doi: 10.1111/irv.12483
33. Brownstein JS, Pena BG, Mandl KD. Characterizing socioeconomic disparities in the burden of influenza and RSV using surveillance data. *Adv Dis Surveill.* (2007) 2:94.
34. González-Candelas F, Astray J, Alonso J, Castro A, Cantón R, Galán JC, et al. Sociodemographic Factors and Clinical Conditions Associated to Hospitalization in Influenza A (H1N1) 2009 Virus Infected Patients in Spain, 2009–2010. *PLoS ONE.* (2012) 7:e33139. doi: 10.1371/journal.pone.0033139
35. Levy NS, Nguyen TQ, Westheimer E, Layton M. Disparities in the severity of influenza illness: a descriptive study of hospitalized and nonhospitalized novel H1N1 influenza–positive patients in New York City 2009–2010 Influenza Season. *J Public Health Manag Pract.* (2013) 19:16–24. doi: 10.1097/PHH.0b013e31824155a2
36. Cavaliere A, De Marchi E, Banterle A. Exploring the adherence to the Mediterranean diet and its relationship with individual lifestyle: the role of healthy behaviors, pro-environmental behaviors, income, and education. *Nutrients.* (2018) 10:141. doi: 10.3390/nu10020141
37. Knight CJ, Jackson O, Rahman I, Burnett DO, Frugé AD, Greene MW. The Mediterranean diet in the Stroke Belt: a cross-sectional study on adherence and perceived knowledge, barriers, and benefits. *Nutrients.* (2019) 11:1847. doi: 10.3390/nu11081847
38. Ruggiero E, Di Castelnuovo A, Costanzo S, Persichillo M, Bracone F, Cerletti C, et al. Socioeconomic and psychosocial determinants of adherence to the Mediterranean diet in a general adult Italian population. *Eur J Public Health.* (2019) 29:328–35. doi: 10.1093/eurpub/cky127
39. Tong TY, Imamura F, Monsivais P, Brage S, Griffin SJ, Wareham NJ, et al. Dietary cost associated with adherence to the Mediterranean diet, and its variation by socio-economic factors in the UK Fenland Study. *Br J Nutr.* (2018) 119:685–94. doi: 10.1017/S0007114517003993
40. Rodrigues S, Caraher M, Trichopoulou A, De Almeida M. Portuguese households' diet quality (adherence to Mediterranean food pattern and compliance with WHO population dietary goals): trends, regional disparities and socioeconomic determinants. *Eur J Clin Nutr.* (2008) 62:1263–72. doi: 10.1038/sj.ejcn.1602852
41. Koivumaa-Honkanen H, Honkanen R, Viinamäki H, Heikkilä K, Kaprio J, Koskenvuo M. Self-reported life satisfaction and 20-year mortality in healthy finnish adults. *Am J Epidemiol.* (2000) 152:983–91. doi: 10.1093/aje/152.10.983
42. Diener E, Chan MY. Happy people live longer: subjective well-being contributes to health and longevity. *Appl Psychol Health Well-Being.* (2011) 3:1–43. doi: 10.1111/j.1758-0854.2010.01045.x
43. Rosella LC, Fu L, Buajitti E, Goel V. Death and chronic disease risk associated with poor life satisfaction: a population-based cohort study. *Am J Epidemiol.* (2018) 188:323–31. doi: 10.1093/aje/kwy245
44. World Health Organization. *Clinical Management of COVID-19: Interim Guidance*. Geneva: World Health Organization (2020).
45. Khosrawipour V, Lau H, Khosrawipour T, Kocbach P, Ichii H, Bania J, et al. Failure in initial stage containment of global COVID-19 epicenters. *J Med Virol.* (2020) 92:863–7. doi: 10.1002/jmv.25883
46. Degiuli F. *Caring for a Living: Migrant Women, Aging Citizens, and Italian Families*. New York, NY: Oxford University Press (2016).
47. Legido-Quigley H, Mateos-García JT, Campos VR, Gea-Sánchez M, Muntaner C, McKee M. The resilience of the Spanish health system against the COVID-19 pandemic. *lancet Public Health.* (2020) 5:e251–2. doi: 10.1016/S2468-2667(20)30060-8
48. Armocida B, Formenti B, Ussai S, Palestra F, Missoni E. The Italian health system and the COVID-19 challenge. *Lancet Public Health.* (2020) 5:e253. doi: 10.1016/S2468-2667(20)30074-8
49. Noale M, Nardi M, Limongi F, Siviero P, Caregaro L, Crepaldi G, et al. Adolescents in southern regions of Italy adhere to the Mediterranean diet more than those in the northern regions. *Nutr Res.* (2014) 34:771–9. doi: 10.1016/j.nutres.2014.08.001
50. Pelucchi C, Galeone C, Negri E, La Vecchia C. Trends in adherence to the Mediterranean diet in an Italian population between 1991 and 2006. *Eur J Clin Nutr.* (2010) 64:1052–6. doi: 10.1038/ejcn.2010.158
51. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LF. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol.* (2020) 20:363–74. doi: 10.1038/s41577-020-0311-8
52. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol.* (2020) 20:355–62. doi: 10.1038/s41577-020-0331-4
53. de Lucena TMC, da Silva Santos AF, de Lima Fabrício BR, de Albuquerque Borborema ME, de Azevêdo Silva J. Mechanism of inflammatory response in associated comorbidities in COVID-19. *Diabetes Metab Syndr Clin Res Rev.* (2020) 14:597–600. doi: 10.1016/j.dsx.2020.05.025
54. Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *Am J Clin Nutr.* (2010) 92:1189–96. doi: 10.3945/ajcn.2010.29673
55. Estruch R, Ros E. The role of the Mediterranean diet on weight loss and obesity-related diseases. *Rev Endocr Metab Disord.* (2020) 21:315–27. doi: 10.1007/s11154-020-09579-0
56. Salas-Salvadó J, Becerra-Tomás N, Papandreou C, Bulló M. Dietary patterns emphasizing the consumption of plant foods in the management of type 2 diabetes: a narrative review. *Adv Nutr.* (2019) 10:S320–31. doi: 10.1093/advances/nmy102
57. Turner-McGrievy GM, Davidson CR, Wingard EE, Wilcox S, Frongillo EA. Comparative effectiveness of plant-based diets for weight loss: a randomized controlled trial of five different diets. *Nutrition.* (2015) 31:350–8. doi: 10.1016/j.nut.2014.09.002
58. Wright N, Wilson L, Smith M, Duncan B, McHugh P. The BROAD study: a randomised controlled trial using a whole food plant-based diet in the community for obesity, ischaemic heart disease or diabetes. *Nutr Diabetes.* (2017) 7:e256. doi: 10.1038/nutd.2017.3
59. Buckland G, Bach A, Serra-Majem L. Obesity and the Mediterranean diet: a systematic review of observational and intervention studies. *Obes Rev.* (2008) 9:582–93. doi: 10.1111/j.1467-789X.2008.00503.x
60. Scrivo R, Vasile M, Bartosiewicz I, Valesini G. Inflammation as "common soil" of the multifactorial diseases. *Autoimmun Rev.* (2011) 10:369–74. doi: 10.1016/j.autrev.2010.12.006
61. Chrysohoou C, Panagiotakos DB, Pitsavos C, Das UN, Stefanadis C. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: the ATTICA study. *J Am Coll Cardiol.* (2004) 44:152–8. doi: 10.1016/j.jacc.2004.03.039
62. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann*

Int Med. (2006) 145:1–11. doi: 10.7326/0003-4819-145-1-200607040-00004
63. Fung TT, McCullough ML, Newby P, Manson JE, Meigs JB, Rifai N, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr.* (2005) 82:163–73. doi: 10.1093/ajcn/82.1.163
64. Eichelmann F, Schwingshackl L, Fedirko V, Aleksandrova K. Effect of plant-based diets on obesity-related inflammatory profiles: a systematic review and meta-analysis of intervention trials. *Obes Rev.* (2016) 17:1067–79. doi: 10.1111/obr.12439
65. Cavicchia PP, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS, et al. A new dietary inflammatory index predicts interval changes in serum high-sensitivity c-reactive protein. *J Nutr.* (2009) 139:2365–72. doi: 10.3945/jn.109.114025
66. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr.* (2013) 17:1689–96. doi: 10.1017/S1368980013002115
67. Wirth MD, Robinson C, Murphy EA, Shivappa N, Hébert JR. The dietary inflammatory index is associated with gastrointestinal infection symptoms in the national health and nutrition examination survey. *Int J Food Sci Nutr.* (2020) 71:106–15. doi: 10.1080/09637486.2019.1614539
68. Wood LG, Shivappa N, Berthon BS, Gibson PG, Hebert JR. Dietary inflammatory index is related to asthma risk, lung function and systemic inflammation in asthma. *Clin Exp Allergy.* (2015) 45:177–83. doi: 10.1111/cea.12323
69. Shivappa N, Bonaccio M, Hebert JR, Di Castelnuovo A, Costanzo S, Ruggiero E, et al. Association of proinflammatory diet with low-grade inflammation: results from the moli-sani study. *Nutrition.* (2018) 54:182–8. doi: 10.1016/j.nut.2018.04.004
70. Razquin C, Martinez-Gonzalez MA. A traditional Mediterranean diet effectively reduces inflammation and improves cardiovascular health. *Nutrients.* (2019) 11:1842. doi: 10.3390/nu11081842
71. Bonaccio M, Pounis G, Cerletti C, Donati MB, Iacoviello L, de Gaetano G. Mediterranean diet, dietary polyphenols and low grade inflammation: results from the moli-sani study. *Br J Clin Pharmacol.* (2017) 83:107–13. doi: 10.1111/bcp.12924
72. Finicelli M, Squillaro T, Di Cristo F, Di Salle A, Melone MAB, Galderisi U, et al. Metabolic syndrome, Mediterranean diet, and polyphenols: evidence and perspectives. *J Cell Physiol.* (2019) 234:5807–26. doi: 10.1002/jcp.27506
73. Martínez-González MA, Salas-Salvadó J, Estruch R, Corella D, Fitó M, Ros E, et al. Benefits of the Mediterranean diet: insights from the PREDIMED study. *Prog Cardiovasc Dis.* (2015) 58:50–60. doi: 10.1016/j.pcad.2015.04.003
74. Mendonça RD, Carvalho NC, Martin-Moreno JM, Pimenta AM, Lopes ACS, Gea A, et al. Total polyphenol intake, polyphenol subtypes and incidence of cardiovascular disease: the SUN cohort study. *Nutr Metab Cardiovasc Dis.* (2019) 29:69–78. doi: 10.1016/j.numecd.2018.09.012
75. Medina-Remón A, Casas R, Tressserra-Rimbau A, Ros E, Martínez-González MA, Fitó M, et al. Polyphenol intake from a Mediterranean diet decreases inflammatory biomarkers related to atherosclerosis: a substudy of the PREDIMED trial. *Br J Clin Pharmacol.* (2017) 83:114–28. doi: 10.1111/bcp.12986
76. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a mediterranean diet supplemented with extra-virgin olive oil or nuts. *New Eng J Med.* (2018) 378:e34. doi: 10.1056/NEJMoa1800389

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Greene, Roberts and Frugé. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

MINI REVIEW
published: 08 March 2021
doi: 10.3389/fimmu.2021.648546

Vitamin D in Corona Virus Disease 2019 (COVID-19) Related Multisystem Inflammatory Syndrome in Children (MIS-C)

Gavriela Feketea[1,2†], Vasiliki Vlacha[3†], Ioana Corina Bocsan[4], Emilia Vassilopoulou[5], Luminita Aurelia Stanciu[6] and Mihnea Zdrenghea[1,7]*

[1] Department of Hematology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, [2] Department of Pediatrics, Pediatric Allergy Outpatient Clinic, "Karamandaneio", Children Hospital, Patras, Greece, [3] Department of Early Years Learning and Care, University of Ioannina, Ioannina, Greece, [4] Department of Pharmacology, Toxicology and Clinical Pharmacology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, [5] Department of Nutritional Sciences and Dietetics International Hellenic University, Thessaloniki, Greece, [6] National Heart and Lung Institute, Imperial College London, London, United Kingdom, [7] Ion Chiricuta Oncology Institute, Cluj-Napoca, Romania

OPEN ACCESS

Edited by:
Ioannis Zabetakis, University of Limerick, Ireland

Reviewed by:
Andres E. Carrillo, Chatham University, United States
Adele Costabile, University of Roehampton London, United Kingdom

***Correspondence:**
Gavriela Feketea
fechetea.gabriela@umfcluj.ro

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: *31 December 2020*
Accepted: *16 February 2021*
Published: *08 March 2021*

Citation:
Feketea G, Vlacha V, Bocsan IC, Vassilopoulou E, Stanciu LA and Zdrenghea M (2021) Vitamin D in Corona Virus Disease 2019 (COVID-19) Related Multisystem Inflammatory Syndrome in Children (MIS-C). Front. Immunol. 12:648546. doi: 10.3389/fimmu.2021.648546

Multisystem Inflammatory Syndrome in children (MIS-C) is a rare but devastating complication of coronavirus disease 19 (COVID-19). The development of prognostic biomarkers and more importantly the implementation of new treatment modalities would have a significant impact in clinical practice regarding the outcome of MIS-C. Vitamin D could be a potential candidate. In this mini review we analyze the immunomodulatory role of vitamin D in viral infections and specifically in COVID-19. We also examine the current literature regarding the association of vitamin D with MIS-C and Kawasaki disease. The vitamin D was evaluated not only as a biomarker but also as a nutritional supplement. We concluded that vitamin D levels could be valuable in predicting severe forms of MIS-C and correction of abnormal levels in severe MIS-C may influences its evolution. 25-hydroxyvitamin D3 [25(OH)D3] supplementation raising serum [25(OH)D] concentrations potentially have a favorable effect in reducing the severity of MIS-C in certain circumstances. Further studies are needed to confirm these results.

Keywords: vitamin D, 25(OH)D3, SARS-CoV-2 infection, COVID-19, multisystem, Kawasaki syndrome-like

KEY POINTS—QUESTIONS

Is vitamin D level a potential biomarker associated with prevalence and severity of Multisystem Inflammatory Syndrome in children (MIS-C)?

Could vitamin D supplementation affect severity and/or clinical course of MIS-C?

MEANING

The Multisystemic Inflammatory Syndrome in Children (MIS-C) is a devastating complication of COVID-19. Vitamin D as an easily measurable compound and also readily available and without significant side effects when it is administered could have a significant impact not only as a biomarker of the disease progression but also as a supplement for favorable evolution of patients with MIS-C.

INTRODUCTION

Coronavirus disease 19 (COVID-19) morbidity and severity in children as well as the incidence of Multisystem Inflammatory Syndrome in Children (MIS-C) clearly vary around the world. A number of factors may account for those differences. Several studies have reported a variety of comorbidities including overweight, asthma and ethnic origin black or Asian, and minority ethnic (BAME) groups as risk factors for MIS-C (1). These conditions are independently associated with Vitamin D (vit D) deficiency and that could be a possible explication for higher MIS-C incidence in these children. Vit D can reduce the risk of infections, through several mechanisms: (i) inducing cathelicidins and defensins that can lower viral replication rates, (ii). reducing concentrations of pro-inflammatory cytokines and (iii) increasing concentrations of anti-inflammatory cytokines (2). A negative correlation has been observed between mean level of vit D and the number of COVID-19 cases/1 M, in each country (3).

Identification of the children who are at risk to develop MIS-C after an asymptomatic/ mild COVID-19 or the ones exposed to severe acute respirator syndrome coronavirus 2 (SARS-CoV-2), and those at risk for a severe MIS-C, could help pediatricians to improve their medical management.

Observational studies have resulted in a proposed link between reduced levels of the circulating form of vit D, 25-hydroxyvitamin D [25(OH)D], and severe or critical infection of SARS-CoV-2 in adults (4).

Additionally, there is evidence of association between low levels of vit D and Kawasaki diseases in children (5). Kawasaki disease is a syndrome resulted from an excessive immune response to an infection. During the COVID-19 pandemic several cases of children with Kawasaki-like syndrome or MIS-C have been identified (6–8).

The objective of this mini-review was to evaluate the evidence regarding the association of vit D status and MIS-C.

Literature Review Strategy and Methods

A literature search has been performed in PubMed, Embase, Web of science and MedRxiv for relevant publications about the vit D and Coronavirus infection and Kawasaki disease published up to 30th December 2020. Keyword used were vit D in combination with coronavirus infection, SARS-CoV-2 infection, COVID-19, MIS-C, multisystem inflammatory syndrome, Kawasaki disease, Kawasaki-like syndrome. Information was derived from selected reviews and original articles published in peer-reviewed journals, from preliminary reports of works that have not yet been certified by peer review. Also, reference tracking was carried out to identify other relevant articles, which were not found during the initial searching. Additionally, current guidelines of International and National Health Organizations were retrieved and summarized. Almost all the published studies until now, regarding relationship between COVID-19 and vit D status included adult patients, and data referring to children and adolescents are extremely limited. Due to a reduce number of publications referring to vit D status in children infected by SARS-CoV-2, the authors also collected information from adults. The search using the words MIS-C and vitamin D revealed no studies in PubMed, Web of science, Embase and MedRxiv. Using the other keywords, the initial search yielded 73 articles, of which, after elimination of duplicates and screening of their titles and abstracts, 7 studies were considered relevant to this review.

Multisystem Inflammatory Syndrome in Children

Despite of low susceptibility to SARS-CoV-2 infection, especially those younger than 14 years, children are still at risk of infection (9).The prevalence of SARS-CoV-2 in children who are asymptomatic varied from 0 to 2.2% but there is a strong association between this prevalence and contemporaneous weekly incidence of COVID-19 in the general population (10). The children with SARS-CoV-2 infection usually are asymptomatic or suffer mild or moderate illness. It has been reported that the prevalence of severe or critical disease is 10.6, 7.3% and about 4% in children aged <1 year, 1–5years and 6–15 year, respectively (11). Few months after the onset of the pandemic, a series of reports around the world described clusters of children and adolescents presenting with a life-threatening, hyperinflammatory syndrome, named Kawasaki-like syndrome (8, 12), Pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) (13) or Multisystem Inflammatory Syndrome in Children (MIS-C) (14). The pathogenesis of MIS-C is still unknown, although it has been suggested that this syndrome occurs while the immune system is activated against the SARS-CoV-2 virus (15). The host immune system in pediatric patients responses excessively to SARS-CoV-2 infections and lead to the multisystem inflammatory syndrome in children (MIS-C). MIS-C is a new childhood disease linked to SARS-CoV-2 infection. It is a dangerous systemic inflammation characterized by fever, abdominal symptoms, conjunctivitis, and rash which appear three to 4 weeks after the initial infection (16). The laboratory evidence of inflammation has been defined by one or more of the following biomarkers: an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, d-dimer, ferritin, lactic acid dehydrogenase (LDH), or interleukin 6 (IL-6), elevated neutrophils, reduced lymphocytes and low albumin (17). Testing for SARS-CoV-2 infection including RT-PCR, antigen test and IgM/IgG antibody is suggested when COVID-19 or MIS-C is suspected (9).

A recent published study demonstrated that MIS-C hyperinflammation differs from severe acute COVID-19 hyperinflammation of adults, as well from the original Kawasaki disease (18). Consiglio et al. found that elevated IL-6, IL-17A, CXCL10 contributed the most to the cytokine storm of MIS-C, while IL-17A an important cytokine in Kawasaki disease, was significantly lower in MIS-C (18).

A systematic review compared characteristics between MIS-C and pediatric confirmed COVID-19 cases, found that the level of inflammation experienced in MIS-C outweigh the inflammation related to COVID-19 (16).

Vitamin D: Vitamin—Hormone. Similarity With Other Steroid Hormones

Despite that its name is "vitamin," in fact vit D is a fat-soluble steroid pre-hormone produced in skin from UV-B exposure (19). Hormones are defined as chemical signals synthesized and secreted into the bloodstream. They act on distant tissues usually in a regulatory fashion. Their action is often associated with binding soluble proteins (20). Steroid hormones are the sex steroids (estrogens, progesterone -female and androgens -male), the mineralo-corticoids, the glucocorticoids, and the vit D with its daughter metabolites (21).

Vit D3 (cholecalciferol) is synthetized in the skin under UV-B radiation from the sun but also derived from intake of animal food and vit D3 supplements, while vit D2 (ergochalciferol) is derived only from intake of vegetable food and vit D2 supplementation. Both are initially hydroxylated in the liver by (CYP2R1, CYP27A1, CYP3A4), resulting the inactive circulating form 25-hydroxyvitamin D [25(OH)D, calcidiol]. This form is then hydroxylated in the kidney by the 1α-hydroxylase enzyme [1α(OH)ase, CYP27B1] into calcitriol, the active form of vit D (22). The immune cells are able to locally convert 25(OH)D3 into its active form—1,25(OH)2D3 (19). 1,25(OH)2D3 (1,25-dihydroxyvitamin D3, calcitriol) acts as a circulating hormone and is the most potent natural ligand of the vit D receptor (VDR) of target cells. Also, 1,25(OH)2D3 has been described as an immunomodulator targeting various immune cells, including monocytes, macrophages, dendritic cells (DCs), as well as T-lymphocytes and B-lymphocytes. For that reason, 1,25(OH)2D3 has characterized as modulator of both innate and adaptive immune responses (23). Severe infections, including COVID-19, can lead to consumption of vit D intracellularly during the immune processes. As a result of that the utilization is faster than the production.

Vitamin D and Infections in Children

Relationship Between Vitamin D and Viral Infections in Children

Kassas et al. shown that lower concentration of serum vit D may be significantly associated with neonatal pneumonia. The authors concluded that the vit D level could predict the need for mechanical ventilation and the duration of hospitalization in neonates with pneumonia (24). In a randomized clinical trial (RCT) conducted among children aged 1 through 5 years, the participants were randomized to receive 2,000 IU/d of vitamin D oral supplementation (high-dose group) or 400 IU/d (standard-dose group) for a minimum of 4 months during the winter and has been found no statistically significant difference in several laboratory-confirmed upper respiratory tract infections between the two groups (25). Recently, an updated meta-analysis of data from RCTs of vit D for the prevention of respiratory infections in children and adults reported an overall protective effect of the intervention with modest statistically significance and found that the daily administration of standard doses for up to 12 months was the most beneficial regime (26). While previous meta-analysis shown a protective effect of vit D supplementation among those with the lowest baseline vit D level, this was not observed in this update (27). Forno et al. performed a randomized, double-blind, placebo-controlled clinical trial of vit D_3 supplementation in high-risk children with asthma aged 6 to 16 years taking low-dose inhaled corticosteroids. The subjects had serum 25(OH)D levels <30 ng/ml. Compared to placebo, vit D_3 supplementation did not significantly improve the time to a first viral-induced severe exacerbation (28).

Relationship Between Vitamin D and SARS-CoV-2 Infection in Children

Since the beginning of the pandemic, despite the lack of direct evidence of an effect of vit D on SARS-CoV-2 infections, it had been suggested the vit D supplementation could be a safe, inexpensive and readily available modifiable intervention for COVID-19 (29). Subsequently, a growing evidence has been published regarding adults, suggesting that serum vit D levels could be valuable in preventing SARS-CoV-2 infection and predicting not only COVID-19 mortality rates but also linearly predicting COVID-19 illness severity (30). However, according to others, there is not enough evidence on the association between vit D levels and COVID-19 severity and mortality (31).

An association but not necessarily a causation, between vit D levels and SARS-CoV-2 infection in adults were found in a number of studies. Ilie et al. searched the potential association between mean levels of vit D in 20 European countries and morbidity and mortality caused by COVID-19. It has been showed that the higher the vit D levels in a specific country was associated not only with lower number of cases diagnosed with COVID-19 but also with lower mortality per million population in that country (3). Additionally, vit D levels seems to be associated with a worse progression of the course of COVID-19 in the hospitalized patients (32). It still remains unclear the exact mechanism of the relationship between vitamin D and SARS-CoV-2 in younger people.

Multisystem Inflammatory Syndrome in Children (MIS-C) and Vitamin D

The risk factors for development of MIS-C (16) correspond with those for occurrence of low vit D levels in children of African American, Afro-Caribbean descent and those related to the children's obesity (33–35).

Proposed Mechanism

In adults, retrospective studies demonstrated a correlation between vit D status and COVID-19 severity and mortality. Whether the vit D deficiency in COVID-19 is a cause or a result is still difficult to postulate based on the present evidence. Since vit D has been shown to have immunomodulatory activity on IL-6, and anti-IL-6 agents has already demonstrated beneficial role in the course of COVID-19, it has been speculated that administration of vit D supplementation could be beneficial for COVID-19 progression (36, 37). Up to date, no published research has addressed the relationship between vit D and MIS-C, compared with the extended number of published works regarding SARS-CoV-2 infection in adults.

The possible role of vit D in SARS-CoV-2 infection in children could be explained initially by its antiviral activities.

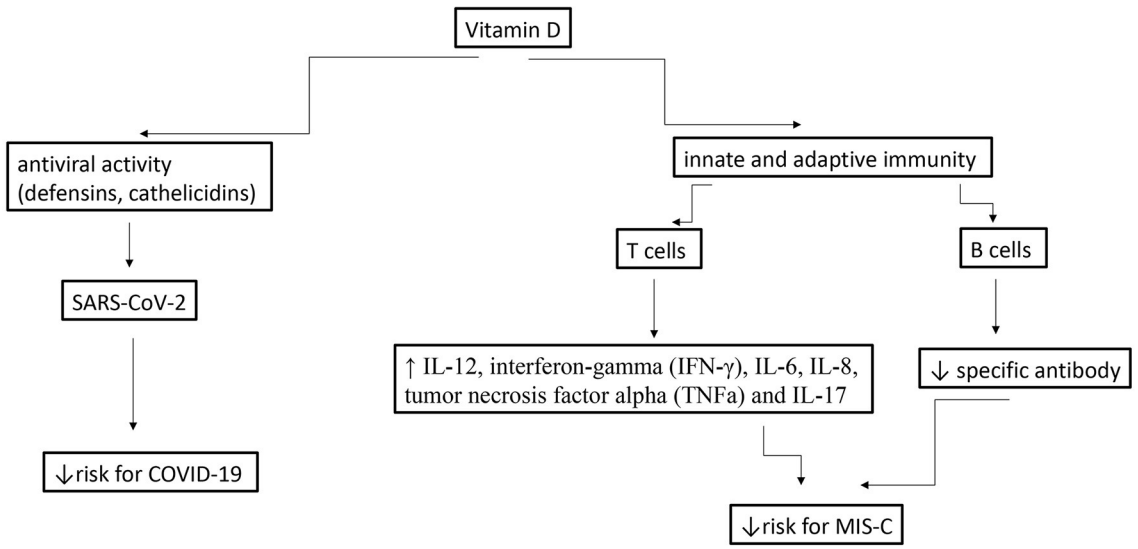


FIGURE 1 | Potential effects of vitamins D during a SARS-CoV-2 infection in children and MIS-C [modified from (46)].

The antiviral properties of vit D have successfully been tested in a wide range of viral species and in different settings. Vit D potency has been investigated against influenza infection in children (38). The administration of vit D effectively reduced cytokine levels and mortality in severe critical-ill patients (30). Furthermore, vit D significantly increased rhinovirus 1B-, rhinovirus 16- and RSV-induced interferons and interferon-stimulated gene mRNA expression and protein production. It also substantially diminished rhinovirus replication and release (39). Even though a specific antiviral role of vit D against SARS-CoV2 has not been established up to now, it can possibly has an adverse effect against viral coinfections. It has been recently reported that the rate of coinfection in COVID-19 reaches 20.5%. The most common co-infections were rhinovirus/enterovirus (6.9%), RSV (5.2%), and non–SARS-CoV-2 Coronaviridae (4.3%) (40).

Anti-inflammatory Action of Vitamin D

Since MIS-C generally occurs 3 to 4 weeks after a SARS-CoV-2 infection, consists a host-dependent reaction to a past infections (41), and this is compatible with antibody- and/or immunocomplex-mediated disease (42), we speculate that in this condition, vit D most likely plays an immunomodulatory role rather than an antiviral one. Furthermore, in the acute phase of MIS-C has been observed high levels of interleukin-1β (IL-1β), IL-6, IL-8, IL-10, IL-17, interferon-gamma (IFN-γ) and differential T and B cell subset lymphopenia (43). Vit D modulates both innate and adaptive immunity and may potentially prevents or reduces the complications associated with SARS-CoV-2 infection by increasing concentrations of anti-inflammatory cytokines (IL-10), Th2 cytokines IL-4 and IL-5, as well as by reducing concentrations of pro-inflammatory cytokines: IL-12, interferon-gamma (IFN-g), IL-6, IL-8, tumor necrosis factor alpha (TNFa) and IL-17 (44), (45). [**Figure 1**- modified from (46)].

Several studies shown that vit D can reduce the risk and/or the gravity of certain inflammatory reactions. Meyer et al. showed that vit D supplementation had a protective effect in association with the occurrence of Kawasaki disease in a German population (47). In a Korean retrospective review, the medical records of 91 patients with Kawasaki disease were analyzed. The patients were grouped according to vit D serum level (above and below 20ng/ml). The vit D deficient group showed statistical significant resistance to intravenous immunoglobulin ($p = 0.023$) (48). Stagi et al. assessing serum levels of 25(OH)D in 79 children with Kawasaki disease found significantly lower levels in comparison with controls (9.17 ± 4.94 vs. 23.3 ± 10.6 ng/ml, $p < 0.0001$). Vit D levels were correlated with erythrocyte-sedimentation rate ($p < 0.0001$), C-reactive protein ($p < 0.0001$), and hemoglobin level ($p < 0.0001$). Those patients who developed coronary artery abnormalities had further significantly lower vit D levels (49). Moreover, low 25(OH)D levels might contribute to the development of coronary arterial lesions (50), severity of coronary aneurysms in patients with Kawasaki disease (5) and it seems to be associated with resistance to intravenous immunoglobulin therapy (48). Activation of the STING (stimulator of interferon genes) pathway which contribute to over-immune response observed in Kawasaki disease and COVID-19, is inhibited by aspirin, intravenous immunoglobulins, vit D and anti-IL-6, potentially being beneficial in these conditions (51). Also, Boonstra et al. demonstrated that vit D3 affects Th cell polarization by inhibiting Th1 (IFN-gamma production) and augmenting Th2 cell development and consequently that results in production of their specific anti-inflammatory cytokines (52). Thus, vit D would limit the production and damaging effect of the pro-inflamatory cytokines during the MIS-C. Despite that the proof of this theory is currently insufficient, we propose measuring the vit D level at diagnosis and subsequently during the course of MIS-C in order to monitor the disease progression.

However, it is unknown as to whether vit D deficiency contributes to, or is a consequence of MIS-C. The systemic inflammation lowers circulating 25(OH)D levels (53) and this mechanism may contribute to the 25(OH)D deficiency observed in patients suffering from infectious diseases, including COVID-19 (54).

Vitamin D as Biomarker in MIS-C

The more severe is the disease, the greater is the inflammatory process and subsequently the greater is the need for vit D (active form) with an anti-inflammatory role. Therefore, the consumption of vit D in the cells involved in immunomodulation is increased, resulting in reduced vit D level in serum (inactive form). Through this perspective, the low level of serum vit D in severe disease is a result of severity and not a predisposing factor for. Inflammatory reactions involved in the process of the diseases would reduce 25(OH)D, which would explain why low vit D levels are reported in a wide range of disorders (55, 56).

A recent study has been shown that the profiles of seven cytokines, indicated treatment responses, could be useful markers to monitor MIS-C patients undergoing treatment (18). Several of these cytokines could be decreased by vit D (45). It has yet to be shown in the clinical practice whether vit D level could be used as a biomarker for the severity of COVID-19 and MIS-C. Serial measurements during the progression of the disease could be useful in clinical practice. Additionally, the association of vit D levels with other inflammatory markers (CRP, ERS) and inflammatory cytokines (IL-6) would help clinicians to manage these.

Vitamin D Supplementation in MIS-C

The rational of vit D supplementation in SARS-CoV-2 infection in children is based on the impact of vit D status on infections due to influenza viruses. Studying the effect of 1,200 IU/day of vit D vs. placebo on the incidence of seasonal influenza A and B among children aged 6–15 years, Urashima et al. showed reduced incidence of influenza A but not of influenza B infection (38). It seems that depending on the type of virus, the alterations at the molecular level may differ, and consequently the effect of vit D supplementation. During the 2009 pandemic of the H1N1, vit D3 supplementation did not lower the overall incidence of influenza A despite the initial benefit during the first month of treatment (57). The relationship between vit D and MIS-C and the significance of the nutritional supplementation must be proven. Vit D status has already recommended to be determined (58) and correction of low levels has been suggested (59). For this purpose, it would be useful to measure a baseline vit D level as soon as the SARS-CoV-2 infection has been detected. Then, sequential measurements, during the disease would be performed and be compared to the other inflammatory indices and to the evolution of the disease.

Increased liver enzymes during MIS-C suggests to some degree that the disturbance of liver function may reflect in the reduction of vit D 25 hydroxylation at this level. Therefore, the direct administration of the 25(OH)D3 (calcifediol) rather than vit D3 can offer a rapidly improvement of vitamin D status overcoming this impediment. Providing 25(OH)D3 to cells of immune system may contribute to reducing the severity and favorable evolution of MIS-C, particularly in children with low vit D levels. Of 50 adult patients hospitalized with COVID-19 infection and treated with of a high dose of calcifediol, one required admission to the ICU, while of 26 untreated patients, 13 required ICU admission. All patients in this study received a combination of hydroxychloroquine and azithromycin (60). The therapeutic efficacy of rapidly correcting vit D deficiency with the use of 25-hydroxyvitamin D3 [25(OH)D3] is under investigation in a clinical trial (NCT04386850) in adult patients with COVID-19. It remains to be demonstrated whether the administration of vit D supplementation is related to a potentially favorable evolution of MIS-C.

CONCLUSIONS

In clinical practice, new biomarkers are needed for early identification of those children with MIS-C who are at increased risk of severe forms. Vit D levels could be an appropriate candidate. Additionally, correction of abnormal levels of vit D in these severe forms of MIS-C may possibly influences their evolution. 25(OH)D3 supplementation in order to raise promptly serum [25(OH)D] concentrations potentially contributes in reducing the severity of MIS-C in certain circumstance.

FURTHER RESEARCH

Further research is needed in this area to confirm the role vit D in MIS-C. We have to establish, through randomized controlled trials, the importance of vit D levels in prediction of MIS-C evolution. Additionally, is needed to be confirmed whether supplementation with vit D3 or 25(OH)D3 could prevent the development, progression or severity of MIS-C.

AUTHOR CONTRIBUTIONS

GF and VV had the conception, designed the work, and collected the data. GF, VV, IB, and EV contributed to the data analysis, its interpretation and to the article's writing and editing. LS, EV, and MZ made the critical revision of the article. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Tsabouri S, Makis A, Kosmeri C, Siomou E. Risk factors for severity in children with coronavirus disease 2019: a comprehensive literature review. *Pediatr Clin North Am.* (2021) 68:321–38. doi: 10.1016/j.pcl.2020.07.014
2. Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, et al. Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. *Nutrients*. (2020) 12:988. doi: 10.3390/nu12040988
3. Ilie PC, Stefanescu S, Smith L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. *Aging Clin Exp Res.* (2020) 32:1195–8. doi: 10.1007/s40520-020-01570-8
4. Vassiliou AG, Jahaj E, Pratikaki M, Orfanos SE, Dimopoulou I, Kotanidou A. Low 25-Hydroxyvitamin D levels on admission to the intensive care

unit may predispose COVID-19 pneumonia patients to a higher 28-day mortality risk: a pilot study on a Greek ICU cohort. *Nutrients.* (2020) 12:3773. doi: 10.3390/nu12123773
5. Falcini F, Stagi S, Lepri G, Casalini E, Rigante D, Matucci-Cerinic M. SAT0505 severe vitamin D deficiency in patients with kawasaki disease: its possible role in the risk to develop coronary artery damage. *Ann Rheum Dis.* (2015) 74(Suppl 2):843. doi: 10.1136/annrheumdis-2015-eular.5784
6. Davies P, Evans C, Kanthimathinathan HK, Lillie J, Brierley J, Waters G, et al. Intensive care admissions of children with paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) in the UK: a multicentre observational study. *Lancet Child Adolesc Health.* (2020) 4:669–77. doi: 10.1016/S2352-4642(20)30215-7
7. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet.* (2020) 395:1607–8. doi: 10.1016/S0140-6736(20)31094-1
8. Verdoni L, Mazza A, Gervasoni A, Martelli L, Ruggeri M, Ciuffreda M, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet.* (2020) 395:1771–8. doi: 10.1016/s0140-6736(20)31103-x
9. Viner RM, Mytton OT, Bonell C, Melendez-Torres GJ, Ward J, Hudson L, et al. Susceptibility to SARS-CoV-2 infection among children and adolescents compared with adults: a systematic review and meta-analysis. *JAMA Pediatr.* (2020)175:143–56. doi: 10.1001/jamapediatrics.2020.4573
10. Sola AM, David AP, Rosbe KW, Baba A, Ramirez-Avila L, Chan DK. Prevalence of SARS-CoV-2 infection in children without symptoms of coronavirus disease 2019. *JAMA Pediatr.* (2020) 175:198–201. doi: 10.1001/jamapediatrics.2020.4095
11. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in china. *Pediatrics.* (2020) 145:e20200702. doi: 10.1542/peds.2020-0702
12. Viner RM, Whittaker E. Kawasaki-like disease: emerging complication during the COVID-19 pandemic. *Lancet.* (2020) 395:1741–3. doi: 10.1016/s0140-6736(20)31129-6
13. Whittaker E, Bamford A, Kenny J, Kaforou M, Jones CE, Shah P, et al. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. *JAMA.* (2020) 324:259–69. doi: 10.1001/jama.2020.10369
14. Ashkan B, Abdolreza M, Nasrin M, Zahra Abbasi S. COVID-19 associated multisystem inflammatory syndrome: a systematic review and meta-analysis. *Iran J Allergy Asthma Immunol.* (2020) 19:570–88. doi: 10.18502/ijaai.v19i6.4927
15. Licciardi F, Baldini L, Denina M, Ricotti E, Covizzi C, Dellepiane M, et al. Peculiar immunophenotypic signature in MIS-C affected children. *Pediatr Allergy Immunol.* (2020). doi: 10.1111/pai.13434
16. Ahmed M, Advani S, Moreira A, Zoretic S, Martinez J, Chorath K, et al. Multisystem inflammatory syndrome in children: a systematic review. *EClinMed.* (2020) 26:100527. doi: 10.1016/j.eclinm.2020.100527
17. C. f. DC. a. Prevention: Information for Healthcare Providers about Multisystem Inflammatory Syndrome in Children (MIS-C). In: *Multisystem Inflammatory Syndrome (MIS-C)* (2020).
18. Consiglio CR, Cotugno N, Sardh F, Pou C, Amodio D, Rodriguez L, et al. The immunology of multisystem inflammatory syndrome in children with COVID-19. *Cell.* (2020) 183:968–81.e7. doi: 10.1016/j.cell.2020.09.016
19. Sassi F, Tamone C, D'Amelio P. Vitamin D: nutrient, hormone, and immunomodulator. *Nutrients.* (2018) 10:1656. doi: 10.3390/nu10111656
20. Melmed S, Auchus, RJ, Goldfine AB, Koenig RJ, Rosen CJ. *Williams Textbook of Endocrinology, 14th ed.*. Philadelphia, PA: Elsevier-Health Science Division (2019) p. 1792.
21. Norman W. Hormones steroid hormones. In: Caballero B, editor. *Encyclopedia of Food Sciences Nutrition*. 2nd ed. Oxford: Academic Press (2003).
22. Ohyama Y, Yamasaki T. Eight cytochrome P450s catalyze vitamin D metabolism. *Front Biosci.* (2004) 9:3007–18. doi: 10.2741/1455
23. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. *Curr Opin Pharmacol.* (2010) 10:482–96. doi: 10.1016/j.coph.2010.04.001
24. El-Kassas GM, El Wakeel MA, Elabd MA, Kamhawy AH, Atti MA, El-Gaffar S, et al. *Vitamin D status in neonatal pulmonary infections: relationship to inflammatory indicators. Open Access Maced J Med Sci.* (2019) 7:3970–4. doi: 10.3889/oamjms.2019.592
25. Aglipay M, Birken CS, Parkin PC, Loeb MB, Thorpe K, Chen Y, et al. Effect of high-dose vs standard-dose wintertime vitamin D supplementation on viral upper respiratory tract infections in young healthy children. *JAMA.* (2017) 318:245–54. doi: 10.1001/jama.2017.8708
26. Jolliffe DA, Camargo CA, Sluyter JD, Aglipay M, Aloia JF, Ganmaa D, et al. Vitamin D supplementation to prevent acute respiratory infections: systematic review and meta-analysis of aggregate data from randomised controlled trials. *medRxiv [Preprint].* (2020). doi: 10.1101/2020.07.14.20152728
27. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ.* (2017) 356:i6583. doi: 10.1136/bmj.i6583
28. Forno E, Bacharier LB, Phipatanakul W, Guilbert TW, Cabana MD, Ross K, et al. Celedón: effect of vitamin D3 supplementation on severe asthma exacerbations in children with asthma and low vitamin D levels: the VDKA randomized clinical trial. *JAMA.* (2020) 324:752–60. doi: 10.1001/jama.2020.12384
29. Zemb P, Bergman P, Camargo CA, Jr Cavalier E, Cormier C, Courbebaisse M, et al. Vitamin D deficiency and the COVID-19 pandemic. *J Glob Antimicrob Resist.* (2020) 22:133–4. doi: 10.1016/j.jgar.2020.05.006
30. Benskin LL. A basic review of the preliminary evidence that COVID-19 risk and severity is increased in vitamin D deficiency. *Front Public Health.* (2020) 8:513. doi: 10.3389/fpubh.2020.00513
31. Ali N. Role of vitamin D in preventing of COVID-19 infection, progression and severity. *J Infect Public Health.* (2020)13:1373–80. doi: 10.1016/j.jiph.2020.06.021
32. Radujkovic A, Hippchen T, Tiwari-Heckler S, Dreher S, Boxberger M, Merle U. Vitamin D deficiency and outcome of COVID-19 patients. *Nutrients.* (2020) 12:2757. doi: 10.3390/nu12092757
33. Holick MF. Bioavailability of vitamin D and its metabolites in black and white adults. *N Engl J Med.* (2013) 369:2047–8. doi: 10.1056/NEJMe1312291
34. Hibbs AM, Ross K, Kerns LA, Wagner C, Fuloria M, Groh-Wargo S, et al. Effect of vitamin D supplementation on recurrent wheezing in black infants who were born preterm: the D-wheeze randomized clinical trial. *JAMA.* (2018) 319:2086–94. doi: 10.1001/jama.2018.5729
35. Zakharova I, Klimov L, Kuryaninova V, Nikitina I, Malyavskaya S, Dolbnya S, et al. Vitamin D Insufficiency in overweight and obese children and adolescents. *Front Endocrinol.* (2019) 10:103. doi: 10.3389/fendo.2019.00103
36. Jain A, Chaurasia R, Sengar NS, Singh M, Mahor S, Narain S. Analysis of vitamin D level among asymptomatic and critically ill COVID-19 patients and its correlation with inflammatory markers. *Sci Rep.* (2020) 10:20191. doi: 10.1038/s41598-020-77093-z
37. Mercola J, Grant WB, Wagner CL. Evidence regarding vitamin D and risk of COVID-19 and its severity. *Nutrients.* (2020) 12:3361. doi: 10.3390/nu12113361
38. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am J Clin Nutr.* (2010) 91:1255–60. doi: 10.3945/ajcn.2009.29094
39. Telcian AG, Zdrenghea MT, Edwards MR, Laza-Stanca V, Mallia P, Johnston SL, et al. Vitamin D increases the antiviral activity of bronchial epithelial cells in vitro. *Antiviral Res.* (2017) 137:93–101. doi: 10.1016/j.antiviral.2016.11.004
40. Kim D, Quinn J, Pinsky B, Shah NH, Brown I. Rates of co-infection between SARS-CoV-2 and other respiratory pathogens. *JAMA.* (2020) 323:2085–6. doi: 10.1001/jama.2020.6266
41. Nijman RG, De Guchtenaere A, Koletzko B, Ross Russell R, Copley S, Titomanlio L, et al. Pediatric inflammatory multisystem syndrome: statement by the pediatric section of the European society for emergency medicine and European academy of pediatrics. *Front Pediatr.* (2020) 8:490. doi: 10.3389/fped.2020.00490
42. Martinez OM, Bridges ND, Goldmuntz E, Pascual V. The immune roadmap for understanding multi-system inflammatory syndrome

in children: opportunities and challenges. *Nat Med.* (2020) 26:1819–24. doi: 10.1038/s41591-020-1140-9
43. Carter MJ, Fish M, Jennings A, Doores KJ, Wellman P, Seow J, et al. Shankar-Hari: Peripheral immunophenotypes in children with multisystem inflammatory syndrome associated with SARS-CoV-2 infection. *Nat Med.* (2020) 26:1701–7. doi: 10.1038/s41591-020-1054-6
44. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol.* (2012) 188:2127–35 doi: 10.4049/jimmunol.1102412
45. Greiller CL, Martineau AR. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients.* (2015) 7:4240–70. doi: 10.3390/nu7064240
46. Feketea G, Bocsan CI, Stanciu LA, Buzoianu AD, Zdrenghea MT. The role of vitamin D deficiency in children with recurrent wheezing—clinical significance. *Front Pediatr.* (2020) 8:344. doi: 10.3389/fped.2020.00344
47. Meyer K, Volkmann A, Hufnagel M, Schachinger E, Klau S, Horstmann J, et al. Breastfeeding and vitamin D supplementation reduce the risk of Kawasaki disease in a German population-based case-control study. *BMC Pediatrics.* (2019) 19:66. doi: 10.1186/s12887-019-1438-2
48. Jun JS, Jung YK, Lee DW. Relationship between vitamin D levels and intravenous immunoglobulin resistance in Kawasaki disease. *Korean J Pediatr.* (2017) 60:216–20. doi: 10.3345/kjp.2017.60.7.216
49. Stagi S, Rigante D, Lepri G, Matucci Cerinic M, Falcini F. Severe vitamin D deficiency in patients with Kawasaki disease: a potential role in the risk to develop heart vascular abnormalities? *Clin Rheumatol.* (2016) 35:1865–72. doi: 10.1007/s10067-015-2970-6
50. Chen YL, Wang JL, Li WQ. Prediction of the risk of coronary arterial lesions in Kawasaki disease by serum 25-hydroxyvitamin D3. *Eur J Pediatr.* (2014) 173:1467–71. doi: 10.1007/s00431-014-2346-y
51. Berthelot JM, Drouet LF. Lioté: Kawasaki-like diseases and thrombotic coagulopathy in COVID-19: delayed over-activation of the STING pathway? *Emerg Microbes Infect.* (2020) 9:1514–22. doi: 10.1080/22221751.2020.1785336
52. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF A. O'Garra: 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J Immunol.* (2001) 167:4974–80. doi: 10.4049/jimmunol.167.9.4974
53. Smolders JJ. van den Ouweland, Geven C, Pickkers P, Kox M. *Letter to the Editor: Vitamin D deficiency in COVID-19: mixing up cause and consequence. Metabolism.* (2020) 154434. doi: 10.1016/j.metabol.2020.154434
54. Yilmaz K, Sen V. Is vitamin D deficiency a risk factor for COVID-19 in children? *Pediatr Pulmonol.* (2020) 55:3595–601. doi: 10.1002/ppul.25106
55. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. *Lancet Diabetes Endocrinol.* (2014) 2:76–89. doi: 10.1016/s2213-8587(13)70165-7
56. Autier P, Mullie P, Macacu A, Dragomir M, Boniol M, Coppens K, et al. Effect of vitamin D supplementation on non-skeletal disorders: a systematic review of meta-analyses and randomised trials. *Lancet Diabetes Endocrinol.* (2017) 5:986–1004. doi: 10.1016/s2213-8587(17)30357-1
57. Urashima M, Mezawa H, Noya M, Camargo CA. Jr. Effects of vitamin D supplements on influenza A illness during the 2009 H1N1 pandemic: a randomized controlled trial. *Food Funct.* (2014) 5:2365–70. doi: 10.1039/c4fo00371c
58. Ramcharan T, Nolan O, Lai CY, Prabhu N, Krishnamurthy R, Richter AG, et al. Paediatric inflammatory multisystem syndrome: temporally associated with SARS-CoV-2 (PIMS-TS): cardiac features, management and short-term outcomes at a UK tertiary paediatric hospital. *Pediatr Cardiol.* (2020) 41:1391–1401. doi: 10.1007/s00246-020-02391-2
59. Panfili FM, Roversi M, D'Argenio P, Rossi P, Cappa M, Fintini D. Possible role of vitamin D in Covid-19 infection in pediatric population. *J Endocrinol Investig.* (2020) 44:1–9. doi: 10.1007/s40618-020-01327-0
60. Entrenas Castillo M, Entrenas Costa LM, Vaquero Barrios JM, Alcalá Díaz JF, López Miranda J, Bouillon RJM. Quesada Gomez: "Effect of calcifediol treatment and best available therapy versus best available therapy on intensive care unit admission and mortality among patients hospitalized for COVID-19: a pilot randomized clinical study. *J Steroid Biochem Mol Biol.* (2020) 203:105751. doi: 10.1016/j.jsbmb.2020.105751

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Feketea, Vlacha, Bocsan, Vassilopoulou, Stanciu and Zdrenghea. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

MINI REVIEW
published: 18 March 2021
doi: 10.3389/fnut.2021.649128

The Looming Effects of Estrogen in Covid-19: A Rocky Rollout

Hayder M. Al-kuraishy[1], Ali I. Al-Gareeb[1], Hani Faidah[2], Thabat J. Al-Maiahy[3], Natália Cruz-Martins[4,5,6] and Gaber El-Saber Batiha[7]**

[1] Department of Clinical Pharmacology and Therapeutic Medicine, College of Medicine, Al-Mustansiriyiah University, Baghdad, Iraq, [2] Microbiology, Faculty of Medicine, Umm Al Qura University, Mecca, Saudi Arabia, [3] Department of Gynecology and Obstetrics, College of Medicine, Al-Mustansiriyiah University, Baghdad, Iraq, [4] Faculty of Medicine, University of Porto, Porto, Portugal, [5] Institute for Research and Innovation in Health (i3S), University of Porto, Porto, Portugal, [6] Laboratory of Neuropsychophysiology, Faculty of Psychology and Education Sciences, University of Porto, Porto, Portugal, [7] Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt

In the face of the Covid-19 pandemic, an intensive number of studies have been performed to understand in a deeper way the mechanisms behind better or worse clinical outcomes. Epidemiologically, men subjects are more prone to severe acute respiratory syndrome-coronavirus type 2 (SARS-CoV-2) infections than women, with a similar scenario being also stated to the previous coronavirus diseases, namely, SARS-CoV in 2003 and Middle East Respiratory Syndrome coronavirus diseases (MERS-CoV) in 2012. In addition, and despite that aging is regarded as an independent risk factor for the severe form of the disease, even so, women protection is evident. In this way, it has been expected that sex hormones are the main determinant factors in gender differences, with the immunomodulatory effects of estrogen in different viral infections, chiefly in Covid-19, attracting more attention as it might explain the case-fatality rate and predisposition of men for Covid-19 severity. Here, we aim to provide a mini-review and an overview on the protective effects of estrogen in Covid-19. Different search strategies were performed including Scopus, Web of Science, Medline, Pubmed, and Google Scholar database to find relative studies. Findings of the present study illustrated that women have a powerful immunomodulating effect against Covid-19 through the effect of estrogen. This study illustrates that estrogens have noteworthy anti-inflammatory and immuno-modulatory effects in Covid-19. Also, estrogen hormone reduces SARS-CoV-2 infectivity through modulation of pro-inflammatory signaling pathways. This study highlighted the potential protective effect of estrogen against Covid-19 and recommended for future clinical trial and prospective studies to elucidate and confirm this protective effect.

Keywords: Covid-19, estrogen, angiotensin converting enzyme-2, SARS-CoV-2, patients outcomes

OPEN ACCESS

Edited by:
Ioannis Zabetakis, University of Limerick, Ireland

Reviewed by:
Markus Xie, Genentech, United States
Shima Shahjouei, Geisinger Health System, United States

****Correspondence:***
Natália Cruz-Martins
ncmartins@med.up.pt
Gaber El-Saber Batiha
gaberbatiha@gmail.com

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Nutrition

Received: *03 January 2021*
Accepted: *15 February 2021*
Published: *18 March 2021*

Citation:
Al-kuraishy HM, Al-Gareeb AI, Faidah H, Al-Maiahy TJ, Cruz-Martins N and Batiha G-S (2021) The Looming Effects of Estrogen in Covid-19: A Rocky Rollout. Front. Nutr. 8:649128. doi: 10.3389/fnut.2021.649128

BACKGROUND

Coronavirus disease 19 (Covid-19) is a global pandemic danger caused by the severe acute respiratory syndrome-coronavirus type 2 (SARS-CoV-2). SARS-CoV-2 spike protein binds to angiotensin-converting enzyme 2 (ACE2) receptors (1), which are involved in the viral entry. Such receptors are highly expressed in different tissues, mainly in lung pneumocyte type II cells, with SARS-CoV-2 bindings to ACE2 leading to downregulation of protective ACE2 and induction of

hyper-inflammation and oxidative stress, with consequent progress of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (2). Also, reduction of ACE2 leads to vasoconstriction, hypertension, coagulopathy, and induction of inflammatory reactions that together increase the risk of ALI and Covid-19 severity (3).

From an epidemiological point of view, and specifically looking at gender differences, men subjects are more prone and vulnerable to SARS-CoV-2 infection than women, about 2.4 times higher due to hormonal differences and expression, as well as distribution of ACE2 (4). Previous coronavirus diseases, including severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and the Middle East Respiratory Syndrome coronavirus disease (MERS-CoV) in 2012, showed the same pattern in gender susceptibility. For instance, in SARS-CoV, the case-fatality rate was 22% for men compared to 13% for women, while in MERS-CoV the case-fatality rate was 52% for men compared to 23% for women (5). In SARS-CoV-2, different studies have underlined that females are less prone to infection, contributing for 18% of total Covid-19 cases compared to the affected matched male subjects (6). In United Kingdom, a large-prospective cohort study illustrated that women account for 40% of Covid-19 cases with 20% lower case-fatality rate compared to men (7). However, aging is regarded as an independent risk factor for both genders in the development of severe Covid-19; even so, women protection is still evident (8). In fact, women differ from men in both nutritional requirements and energy consumption based on sex hormone differences. Also, the rate of infection in men compared to women might be due to different other reasons, including the following: men more prevalently work outside the house or work in places that put them to virus exposure; women, due to the low rate of health insurance or other social issues, less frequently go for a test and seek medical attention unless the symptoms are serious. Also, statistical bias in different studies may affect the prevalence and incidence of various infections in regard to gender difference (9). In general, women have a higher immune response against different pathogens due to underlying genetic (two X chromosomes) and hormonal differences (10). The presence of two X chromosomes in women affects the immune system even if one is inactive. The X chromosome acts on various elements of the immune system such as Toll-like receptors (TLRs) and chemokines which can be overexpressed in women and influence the response to viral infections and vaccinations (11). Likewise, the levels of activation of the immune cells affected by sex hormone are higher in women than in males, and this is linked with the stimulation of TLR7 and the production of interferon gamma (INF-γ). Hence, related to the X chromosomes, TLR7 is higher in women than in men and its expression leads to higher immune responses, although these reactions can cause autoimmune phenomena (12). Also, both immunoglobulins and circulating T cells are higher in women compared to men, so that women's immune reactivity is more active against different viral infections, which predispose them for risk of autoimmunity (13). Furthermore, women have an average higher frequency of circulating CD4 T cells than men and clinical studies reveal that men have lower CD3 and CD4 T cell counts, CD4/CD8 T cell ratios, and helper T cell type 1 (Th1) responses than women; thus, cytokine productions in response to infections are enhanced in women compared with men (13).

On the other hand, it has been proposed that pathogen-associated molecular patterns (PAMPs) bind to TLRs. TLR2 and TLR4 which bind bacterial cell wall proteins are more evident in men compared to TLR3, TLR7, and TLR9 which bind viral proteins in women (14). Thus, the production of interferon (mediated by TLR7) and IL-10 (mediated by TLR9) are lower in men during viral infections (15, 16). Also, women tend to activate type 2 immune response, which engaged with the production of anti-inflammatory cytokines, while men tend to activate type 1 immune response, which are involved in the production of pro-inflammatory cytokines (17). Taken together, men subjects have a lower level of regulatory T cells (Treg) during cytomegalovirus infections compared to women, suggesting a propensity for activation of type 1 immune response in men with production of pro-inflammatory cytokines (18, 19). Different studies have documented that sex steroids are the main determinant factors in gender immune differences mainly at the reproductive age (20), with the immunomodulatory effects of estrogen, progesterone, and androgen in different viral infections, chiefly in Covid-19, attracting more attention as it might explain the case-fatality rate and men's predisposition for Covid-19 severity, since serum concentrations of sex hormones change across life span (21). Here, we aim to provide an overview on the gender-specific immunological profile and the protective effects of estrogen in Covid-19.

METHOD AND SEARCH STRATEGY

The literature and study search were performed according to the guideline of systemic review. We search PubMed, Scopus, Web of Science, and Google Scholar database by using sequence keywords including (SARS-CoV-2 OR Covid-19) AND (acute respiratory syndrome OR acute lung injury), (SARS-CoV-2 OR Covid-19) AND (Gender differences OR hormonal effects), (SARS-CoV-2 OR Covid-19) AND (Estrogen OR estrogen agonists), (SARS-CoV-2 OR Covid-19) AND (women OR men), and (SARS-CoV-2 OR Covid-19) AND (immunological differences OR Covid-19 manifestations). The literature search was organized independently by all authors throughout searching the titles and abstracts of regained articles. All of the published and preprinted studies were integrated in this study without limitation of languages. Following the preliminary search and screening, the selected articles were experienced for eligibility and summarized in a mini-review (**Figure 1**).

ESTROGEN AND COVID-19

Estradiol (E2) binds to the cytoplasmic estrogenic receptors (ER-α and ER-β) that are expressed on T cells and B cells, respectively (22). Momentarily, E2 activates humoral immunity and antibody production against different viral infections (23). High E2 concentration is correlated with immune-reactivity around ovulation (24). Both hormone replacement therapy after menopause and use of combined contraceptive pills produce a

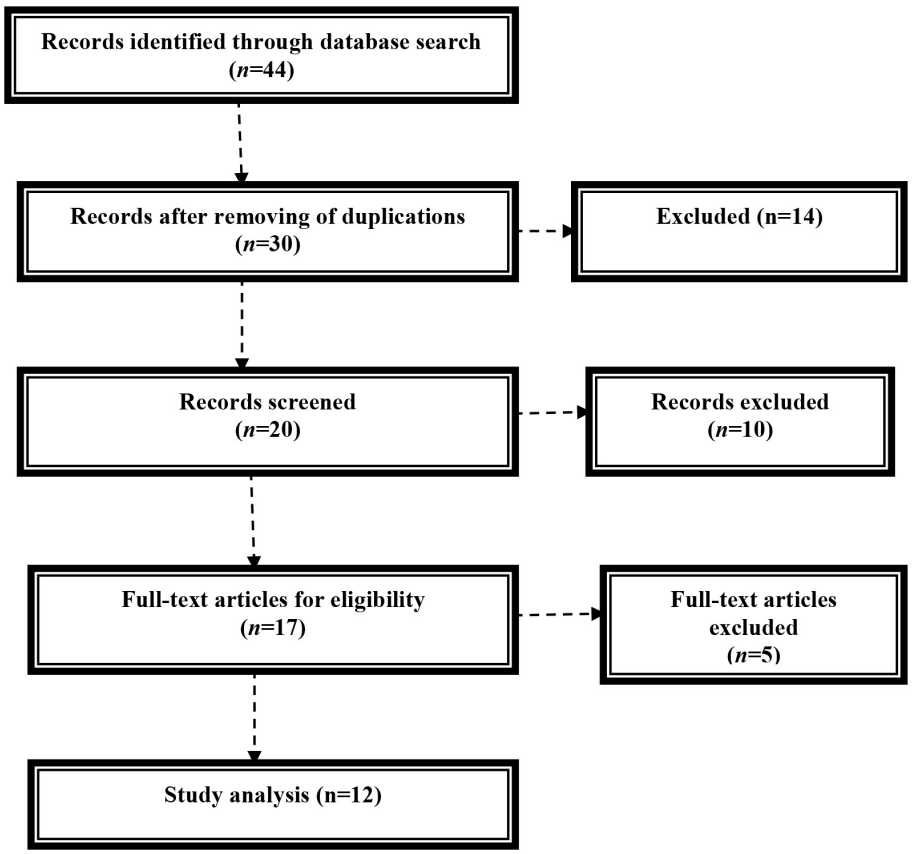


FIGURE 1 | Consort flowchart of the present study.

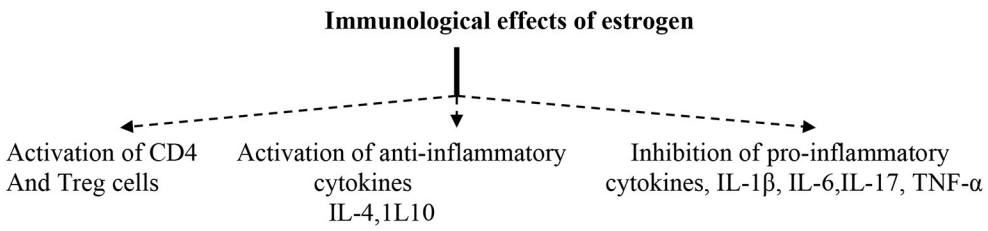


FIGURE 2 | Immunological effects of estrogens.

similar potent immune response and protection against viral infections (25). Besides, progesterone and testosterone have immunosuppressive effects on cell-mediated response and innate immunity. However, progesterone inhibits type 1 immune response as well as cytotoxic T and natural kill cells with activation of type 2 immune response, and this might explain the association between the use of progestin-only contraceptive depot and the risk of human immune deficiency virus (HIV) (26). Ruggieri et al. (27) found the association between testosterone use and the risk of viral infections including hepatitis C and B due to suppression of T cells and production of interferon gamma (IFN-γ). E2 has potent anti-inflammatory and immunomodulation effects in both humans and animals. E2 inhibits innate immunity through suppression of immune cell migration, mainly neutrophils and monocytes to the inflamed area. This effect attenuates the development and progression of cytokine storm (CS) via inhibition of pro-inflammatory cytokine release (i.e., IL-1β, IL-6, IL-17, TNF-α, and chemokines) (28). CS is regarded as a major cause for the development of ALI, ARDS, multiorgan failure, and death in Covid-19 (29). In addition, physiological E2 activates the production of anti-inflammatory cytokines (IL-4, IL-10) and promotes immune tolerance through stimulation of CD4 and regulatory T cells (Treg), although low dose of E2 promotes pro-inflammatory activation (30). However, Bommer et al. (31) showed that higher E2 concentration during pregnancy inhibits the anti-inflammatory IL-10 without effect on

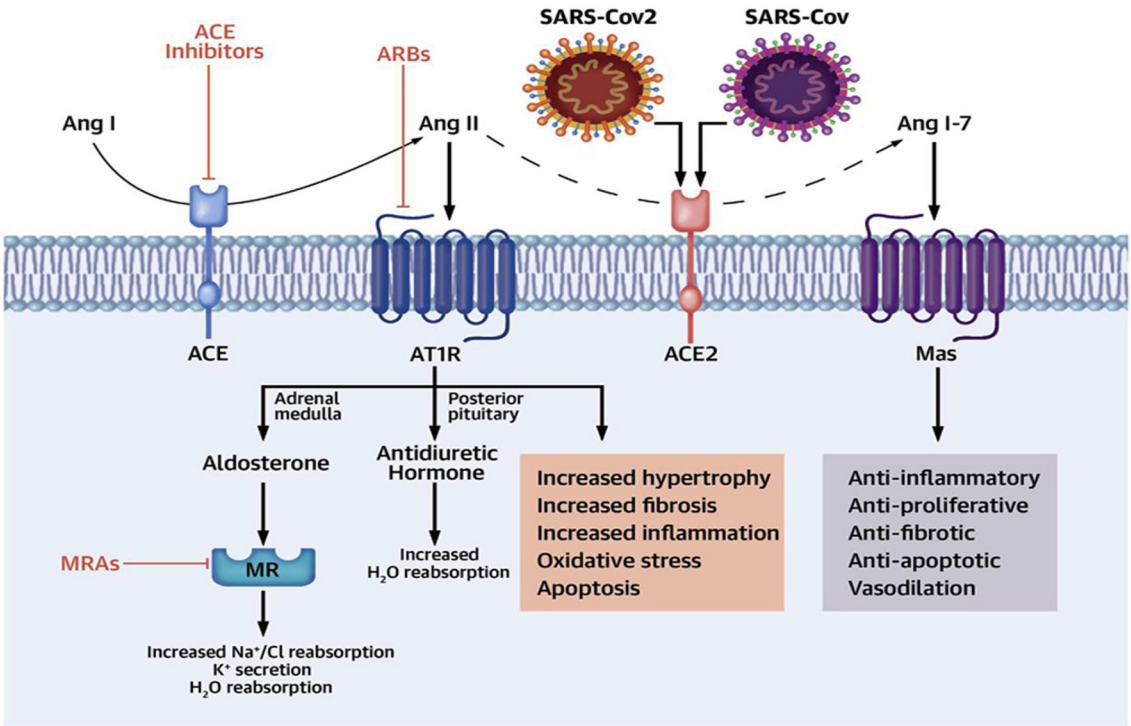


FIGURE 3 | Renin–angiotensin system (RAS) and SARS-CoV-2 infection [adopted from Yehualashet et al. (44)].

the pro-inflammatory profile. In Covid-19, a higher circulating Treg and anti-inflammatory cytokines have been linked to a rapid recovery and good clinical outcomes (32). In fact, a case–control study by Neumann et al. (33) proposed that higher IL-10-producing Treg cells are linked to Covid-19 severity. However, high IL-10-producing Treg cells might be a compensatory mechanism to overcome high pro-inflammatory cytokines (34). In addition, E2 inhibits the synthesis and release of IL-6 in different acute and chronic inflammatory disorders, like ALI by inhibiting IL-6 (35). Indeed, IL-6 antagonist tocilizumab is an effective agent in the ARDS management of Covid-19, suggesting the critical role of IL-6 in ALI pathogenesis and Covid-19 severity (36). Experimental studies have shown that lipopolysaccharide (LPS)-induced ALI and high IL-6 levels are higher in ovariectomized female and male mice (37).

Different clinical studies documented the protective role through various pathways. In Italy, the case-fatality rate is higher in men at about 63.9% compared with affected women due to hormonal changes and lifestyle diversity (38). Shabbir et al.'s (39) observational study involved 162,392 Covid-19 patients which showed that 74% of affected patients were men compared to 26% women due to the protective effect of estrogen. Moreover, Garg et al. (40) revealed that the mortality rate was higher in postmenopausal (12.8%) compared with premenopausal women (8.6%), highlighting the protective role of estrogen against Covid-19.

On the other hand, it has been reported that endogenous E2 leads to significant pulmonary protection against influenza and SARS viruses in previous preclinical studies through anti-inflammatory effects. Also, the use of fulvestrant (ER full antagonist) in women increases the risk of SARS-induced ALI comparable to men, suggesting of a potential lung-protective effect of endogenous estrogen (41). Likewise, selective estrogen receptor modulators (SERMs), like tamoxifen and toremifene, have noteworthy protective effects against ALI in SARS-CoV and MERS-CoV. Also, these drugs have antiviral effects against different coronaviruses through inhibition of viral entry and replication, along with cytopathic effects (42). Specifically, tamoxifen blocks the mitochondrial complex and oxygen consumption of infected cells through activation of AMPK signaling. In addition, endogenous estrogen and SERMs modulate the SARS-CoV-2 binding to the entry point ACE2 (43). Since SARS-CoV has a higher similarity with SARS-CoV-2, thus estrogen may be an effective agent against Covid-19-induced ARDS. Therefore, the net effects of estrogen on the inflammatory profile during SARS-CoV-2 are summarized (**Figure 2**).

ESTROGEN AND RENIN–ANGIOTENSIN SYSTEM IN COVID-19

The renin–angiotensin system (RAS) is highly deteriorated during SARS-CoV-2 infection, as SARS-CoV-2 spike protein binds to ACE2, leading to a significant downregulation of protective ACE2. ACE2 is involved in the metabolism and conversion of angiotensin I (Ang I) to Ang1–9 and Ang

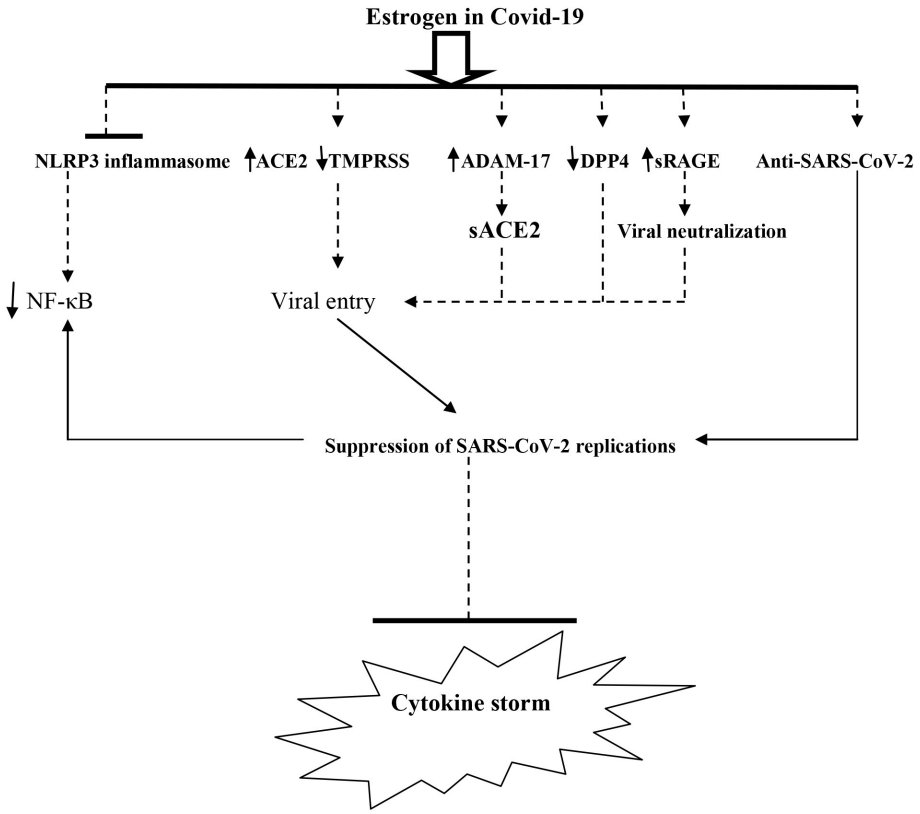


FIGURE 4 | The potential role of estrogen against SARS-CoV-2 pathogenesis and cytokine storm. TMPRRSS, type II transmembrane protease serine; ADAMA-17, A Disintegrin And Metalloproteinase-17; DPP4, Dipeptidyl Peptidase 4; sRAGE, soluble receptor for advanced glycation end-Product; sACE2, soluble angiotensin-converting enzyme 2.

II to Ang1–7. Both Ang1–9 and Ang1–7 have protective effects, exerting anti-inflammatory, anti-apoptotic, and anti-fibrotic effects through activation of Mas receptors (**Figure 3**) (45). Therefore, reduction and downregulation of ACE2 by SARS-CoV-2 lead to an elevation of vasoconstrictor Ang II causing vasoconstriction, hypertension, and induction of inflammatory and pro-inflammatory activations. A high Ang II concentration is directly linked to ALI and ARDS in Covid-19, so that both angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are able to reduce the harmful effect of Ang II on the lung through ACE2 upregulation (44).

It has been reported that E2 increases the expression of ACE2 and production of Ang1-7 (46) so it may counteract the deleterious effect of high Ang II-induced-ALI and ARDS. Tao et al. (47) confirmed the association between high Ang II concentration and ALI in mice. Thereby, estrogen therapy may have an important step in the management of Covid-19 patients. Shabbir et al. (39) illustrated that estrogen therapy mitigates endoplasmic reticulum stress (ER) induced by SARS-CoV-2 invasion through activation of cellular unfold protein response and regulation of inositol triphosphate (IP3) and phospholipase C. Baran-Gale et al. (48) reported that E2 inhibits type II transmembrane protease serine (TMPRSS2), necessary for trimming and activation of SARS-CoV-2 spike protein to bind ACE2. Indeed, it has been reported that TMPRSS2 inhibitors, such as camostat and nafamostat, block SARS-CoV-2 entry, mainly in lung cells (49). Different studies have also illustrated that E2 increases the expression of A Disintegrin And Metalloproteinase (ADAMs) mainly ADAM-17, which is involved in cleaving of the ACE2 ectodomain, leading to high-circulating soluble ACE2 that neutralizes SARS-CoV-2 and prevents its binding to the ACE2 membrane (50). Thus, E2 reduces SARS-CoV-2 infectivity through modulation of cellular ACE2/TMPRSS2/ADAM-17 axis expression.

ESTROGEN AND SIGNALING PATHWAY IN COVID-19

The Covid-19 severity is linked to the activation of inflammasomes, which are multi-protein complexes that control the activation of pro-inflammatory cytokines through intracellular caspase-1. The nod-like receptor pyrin domain 3 (NLRP3) is the most common inflammasome engaged with immunity against different pathogens (51). Raut et al. (52) illustrated that estrogen therapy inhibits the activation of NLRP3 inflammasomes, so E2 ameliorates airway

inflammation and hyper-responsiveness through inhibition of NLRP3 inflammasome-induced proinflammatory cytokine release (53), therefore attenuating the SARS-CoV-2-induced hyper-inflammation and ALI.

Furthermore, protease dipeptidyl peptidase 4 (DPP4) is also regarded as an entry point for SARS-CoV-2 and linked to viral pathogenesis and hyper-inflammation. Besides, DPP4 inhibitors mitigate SARS-CoV-2 infections and reduce the mortality in severe Covid-19 patients (54). It has been shown that E2 by ER-α inhibits DPP4 gene transcription through suppression of NF-kB signaling (55), and for that reason, high E2 levels in women may reduce DPP4 expression in lung and adipose tissues, thereby lessening the infectivity and pathogenicity of SARS-CoV-2.

Moreover, the active site of DPP4 (CD26) contains adenosine deaminase (ADA) which is involved in the metabolism of adenosine to inosine. Adenosine has anti-inflammatory and immunosuppressive effects and can protect against ALI and ARDS development (56). Therefore, ADA inhibitors, such as pentostatin, may have a role in preventing and attenuating SARS-CoV-2 infection and associated hyper-inflammation in the late phase of Covid-19 (57). Mohamadi et al. (58) illustrated that E2 through ER-α activates adenosine receptors, so that estrogen might be of value in repressing ALI and ARDS in the late phase of Covid-19 through modulation of the DPP4/CD26/adenosine signaling pathway.

Moreover, the receptor for advanced glycation end products (RAGE) is a member of immunoglobulin superfamily proteins, present in two forms, membrane RAGE (mRAGE) and soluble RAGE (sRAGE). mRAGE has inflammatory effects through activation of NF-kB, while sRAGE has anti-inflammatory effects through upregulation of ACE2 and anti-inflammatory cytokines. The RAGE pathway is mainly expressed in lung tissue and linked to development of acute and chronic lung injuries (59). In Covid-19, SARS-CoV-2 activates mRAGE at pulmonary alveolar cells leading to induction of severe inflammatory reactions (60). Indeed, it has been reported that sRAGE concentration is reduced with aging, which might explain the susceptibility of the elderly to Covid-19. However, in young and asymptomatic Covid-19 patients, the sRAGE concentration is high, while in severe Covid-19, the sRAGE level is significantly reduced, and so a low sRAGE level is linked to the development of ALI and ARDS (61). Finally, it has been shown that estrogen modulates the RAGE pathway through upregulation of sRAGE and reduction of mRAGE (62), so that the administration of E2 in patients with severe Covid-19 may have a lung-protective effect through modulation of the lung RAGE signaling pathway (**Figure 4**).

From the above, the diverse effects of estrogen on SARS-CoV-2 genome replication and associated immunological response in Covid-19 favor the hypothesis of an interplayed action among SARS-CoV-2 infection, immune response, and estrogen hormone which determine and regulate the gender-dependent final outcomes of Covid-19. Up to date, available data on the precise mechanisms determining the diverse predisposition and outcomes of SARS-CoV-2 infection, either hormonal or immunological, are fragmented and not comprehensive, but the disclosure in order to ascertain gender-specific molecular pathways involved is hopeful. Therefore, emergence of future clinical trials and large-scale prospective studies are warranted in this regard to confirm the supreme importance of estrogen therapy in the management of Covid-19 mainly in postmenopausal women.

CONCLUSION

This study illustrates that estrogens have noteworthy anti-inflammatory and immunomodulation effects in Covid-19. Also, estrogen hormone reduces SARS-CoV-2 infectivity through modulation of pro-inflammatory signaling pathways. This study highlighted the potential protective effect of estrogen against Covid-19 and recommended for future clinical trials and prospective studies to elucidate and confirm this protective effect.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

NC-M acknowledges the Portuguese Foundation for Science and Technology under the Horizon 2020 Program (PTDC/PSI-GER/28076/2017). HA-k acknowledges medical staff members of Al-Shiffa Medical Center, Baghdad, Iraq for their participations.

REFERENCES

1. Al-Kuraishy HM, Al-Naimi MS, Lungnier CM, Al-Gareeb AI. Macrolides and COVID-19: an optimum premise. *Biomed Biotechnol Res J.* (2020) 4:189. doi: 10.4103/bbrj.bbrj_103_20
2. Al-Kuraishy HM, Hussien NR, Al-Naimi MS, Al-Buhadily AK, Al-Gareeb AI, Lungnier C. Is ivermectin–Azithromycin combination the next step for COVID-19?. *Biomed Biotechnol Res J.* (2020) 4:101. doi: 10.4103/bbrj.bbrj_109_20
3. Al-Kuraishy HM, Hussien NR, Al-Naimi MS, Al-Buhadily AK, Al-Gareeb AI, Lungnier C. Renin–Angiotensin system and fibrinolytic pathway in COVID-19: One-way skepticism. *Biomed Biotechnol Res J.* (2020) 4:33. doi: 10.4103/bbrj.bbrj_105_20
4. Conti P, Younes A. Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection. *J Biol Regul Homeost Agents.* (2020) 34:339–43.
5. Ahmadzadeh J, Mobaraki K, Mousavi SJ, Aghazadeh-Attari J, Mirza-Aghazadeh-Attari M, Mohebbi I. The risk factors associated with MERS-CoV patient fatality: a global survey. *Diagn Microbiol Infect Dis.* (2020) 96:114876. doi: 10.1016/j.diagmicrobio.2019.114876
6. Wu Y, Lin H, Xie Q, Chen Q, Huang Y, Zhu Y, et al. COVID-19 in a patient with pre-existing acute lymphoblastic leukaemia. *Br J Haematol.* (2020) 190:e13–5. doi: 10.1111/bjh.16799
7. Perez-Guzman PN, Daunt A, Mukherjee S, Crook P, Forlano R, Kont MD, et al. Clinical characteristics and predictors of outcomes of hospitalized patients with coronavirus disease 2019 in a multiethnic London national

health service trust: a retrospective cohort study. *Clin Infect Dis.* (2020) 7:1–1. doi: 10.1093/cid/ciaa1091
8. Klein SL, Dhakal S, Ursin RL, Deshpande S, Sandberg K, Mauvais-Jarvis F. Biological sex impacts COVID-19 outcomes. *PLoS Pathogens.* (2020) 16:e1008570. doi: 10.1371/journal.ppat.1008570
9. Sun C, Zhai Z. The efficacy of social distance and ventilation effectiveness in preventing COVID-19 transmission. *Sustain Cities Soc.* (2020) 62:102390. doi: 10.1016/j.scs.2020.102390
10. Quakkelaar ED, Melief CJ. Experience with synthetic vaccines for cancer and persistent virus infections in nonhuman primates and patients. *Adv Immunol.* (2012) 114:77–106. doi: 10.1016/B978-0-12-396548-6.00004-4
11. Souyris M, Cenac C, Azar P, Daviaud D, Canivet A, Grunenwald S, et al. TLR7 escapes X chromosome inactivation in immune cells. *Sci Immunol.* (2018) 3:eaap8855. doi: 10.1126/sciimmunol.aap8855
12. Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. *Nat Rev Immunol.* (2020) 20:442–7. doi: 10.1038/s41577-020-0348-8
13. Meester I, Rivera-Silva GF, González-Salazar F. Immune system sex differences may bridge the gap between sex and gender in fibromyalgia. *Front Neurosci.* (2020) 13:1414. doi: 10.3389/fnins.2019.01414
14. Roberts BJ, Dragon JA, Moussawi M, Huber SA. Sex-specific signaling through Toll-like receptors 2 and 4 contributes to survival outcome of Coxsackievirus B3 infection in C57Bl/6 mice. *Biol Sex Diff.* (2012) 3:1–3. doi: 10.1186/2042-6410-3-25
15. Pratap UP, Hima L, Kannan T, Thyagarajan C, Priyanka HP, Vasantharekha R, et al. Sex-based differences in the cytokine production and intracellular signaling pathways in patients with rheumatoid arthritis. *Arch Rheumatol.* (2020) 35:545–57. doi: 10.46497/ArchRheumatol.2020.7481
16. Moore SE, Voss JG, Webel AR. Sex-based differences in plasma cytokine concentrations and sleep disturbance relationships among people living with HIV. *J Assoc Nurs AIDS Care.* (2020) 31:249–54. doi: 10.1097/JNC.0000000000000125
17. Bouman A, Schipper M, Heineman MJ, Faas MM. Gender difference in the non-specific and specific immune response in humans. *Am J Reprod Immunol.* (2004) 52:19. doi: 10.1111/j.1600-0897.2004.00177.x
18. Taneja V. Sex hormones determine immune response. *Front Immunol.* (2018) 9:1931. doi: 10.3389/fimmu.2018.01931
19. van der Heiden M, Berbers GA, Fuentes S, van Zelm MC, Boots AM, Buisman AM. An explorative biomarker study for vaccine responsiveness after a primary meningococcal vaccination in middle-aged adults. *Front Immunol.* (2018) 8:1962. doi: 10.3389/fimmu.2017.01962
20. Foo YZ, Nakagawa S, Rhodes G, Simmons LW. The effects of sex hormones on immune function: a meta-analysis. *Biol Rev.* (2017) 92:551–71. doi: 10.1111/brv.12243
21. Khan D, Ansar Ahmed S. The immune system is a natural target for estrogen action: opposing effects of estrogen in two prototypical autoimmune diseases. *Front Immunol.* (2016) 6:635. doi: 10.3389/fimmu.2015.00635
22. Tecalco-Cruz AC, Pérez-Alvarado IA, Ramírez-Jarquín JO, Rocha-Zavaleta L. Nucleo-cytoplasmic transport of estrogen receptor alpha in breast cancer cells. *Cell Signal.* (2017) 34:121–32. doi: 10.1016/j.cellsig.2017.03.011
23. Littauer EQ, Skountzou I. Hormonal regulation of physiology, innate immunity and antibody response to H1N1 influenza virus infection during pregnancy. *Front Immunol.* (2018) 9:2455. doi: 10.3389/fimmu.2018.02455
24. Aguilar-Pimentel JA, Cho YL, Gerlini R, Calzada-Wack J, Wimmer M, Mayer-Kuckuk P, et al. Increased estrogen to androgen ratio enhances immunoglobulin levels and impairs B cell function in male mice. *Sci Rep.* (2020) 10:1–4. doi: 10.1038/s41598-020-75059-9
25. Graham JH, Yoachim SD, Gould KA. Estrogen receptor alpha signaling is responsible for the female sex bias in the loss of tolerance and immune cell activation induced by the lupus susceptibility locus Sle1b. *Front Immunol.* (2020) 11:582214. doi: 10.3389/fimmu.2020.582214
26. Curtis KM, Hannaford PC, Rodriguez MI, Chipato T, Steyn PS, Kiarie JN. Hormonal contraception and HIV acquisition among women: an updated systematic review. *BMJ Sex Reprod Health.* (2020) 46:8–16. doi: 10.1136/bmjsrh-2019-200509
27. Ruggieri A, Gagliardi MC, Anticoli S. Sex-dependent outcome of hepatitis B and C viruses infections: synergy of sex hormones and immune responses?. *Front Immunol.* (2018) 9:2302. doi: 10.3389/fimmu.2018.02302
28. AbdulHussain G, Azizieh F, Makhseed MA, Raghupathy R. Effects of progesterone, dydrogesterone and estrogen on the production of Th1/Th2/Th17 cytokines by lymphocytes from women with recurrent spontaneous miscarriage. *J Reprod Immunol.* (2020) 140:103132. doi: 10.1016/j.jri.2020.103132
29. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 cytokine storm; what we know so far. *Front Immunol.* (2020) 11:1446. doi: 10.3389/fimmu.2020.01446
30. Maglione A, Rolla S, Mercanti SF, Cutrupi S, Clerico M. The adaptive immune system in multiple sclerosis: an estrogen-mediated point of view. *Cells.* (2019) 8:1280. doi: 10.3390/cells8101280
31. Bommer I, Muzzio DO, Zygmunt M, Jensen F. Progesterone and estradiol exert an inhibitory effect on the production of anti-inflammatory cytokine IL-10 by activated MZ B cells. *J Reprod Immunol.* (2016) 116:113–6. doi: 10.1016/j.jri.2016.05.008
32. Liu Y, Qi G, Bellanti JA, Moser R, Ryffel B, Zheng SG. Regulatory T cells: a potential weapon to combat COVID-19?. *MedComm.* (2020) 1:157–64. doi: 10.1002/mco2.12
33. Neumann J, Prezzemolo T, Vanderbeke L, Roca CP, Gerbaux M, Janssens S, et al. An open resource for T cell phenotype changes in COVID-19 identifies IL-10-producing regulatory T cells as characteristic of severe cases. *medRxiv [Preprint].* (2020). doi: 10.1101/2020.05.31.20112979
34. Brinkhoff A, Siebericks A, Engler H, Dolff S, Benson S, Korth J, et al. Pro-inflammatory Th1 and Th17 cells are suppressed during human experimental endotoxemia whereas anti-inflammatory IL-10 producing T-cells are unaffected. *Front Immunol.* (2018) 9:1133. doi: 10.3389/fimmu.2018.01133
35. Hsieh YC, Frink M, Hsieh CH, Choudhry MA, Schwacha MG, Bland KI, et al. Downregulation of migration inhibitory factor is critical for estrogen-mediated attenuation of lung tissue damage following trauma-hemorrhage. *Am J Physiol Lung Cell Mol Physiol.* (2007) 292:L1227–32. doi: 10.1152/ajplung.00479.2006
36. Scherger S, Henao-Martínez A, Franco-Paredes C, Shapiro L. Rethinking interleukin-6 blockade for treatment of COVID-19. *Med Hypotheses.* (2020) 144:110053. doi: 10.1016/j.mehy.2020.110053
37. Speyer CL, Rancilio NJ, McClintock SD, Crawford JD, Gao H, Sarma JV, et al. Regulatory effects of estrogen on acute lung inflammation in mice. *Am J Physiol Cell Physiol.* (2005) 288:C881–90. doi: 10.1152/ajpcell.00467.2004
38. Froldi G, Dorigo P. Endothelial dysfunction in coronavirus disease (2019). (COVID-19): gender and age influences. *Med Hypotheses*. (2020) 144:110015. doi: 10.1016/j.mehy.2020.110015
39. Shabbir S, Hafeez A, Rafiq MA, Khan MJ. Estrogen shields women from COVID-19 complications by reducing ER stress. *Med Hypotheses.* (2020) 143:110148. doi: 10.1016/j.mehy.2020.110148
40. Garg R, Agrawal P, Gautam A, Pursnani N, Agarwal M, Agarwal A, et al. COVID-19 outcomes in postmenopausal and perimenopausal females: is estrogen hormone attributing to gender differences? *J Midlife Health.* (2020) 11:250. doi: 10.4103/jmh.jmh_287_20
41. Breithaupt-Faloppa AC, Correia CD, Prado CM, Stilhano RS, Ureshino RP, Moreira LF. 17β-Estradiol, a potential ally to alleviate SARS-CoV-2 infection. *Clinics*. (2020) 75L:e1980. doi: 10.6061/clinics/2020/e1980
42. Almosawey HA, AL-Khikani FH, Hameed RM, Abdullah YJ, Al-Ibraheemi MK, Al-Asadi AA. Tamoxifen from chemotherapy to antiviral drug: possible activity against COVID-19. *Biomed Biotechnol Res J.* (2020) 4:108.
43. Vatansev H, Kadiyoran C, Cure MC, Cure E. COVID-19 infection can cause chemotherapy resistance development in patients with breast cancer and tamoxifen may cause susceptibility to COVID-19 infection. *Med Hypotheses.* (2020) 143:110091. doi: 10.1016/j.mehy.2020.110091
44. Yehualashet AS, Belachew TF. ACEIs and ARBs and their correlation with COVID-19: a review. *Infect Drug Resist.* (2020) 13:3217. doi: 10.2147/IDR.S264882
45. Danser AJ, Epstein M, Batlle D. Renin-angiotensin system blockers and the COVID-19 pandemic: at present there is no evidence to abandon renin-angiotensin system blockers. *Hypertension.* (2020) 75:1382–5. doi: 10.1161/HYPERTENSIONAHA.120.15082

46. Mompeón A, Lázaro-Franco M, Bueno-Betí C, Pérez-Cremades D, Vidal-Gómez X, Monsalve E, et al. Estradiol, acting through ERα, induces endothelial non-classic renin-angiotensin system increasing angiotensin 1–7 production. *Mol Cell Endocrinol.* (2016) 422:1–8. doi: 10.1016/j.mce.2015.11.004
47. Tao W, Li PS, Xu G, Luo Y, Shu YS, Tao YZ, et al. Soluble epoxide hydrolase plays a vital role in angiotensin II-induced lung injury in mice. *Shock.* (2018) 50:589–94. doi: 10.1097/SHK.0000000000001067
48. Baran-Gale J, Purvis JE, Sethupathy P. An integrative transcriptomics approach identifies miR-503 as a candidate master regulator of the estrogen response in MCF-7 breast cancer cells. *RNA.* (2016) 22:1592–603. doi: 10.1261/rna.056895.116
49. Rahman N, Basharat Z, Yousuf M, Castaldo G, Rastrelli L, Khan H. Virtual screening of natural products against Type II transmembrane serine protease (TMPRSS2), the priming agent of coronavirus 2 (SARS-CoV-2). *Molecules.* (2020) 25:2271. doi: 10.3390/molecules25102271
50. Ragia G, Manolopoulos VG. Assessing COVID-19 susceptibility through analysis of the genetic and epigenetic diversity of ACE2-mediated SARS-CoV-2 entry. *Pharmacogenomics.* (2020) 21:1311–29. doi: 10.2217/pgs-2020-0092
51. Rodrigues TS, de Sá KS, Ishimoto AY, Becerra A, Oliveira S, Almeida L, et al. Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients. *J Exp Med.* (2021) 218:e20201707. doi: 10.1084/jem.20201707
52. Raut PK, Kim SH, Choi DY, Jeong GS, Park PH. Growth of breast cancer cells by leptin is mediated via activation of the inflammasome: critical roles of estrogen receptor signaling and reactive oxygen species production. *Biochem Pharmacol.* (2019) 161:73–88. doi: 10.1016/j.bcp.2019.01.006
53. Cheng C, Wu H, Wang M, Wang L, Zou H, Li S, et al. Estrogen ameliorates allergic airway inflammation by regulating activation of NLRP3 in mice. *Biosci Rep.* (2019) 39:BSR20181117. doi: 10.1042/BSR20181117
54. Du H, Wang DW, Chen C. The potential effects of DPP-4 inhibitors on cardiovascular system in COVID-19 patients. *J Cell Mol Med.* (2020) 24:10274–8. doi: 10.1111/jcmm.15674
55. Bruzzese L, Fromonot J, By Y, Durand-Gorde JM, Condo J, Kipson N, et al. NF-κB enhances hypoxia-driven T-cell immunosuppression via upregulation of adenosine A2A receptors. *Cell Signal.* (2014) 26:1060–7. doi: 10.1016/j.cellsig.2014.01.024
56. Köhler D, Streißenberger A, Morote-García JC, Granja TF, Schneider M, Straub A, et al. Inhibition of adenosine kinase attenuates acute lung injury. *Crit Care Med.* (2016) 44:e181. doi: 10.1097/CCM.0000000000001370
57. Geiger JD, Khan N, Murugan M, Boison D. Possible role of adenosine in COVID-19 pathogenesis and therapeutic opportunities. *Front Pharmacol.* (2020) 11:594487. doi: 10.3389/fphar.2020.594487
58. Mohamadi A, Aghaei M, Panjehpour M. Estrogen stimulates adenosine receptor expression subtypes in human breast cancer MCF-7 cell line. *Res Pharm Sci.* (2018) 13:57. doi: 10.4103/1735-5362.220968
59. Dozio E, Sitzia C, Pistelli L, Cardani R, Rigolini R, Ranucci M, et al. Soluble receptor for advanced glycation end products and its forms in COVID-19 patients with and without diabetes mellitus: a pilot study on their role as disease biomarkers. *J Clin Med.* (2020) 9:3785. doi: 10.3390/jcm9113785
60. Yalcin Kehribar D, Cihangiroglu M, Sehmen E, Avci B, Capraz A, Yildirim Bilgin A, et al. The receptor for advanced glycation end product (RAGE) pathway in COVID-19. *Biomarkers.* (2020) 26:114–8. doi: 10.1080/1354750X.2020.1861099
61. De Francesco EM, Vella V, Belfiore A. COVID-19 and diabetes: the importance of controlling RAGE. *Front Endocrinol.* (2020) 11:526. doi: 10.3389/fendo.2020.00526
62. Mukherjee TK, Reynolds PR, Hoidal JR. Differential effect of estrogen receptor alpha and beta agonists on the receptor for advanced glycation end product expression in human microvascular endothelial cells. *Biochim Biophys Acta Mol Cell Res.* (2005) 1745:300–9. doi: 10.1016/j.bbamcr.2005.03.012

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Al-kuraishy, Al-Gareeb, Faidah, Al-Maiahy, Cruz-Martins and Batiha. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

HYPOTHESIS AND THEORY
published: 23 March 2021
doi: 10.3389/fimmu.2021.637152

Bovine Coronavirus Immune Milk Against COVID-19

Antonio Arenas, Carmen Borge, Alfonso Carbonero, Ignacio Garcia-Bocanegra, David Cano-Terriza, Javier Caballero and Antonio Arenas-Montes*

Department of Animal Health, University of Cordoba, Córdoba, Spain

After a year of evolution of the SARS-CoV-2 epidemic, there is still no specific effective treatment for the disease. Although the majority of infected people experience mild disease, some patients develop a serious disease, especially when other pathologies concur. For this reason, it would be very convenient to find pharmacological and immunological mechanisms that help control SARS-CoV-2 infection. Since the COVID-19 and BCoV viruses are very close phylogenetically, different studies demonstrate the existence of cross-immunity as they retain shared epitopes in their structure. As a possible control measure against COVID-19, we propose the use of cow's milk immune to BCoV. Thus, the antigenic recognition of some highly conserved structures of viral proteins, particularly M and S2, by anti-BCoV antibodies present in milk would cause a total or partial inactivation of SARS-COV-2 (acting as a particular vaccine) and be addressed more easily by GALT's highly specialized antigen-presenting cells, thus helping the specific immune response.

Keywords: BCoV, SARS-CoV-2, immune milk, COVID-19, control

OPEN ACCESS

Edited by:
Alexandros Tsoupras, University of Limerick, Ireland

Reviewed by:
François J. M. A. Meurens, UMR INRAE-Oniris 1300 Oniris-Nantes Atlantic National College of Veterinary Medicine, France
Markus Xie, Genentech, United States

****Correspondence:***
Antonio Arenas
arenas@uco.es

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: *02 December 2020*
Accepted: *03 March 2021*
Published: *23 March 2021*

Citation:
Arenas A, Borge C, Carbonero A, Garcia-Bocanegra I, Cano-Terriza D, Caballero J and Arenas-Montes A (2021) Bovine Coronavirus Immune Milk Against COVID-19. Front. Immunol. 12:637152. doi: 10.3389/fimmu.2021.637152

INTRODUCTION

COVID-19 is a severe human pandemic caused by the SARS-CoV-2 virus. Since there is currently no specific treatment for severe cases, it is critical to find immune mechanisms and strategies to help control the disease. We propose the use of heterologous passive immunity using Bovine Coronavirus immune milk (BIM) as an immunostimulant therapy to control SARS-CoV-2 infection, helping to activate the intestinal immune system.

The coronaviruses were initially classified according to their antigenic characteristics into three serological groups (1), known as Groups 1, 2, and 3, which were later renamed as the new genera *Alphacoronavirus*, *Betacoronavirus*, and *Gammacoronavirus*, respectively (2). Based on their phylogenetic relationships and genomic structures, the *Betacoronavirus* genus now contains five lines or subgenera, including the *Sarbecovirus* (which includes SARS-CoV and SARS-CoV-2), *Merbecovirus* (MERS-CoV), and *Embecovirus* subgenera.

Notable among the embecoviruses are *HCoV-OC43* (OC43), which causes a mild human endemic respiratory infection, and *Bovine Coronavirus* (BCoV). These are two different biotypes of the same species, since the human virus probably evolved from strains of the bovine coronavirus that jumped the species barrier and caused sustained infection in humans (3). BCoV and OC43 share a global nucleotide identity of 96% (4); in contrast, the SARS-CoV-2 genes shared less than 80% nucleotide sequence identity to other Sarbecovirus as SARS-CoV, and about 50% to

MERS-CoV (5). Viral seroneutralization techniques show that there is a close antigenic relationship between BCoV and OC43 viruses (6).

Structurally, BCoV (also OC43) is an enveloped virus composed of five structural proteins: the spike glycoprotein (S), the envelope (E) protein, the membrane (M) protein, the nucleocapsid (N) protein, and the hemagglutinin-esterase (HE) protein. The SARS-CoV-2 structure is very similar to the other members of Family Coronaviridae; also contains four structural proteins: S, E, M, and N proteins (5), but it lacks the HE protein.

To attach to host cells, BCoV uses 5-N-acetyl-9-O-acetylneuraminic acid as the preferred receptor to cellular binding (7) whereas SARS-CoV-2 binding angiotensin-converting enzyme receptor (5). Then, the fusion peptide is activated triggering the fusion of viral particle to cellular membrane. The described mechanism allows the virus to infect the host cells.

CROSS-IMMUNE REACTIVITY AMONG BETACORONAVIRUSES

The most important immune response generated by coronaviruses is produced against S protein, since it is widely exposed on the viral surface and is an immunodominant structure. S protein is a large class I fusion protein consists of S1 subunit (S1) that contains, among other epitopes the receptor binding domain (RBD), and S2 subunit (S2) that mediates viral membrane fusion (8) contains conserved regions that are necessary for function: the fusion peptide and two conserved repeats (9, 10).

Transmembrane M protein is the most abundant structural protein and is highly conserved among the coronaviruses, but their function is not clearly understood (11). Different M protein epitopes elicit a detectable immune response in the serum of SARS and COVID-19 patients (12, 13). The OC43 M protein is an antagonist of the host antiviral defenses interfering different immune systems (14) and SARS-CoV-2 M protein plays similar effects disabling antiviral signaling cascade (15).

The viral N protein is highly conserved maintaining antigenic cross-reactivity among some coronavirus species, but no between BCoV and SARS-CoV-2 (16). The HE protein, which is not present in other betacoronaviruses (as SARS-CoV-2), enables the BCoV to bind different types of cells. The small E protein is poorly immunogenic for humoral response (17).

Cross-reactivity has been found between OC43 and SARS-CoV (18, 19), which seems to be supported by different antigenic determinants present in N, M, and S2 (a highly conserved region that is almost invariant across the betacoronaviruses), as well as between SARS-CoV and SARS-CoV-2 (20).

SARS-CoV monoclonal antibodies neutralize SARS-CoV-2 through a mechanism, yet unknown, but different from RBD interference. Likewise, alternative mechanisms of coronavirus neutralization by antibodies targeting RBD have been reported, particularly inactivation of the S protein by altering its structure in prefusion conformation (21–23).

A consistent cross-reactivity, but limited cross-neutralization, has been reported between SARS-CoV-2 and other human endemic coronaviruses, including OC43 (24). In addition, recent studies have found that between 40 and 60% of people not previously exposed to SARS-CoV-2 have T helper cells (CD4+) reactive to OC43 (25). This suggests that CD4+ cells specific for endemic betacoronaviruses of the common cold may also recognize the epidemic coronavirus SARS-CoV-2. This hypothesis is reinforced by the seroconversion found in a high proportion (one in three infected) of asymptomatic human SARS-CoV-2 infections (26), some of which could be due to cross-reactivity following exposure to other human coronaviruses (particularly OC43, HCoV-229E, and HECoV viruses).

The formation of immunoglobulin G isotype (IgG) against the RBD domain of the newly emerged SARS-CoV-2 is considered essential for adequate immune protection. These specific IgGs should be absent (or at a low level) in unexposed people, nevertheless, a recent study (27) showed that IgGs reactive to the S2 subunit of SARS-CoV-2 were present in most of the unexposed subjects studied. The authors concluded that cross-reactivity with other human coronaviruses was a plausible explanation. They also found that convalescing COVID-19 patients showed a significant increase in OC43-reactive memory B cells compared with previously unexposed healthy subjects. Consequently, since everything seems to point to strong cross-reactivity between SARS-CoV-2 and OC43 at both the cellular and humoral level, it is likely also to be found with BCoV as reported previously (6). No specific cross-reactivity studies have been performed between SARS-CoV-2 and BCoV, but when the epitope sequence alignment of the SARS-CoV-2 spike proteins was analyzed, a high homology (57 to 83%) with BCoV was found (28).

The phenomenon known as antibody-dependent enhancement (ADE), in which the presence of non-neutralizing antibodies can aggravate the course of the disease, is one of the present general concerns in immunotherapy (and the development of vaccines). The pathophysiology of ADE is not yet well understood, but it appears to be closely related to low levels of neutralizing antibodies against RBD. However, it has been shown that ADE does not occur when antibodies bind to the nucleoprotein or any other structure different from RBD antigens (29).

BCoV causes specific syndromes in cattle with a very high inter- and intra-herd prevalence (30, 31) and has been associated with gastroenteritis and respiratory diseases in lactating calves (32), shipping fever in fattening calves (33) and winter diarrhea in dairy cows (34). Due to their economic, medical, and epidemiological importance, different commercial vaccines against BCoV (including live and inactivated viruses) are currently available and have shown a very good protective effect.

IMMUNE MILK AGAINST BCOV

Functional foods are products that not only satisfy nutritional requirements but also modulate organic functions and perform highly beneficial tasks in some diseases (35). Bovine milk and colostrum contain high levels of bioactive components, including growth factors, immunoglobulins (Igs), lactoperoxidase, lysozyme, lactoferrin, cytokines, nucleosides, vitamins, peptides, and oligosaccharides, all of them beneficial to human health (36).

Hyperimmune cow's milk has been used against human infections for a long time (37, 38). Raw and pasteurized milk can contain specific antibodies against different human pathogens (39), exerting a synergistic effect on the activity of nonspecific antimicrobial factors (40, 41). Bovine IgG is functionally active throughout the gastrointestinal tract and can prevent digestive and respiratory tract infections in humans (42).

Whole cow's milk contains more than 30 g/L of protein, almost 1 g/L of which are immunoglobulins, which can reach more than 200 g/L in colostrum (36). IgGs present in bovine milk survive gastric exposure and resist proteolytic digestion in the stomach and intestinal tract, maintaining their active binding capacity to receptors (43). No evidence for the intestinal absorption of intact IgG has been found. In addition, the ingestion of Ig preparations obtained from serum leads to an increase in anti-inflammatory cytokines and a decrease in pro-inflammatory cytokines (44).

It was recently confirmed that SARS-CoV-2 RNA persisted longer (over a month) in the feces of infected people, mainly children, than in respiratory exudates (45, 46). This shows that the infectious period could be prolonged due to the enteric persistence of the virus, even when a person has been considered negative after a routine diagnostic test. We think that the presence of specific IgG in the intestinal lumen would help control viral excretion.

Passive immunity conferred through breast milk has a remarkable protective effect against most of the infectious diseases of animals and has been well studied in almost all coronaviruses of veterinary concern (47–49). The presence of specific antibodies to SARS-CoV-2 has also been found in human breast milk (50).

Ultra-high temperature (UHT) processing is the most widely used heat treatment for the microbiological safety of milk for human consumption, but this treatment destroys the immunoglobulins present in the milk. However, pasteurization is a less aggressive heat treatment that ensures microbiological safety but also preserves most immunoglobulins in milk. Most commercial pasteurized milk from Spain contains antibodies against BCoV, probably due to the high prevalence of infection and the systematic vaccination of cattle. Nevertheless, the heat treatment process used reduces titers below 30. BIM, which we obtained by cow hyperimmunization using commercial coronavirus vaccine, contains antibodies with titers between 128 and 256 (unpublished results) and human consumption could transfer heterologous antibodies against SARS-CoV-2, thus conferring some passive immunity.

DISCUSSION

The evolution of antibody kinetics after natural infection appears to be very similar between BCoV and SARS-CoV-2. Briefly, after infection, different antibody class (i.e., IgA, IgM, IgG) are produced directed against the different structural proteins of the virus (16). Antibodies directed against RBD are highly neutralizing, but are quite different between the two viruses, as they target different domains. In contrast, antibodies directed towards the M protein may have cross-immunity among coronaviruses, binding selectively to Fc receptors (FcRs) (51). FcRs have interesting immunological functions including phagocytosis, degranulation, antibody-dependent cellular cytotoxicity (ADCC), cytokine formation, lipid mediator, and superoxide production (52).

Animal respiratory coronaviruses often have a pneumoenteric tropism and can persist in carriers who maintain and excrete the virus in respiratory secretions, but mainly in the feces, for long periods of time, even years (30, 53). Fecal excretion has also been demonstrated in SARS-CoV-2 (45, 46) and is even more prolonged than nasal excretion. For BCoV, it has been speculated that, after initial replication, the virus spreads to the gastrointestinal tract by ingestion of large amounts of virus coated in mucous secretions, which would cause the virus to transit to the intestine, causing intestinal infection and subsequent fecal excretion (8).

Gut-associated lymphoid tissue (GALT) (Peyer's patches, cecal and colonic patches) plays a key role in immunity since it discriminates between food antigens, commensal microorganisms, and pathogens. It should therefore have very safe mechanisms for adjusting immune response to the type of antigen that it locates. These immunoregulatory functions include mechanisms for the recruitment of lymphocytes and myeloid cells from the bloodstream to antigen sampling from surrounding tissue or the surface of the luminal epithelium, as well as others for returning activated effector lymphocytes to the bloodstream or remaining on the mucosal surface where they eliminate pathogens or reside as memory cells.

After consumption of BIM, the ingested antibodies would specifically bind to some shared antigenic structures of SARS-CoV-2. This could totally or partially inactivate the virus and facilitate the processing of SARS-CoV-2 antigens by GALT, but also the formation of immune complexes that can activate the antiviral signaling cascade (15), complement fixation, and binding to the FcRs that control humoral immunity and are essential for an adequate response to infections (52).

Bearing in mind that coronaviruses are markedly pneumoenteric viruses, GALT and other mucosa-associated lymphoid tissues are of enormous importance for the control of infection (54). The reduction in the number of active viral particles in the intestinal mucosa and the activation of other immune mechanisms in the GALT could reinforce the specific immune response against infection by SARS-CoV-2, improving the regulation of the immune system.

Thus, recognition of SARS-CoV-2 by heterologous antibodies in BIM could cause complement fixation of the immune complexes, triggering activation of any of its pathways. This would generate specific responses of neutralization, cytolysis, chemotaxis, opsonization, and others (55), as well as the activation of other immune mechanisms that would benefit immune action, such as the production of IL-10, IFN-β (56), or CD27 cells (57) (which would involve the activation, proliferation, and differentiation of B lymphocytes) or even activation of immunoglobulin class switching (58). The complexity of the cytokine network in humans makes immunomodulation essential in the control of severe COVID-19 and could be achieved by the presence of specific immunoglobulins in the GALT and gut lumen.

Considering the arguments presented above, research should be conducted, focused on exploring the effectiveness of BIM and other immune compounds (milk, milk derivatives, egg yolk, among others) in the control of COVID-19 in human patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

ACKNOWLEDGMENTS

We want to thank our colleague Elvira Muñoz for her invaluable aid.

REFERENCES

1. Brian DA, Baric RS. Coronavirus genome structure and replication. *Curr Top Microbiol Immunol* (2005) 287:1–30. doi: 10.1007/3-540-26765-4_1
2. de Groot RJ, Baker S, Barik RS, Holmes KV. Family Coronaviridae. In: Ed: King AMQ, Adams MJ, Cartens EB, Lefkowitz EJ editors. *Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses*. Elsevier (2012). p. 806–28. doi: 10.1016/B978-0-12-384684-6.00068-9
3. Vijgen L, Keyaerts E, Moës E, Thoelen I, Wollants E, Lemey P, et al. Complete Genomic Sequence of Human Coronavirus OC43: Molecular Clock Analysis Suggests a Relatively Recent Zoonotic Coronavirus Transmission Event. *J Virol* (2005) 79(3):1595–604. doi: 10.1128/JVI.79.3.1595-1604.2005
4. Kin N, Miszczak F, Diancourt L, Caro V, Moutou F, Vabret A, et al. Comparative molecular epidemiology of two closely related coronaviruses, bovine coronavirus (BCoV) and human coronavirus OC43 (HCoV-OC43), reveals a different evolutionary pattern. *Infect Genet Evol* (2016) 40:186–91. doi: 10.1016/j.meegid.2016.03.006
5. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor bindings. *Lancet* (2020) 395(10224):565–74. doi: 10.1016/S0140-6736(20)30251-8
6. Pedersen NC, Ward J, Mengeling WL. Antigenic relationship of the feline infectious peritonitis virus to coronaviruses of other species. *Arch Virol* (1978) 58(1):45–53. doi: 10.1007/BF01315534
7. Peng G, Xu L, Lin YL, Chen L, Pasquarella JR, Holmes KV, et al. Crystal structure of bovine coronavirus spike protein lectin domain. *J Biol Chem* (2012) 287(50):41931–8. doi: 10.1074/jbc.M112.418210
8. Saif LJ. Bovine respiratory coronavirus. *Vet Clin North Am Food Anim Pract* (2010) 26(2):349–64. doi: 10.1016/j.cvfa.2010.04.005
9. Alsaadi EA J, Jones IM. Membrane binding proteins of coronaviruses. *Future Virol* (2019) 14(4):275–86. doi: 10.2217/fvl-2018-0144
10. Prévost J, Gasser R, Beaudoin-Bussières G, Richard J, Duerr R, Laumaea A, et al. Cross-Sectional Evaluation of Humoral Responses against SARS-CoV-2 Spike. *Cell Rep Med* (2020) 1(7):100126. doi: 10.1016/j.xcrm.2020.100126
11. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. *Virol J* (2019) 16(1):69. doi: 10.1186/s12985-019-1182-0
12. Du L, He Y, Jiang S, Zheng BJ. Development of subunit vaccines against severe acute respiratory syndrome. *Drugs Today (Barc)* (2008) 44(1):63–73. doi: 10.1358/dot.2008.44.1.1131830
13. Thieme CJ, Anft M, Paniskaki K, Blazquez-Navarro A, Doevelaar A, Seibert FS, et al. Robust T Cell Response Toward Spike, Membrane, and Nucleocapsid SARS-CoV-2 Proteins Is Not Associated with Recovery in Critical COVID-19 Patients. *Cell Rep Med* (2020) 1(6):100092. doi: 10.1016/j.xcrm.2020.100092
14. Beidas M, Chehadeh W. Effect of Human Coronavirus OC43 Structural and Accessory Proteins on the Transcriptional Activation of Antiviral Response Elements. *Intervirology* (2018) 61(1):30–5. doi: 10.1159/000490566
15. Zheng Y, Zhuang MW, Han L, Zhang J, Nan ML, Zhan P, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) membrane (M) protein inhibits type I and III interferon production by targeting RIG-I/MDA-5 signaling. *Signal Transd Target Ther* (2020) 5(1):299. doi: 10.1038/s41392-020-00438-7
16. Huang AT, Garcia-Carreras B, Hitchings MDT, Yang B, Katzelnick LC, Rattigan SM, et al. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nat Commun* (2020) 11(1):4704. doi: 10.1038/s41467-020-18450-4
17. Zhang J, Zeng H, Gu J, Li H, Zheng L, Zou Q. Progress and Prospects on Vaccine Development against SARS-CoV-2. *Vaccines (Basel)* (2020) 8(2):153. doi: 10.3390/vaccines8020153
18. Woo PC, Lau SK, Wong BH, Chan KH, Hui WT, Kwan GS, et al. False-positive results in a recombinant severe acute respiratory syndrome-associated coronavirus (SARS-CoV) nucleocapsid enzyme-linked immunosorbent assay due to HCoV-OC43 and HCoV-229E rectified by Western blotting with recombinant SARS-CoV spike polypeptide. *J Clin Microbiol* (2004) 42(12):5885–8. doi: 10.1128/JCM.42.12.5885-5888.2004
19. Che XY, Qiu LW, Liao ZY, Wang YD, Wen K, Pan YX, et al. Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43. *J Infect Dis* (2005) 191(12):2033–7. doi: 10.1086/430355
20. Lv H, Wu NC, Tsang OT, Yuan M, Perera RAPM, Leung WS, et al. Cross-reactive Antibody Response between SARS-CoV-2 and SARS-CoV Infections. *Cell Rep* (2020) 31(9):107725. doi: 10.1016/j.celrep.2020.107725
21. Wang C, Li W, Drabek D, Okba NMA, Haperen RV, Osterhaus ADME, et al. A human monoclonal antibody blocking SARS-CoV-2 infection. *Nat Commun* (2020) 11(1):1–6. doi: 10.1038/s41467-020-16452-w
22. Walls AC, Xiong X, Park YJ, Tortorici MA, Snijder J, Quispe J, et al. Unexpected Receptor Functional Mimicry Elucidates Activation of Coronavirus Fusion. *Cell* (2019) 176(5):1026–39.e15. doi: 10.1016/j.cell.2018.12.028
23. Kirchdoerfer RN, Cottrell CA, Wang N, Pallesen J, Yassine HM, Turner HL, et al. Pre-fusion structure of a human coronavirus spike protein. *Nature* (2016) 531(7592):118–21. doi: 10.1038/nature17200
24. Post N, Eddy D, Huntley C, van Schalkwyk MCI, Shrotri M, Leeman D, et al. Antibody response to SARS-CoV-2 infection in humans: A systematic review. *PloS One* (2020) 15(12):e0244126. doi: 10.1371/journal.pone.0244126
25. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* (2020) 181(7):1489–501.e15. doi: 10.1016/j.cell.2020.05.015
26. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, Oteo J, Hernán MA, Pérez-Olmeda M, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet* (2020) 396 (10250):535–44. doi: 10.1016/S0140-6736(20)32266-2
27. Nguyen-Contant P, Embong AK, Kanagaiah P, Chaves FA, Yang H, Branche AR, et al. S Protein-Reactive IgG and Memory B Cell Production after Human SARS-CoV-2 Infection Includes Broad Reactivity to the S2 Subunit. *mBio* (2020) 11(5):e01991–20. doi: 10.1101/2020.07.20.213298
28. Tilocca B, Soggiu A, Musella V, Britti D, Sanguinetti M, Urbani A, et al. Molecular basis of COVID-19 relationships in different species: a one health perspective. *Microbes Infect* (2020) 22(4-5):218–20. doi: 10.1016/j.micinf.2020.03.002
29. Wang SF, Tseng SP, Yen CH, Yang JY, Tsao CH, Shen CW, et al. Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins. *Biochem Biophys Res Commun* (2014) 451(2):208–14. doi: 10.1016/j.bbrc.2014.07.090
30. Oma VS, Tråvén M, Alenius S, Myrmel M, Stokstad M. Bovine coronavirus in naturally and experimentally exposed calves; viral shedding and the potential for transmission. *Virol J* (2016) 13(1):1–11. doi: 10.1186/s12985-016-0555-x

31. Saif LJ, Jung K. Comparative Pathogenesis of Bovine and Porcine Respiratory Coronaviruses in the Animal Host Species and SARS-CoV-2 in Humans. *J Clin Microbiol* (2020) 58(8):e01355–20. doi: 10.1128/JCM.01355-20
32. Boileau MJ, Kapil S. Bovine Coronavirus Associated Syndromes. *Vet Clin North Am Food Anim Pract* (2010) 26(1):23–46. doi: 10.1016/j.cvfa.2009.10.003
33. Storz J, Purdy CW, Lin X, Burrell M, Truax RE, Briggs RE, et al. Isolation of respiratory bovine coronavirus, other cytocidal viruses, and Pasteurella spp from cattle involved in two natural outbreaks of shipping fever. *J Am Vet Med Assoc* (2000) 216(10):1599–604. doi: 10.2460/javma.2000.216.1599
34. Natsuaki S, Goto K, Nakamura K, Yamada M, Ueo H, Komori T, et al. Fatal winter dysentery with severe anemia in an adult cow. *J Vet Med Sci* (2007) 69 (9):957–60. doi: 10.1292/jvms.69.957
35. Roberfroid MB. Concepts and strategy of functional food science: the European perspective. *Am J Clin Nutr* (2000) 71(6 Suppl):1660S–4S; discussion 74S-5S. doi: 10.1093/ajcn/71.6.1660S
36. Gapper LW, Copestake DE, Otter DE, Indyk HE. Analysis of bovine immunoglobulin G in milk, colostrum and dietary supplements: a review. *Anal Bioanal Chem* (2007) 389(1):93–109. doi: 10.1007/s00216-007-1391-z
37. Tacket CO, Binion SB, Bostwick E, Losonsky G, Roy MJ, Edelman R. Efficacy of bovine milk immunoglobulin concentrate in preventing illness after Shigella flexneri challenge. *Am J Trop Med Hyg* (1992) 47(3):276–83. doi: 10.4269/ajtmh.1992.47.276
38. Korhonen H, Marnila P, Gill HS. Bovine milk antibodies for health. *Br J Nutr* (2000) 84 Suppl 1:S135–46. doi: 10.1017/S0007114500002361
39. Yolken RH, Losonsky GA, Vonderfecht S, Leister F, Wee SB. Antibody to human rotavirus in cow's milk. *N Engl J Med* (1985) 312(10):605–10. doi: 10.1056/NEJM198503073121002
40. Takahashi N, Eisenhuth G, Lee I, Laible N, Binion S, Schachtele C. Immunoglobulins in milk from cows immunized with oral strains of Actinomyces, Prevotella, Porphyromonas, and Fusobacterium. *J Dent Res* (1992) 71(8):1509–15. doi: 10.1177/00220345920710080901
41. Loimaranta V, Tenovuo J, Korhonen H. Combined inhibitory effect of bovine immune whey and peroxidase-generated hypothiocyanite against glucose uptake by Streptococcus mutans. *Oral Microbiol Immunol* (1998) 13 (6):378–81. doi: 10.1111/j.1399-302X.1998.tb00695.x
42. Ulfman LH, Leusen JHW, Savelkoul HFJ, Warner JO, van Neerven RJJ. Effects of Bovine Immunoglobulins on Immune Function, Allergy, and Infection. *Front Nutr* (2018) 5:52. doi: 10.3389/fnut.2018.00052
43. Jasion VS, Burnett BP. Survival and digestibility of orally-administered immunoglobulin preparations containing IgG through the gastrointestinal tract in humans. *Nutr J* (2015) 14:22. doi: 10.1186/s12937-015-0010-7
44. Pérez-Bosque A, Miró L, Polo J, Russell L, Campbell J, Weaver E, et al. Dietary plasma protein supplements prevent the release of mucosal proinflammatory mediators in intestinal inflammation in rats. *J Nutr* (2010) 140(1):25–30. doi: 10.3945/jn.109.112466
45. Wu YG, Tang C, Hong L, Zhou Z, Dong J, Yin X, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* (2020) 5:434–5. doi: 10.1016/S2468-1253(20)30083-2
46. Xu Y, Li X, Zhu B, Liang H, Fang C, Gong Y, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. *Nat Med* (2020) 26(4):502–5. doi: 10.1038/s41591-020-0817-4
47. Jackwood MW, de Wit S. Infectious Bronchitis. In: Swayne DE, editor. *Diseases of Poultry*. Hoboken, NJ, USA: John Wiley & Sons, Inc (2020). p. 167–88.
48. Tizard IR. Vaccination against coronaviruses in domestic animals. *Vaccine* (2020) 38(33):5123–30. doi: 10.1016/j.vaccine.2020.06.026
49. Homberger FR. Maternally-derived passive immunity to enterotropic mouse hepatitis virus. *Arch Virol* (1992) 122(1-2):133–41. doi: 10.1007/BF01321123
50. Fox A, Marino J, Amanat F, Krammer F, Hahn-Holbrook J, Zolla-Pazner S, et al. Evidence of a significant secretory-IgA-dominant SARS-CoV-2 immune response in human milk following recovery from COVID-19. *medRxiv* (2020) 2020.05.04.20089995. doi: 10.1101/2020.05.04.20089995
51. Loos C, Atyeo C, Fischinger S, Burke J, Slein MD, Streeck H, et al. Evolution of Early SARS-CoV-2 and Cross-Coronavirus Immunity. *mSphere* (2020) 5(5): e00622–20. doi: 10.1128/mSphere.00622-20
52. Ben Mkaddem S, Benhamou M, Monteiro RC. Understanding Fc Receptor Involvement in Inflammatory Diseases: From Mechanisms to New Therapeutic Tools. *Front Immunol* (2019) 10:811. doi: 10.3389/fimmu.2019.00811
53. Crouch CF, Bielefeldt Ohmann H, Watts TC, Babiuk LA. Chronic shedding of bovine enteric coronavirus antigen-antibody complexes by clinically normal cows. *J Gen Virol* (1985) 66(Pt 7):1489–500. doi: 10.1099/0022-1317-66-7-1489
54. Silva-Sanchez A, Randall TD. Anatomical Uniqueness of the Mucosal Immune System (GALT, NALT, iBALT) for the Induction and Regulation of Mucosal Immunity and Tolerance. In: *Mucosal Vaccines*. Elsevier (2020). p. 21–54. doi: 10.1016/B978-0-12-811924-2.00002-X
55. Rodriguez-Argente F, Alba-Dominguez M, Ortiz-Muñoz E, Ortega-Gonzalez A. Oromucosal immunomodulation as clinical spectrum mitigating factor in SARS-CoV-2 infection. *Scand J Immunol* (2020) 00:e12972. doi: 10.1111/sji.12972
56. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ. The complement system and innate immunity. In: *Immunobiology: The Immune System in Health and Disease. 5th edition*. New York: Garland Science (2001).
57. Spiegel M, Pichlmair A, Mühlberger E, Haller O, Weber F. The antiviral effect of interferon-beta against SARS-coronavirus is not mediated by MxA protein. *J Clin Virol* (2004) 30(3):211–3. doi: 10.1016/j.jcv.2003.11.013
58. Agematsu K. Memory B cells and CD27. *Histol Histopathol* (2000) 15(2):573–6. doi: 10.14670/HH-15.573
59. Nielsen SCA, Yang F, Jackson KJL, Hoh RA, Röltgen K, Stevens B, et al. Human B cell clonal expansion and convergent antibody responses to SARS-CoV-2. *Cell Host Microbe* (2020) 28(4):516–25.e5. doi: 10.21203/rs.3.rs-27220/v1

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Arenas, Borge, Carbonero, Garcia-Bocanegra, Cano-Terriza, Caballero and Arenas-Montes. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

SYSTEMATIC REVIEW
published: 29 March 2021
doi: 10.3389/fnut.2021.660420

Low Serum 25-hydroxyvitamin D (Vitamin D) Level Is Associated With Susceptibility to COVID-19, Severity, and Mortality: A Systematic Review and Meta-Analysis

Mohammad Rizki Akbar[1]*, *Arief Wibowo*[1], *Raymond Pranata*[1,2] and *Budi Setiabudiawan*[3]

[1] Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, [2] Faculty of Medicine, Universitas Pelita Harapan, Tangerang, Indonesia, [3] Department of Child Health, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

OPEN ACCESS

Edited by:
Christophe Matthys,
KU Leuven, Belgium

Reviewed by:
Roger Bouillon,
KU Leuven, Belgium
Zhongheng Zhang,
Sir Run Run Shaw Hospital, China

***Correspondence:**
Mohammad Rizki Akbar
m.r.akbar@unpad.ac.id

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Nutrition

Received: 29 January 2021
Accepted: 05 March 2021
Published: 29 March 2021

Citation:
Akbar MR, Wibowo A, Pranata R and Setiabudiawan B (2021) Low Serum 25-hydroxyvitamin D (Vitamin D) Level Is Associated With Susceptibility to COVID-19, Severity, and Mortality: A Systematic Review and Meta-Analysis. Front. Nutr. 8:660420. doi: 10.3389/fnut.2021.660420

Background: This systematic review and meta-analysis aimed to assess whether low serum 25-hydroxyvitamin D (25-OHD) level is associated with susceptibility to COVID-19, severity, and mortality related to COVID-19.

Methods: Systematic literature searches of PubMed, Scopus, and Embase database up until 9 December 2020. We include published observational prospective and retrospective studies with information on 25-OHD that reported main/secondary outcome. Low serum 25-OHD refers to participants with serum 25-OHD level below a cut-off point ranging from 20 to 30 ng/mL. Other cut-off values were excluded to reduce heterogeneity. The main outcome was mortality defined as non-survivor/death. The secondary outcome was susceptibility and severe COVID-19.

Results: There were 14 studies comprising of 999,179 participants. Low serum 25-OHD was associated with higher rate of COVID-19 infection compared to the control group (OR = 2.71 [1.72, 4.29], $p < 0.001$; I^2: 92.6%). Higher rate of severe COVID-19 was observed in patients with low serum 25-OHD (OR = 1.90 [1.24, 2.93], $p = 0.003$; I^2: 55.3%), with a sensitivity of 83%, specificity of 39%, PLR of 1.4, NLR of 0.43, and DOR of 3. Low serum 25-OHD was associated with higher mortality (OR = 3.08 [1.35, 7.00], $p = 0.011$; I^2: 80.3%), with a sensitivity of 85%, specificity of 35%, PLR of 1.3, NLR of 0.44, and DOR of 3. Meta-regression analysis showed that the association between low serum 25-OHD and mortality was affected by male gender (OR = 1.22 [1.08, 1.39], $p = 0.002$), diabetes (OR = 0.88 [0.79, 0.98], $p = 0.019$).

Conclusion: Low serum 25-OHD level was associated with COVID-19 infection, severe presentation, and mortality.

Keywords: coronavirus, COVID-19, immunity, infection, mortality, severity, susceptibility, vitamin D

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is one of the most prevalent diseases to date (1). Although most COVID-19 patients have mild-moderate symptom, a considerable number of patients, especially in patients with pre-existing comorbidities, experiences severe infection that might lead to death (2, 3).

Vitamin D is known to modulate immune response (4) and its deficiency was associated with respiratory distress in patients hospitalized for pneumonia (5). Nevertheless, controversies exist, a study indicates that low serum 25-hydroxyvitamin D (25-OHD) was not associated with lung injury or mortality in severe sepsis and trauma (6). Numerous studies on vitamin D in COVID-19 patients also have conflicting results, similar to other diseases. This systematic review and meta-analysis aimed to assess whether low serum 25-OHD is associated with susceptibility to COVID-19, severity, and mortality related to COVID-19.

MATERIALS AND METHODS

This study follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guideline.

Eligibility Criteria

The inclusion criteria were: (1) published observational retrospective and prospective studies, (2) Information on serum 25-OHD with a clear cut-off value ranging from 20 to 30 ng/mL, (3) comparing patients with COVID-19 vs non-COVID-19 OR severity in COVID-19 patients OR mortality in COVID-19 patients.

The paper was excluded if it fulfils one of the following: (1) reviews, (2) preprints, (3) non-research letters, (4) case reports, (5) commentaries, and (6) language other than English. We excluded preprints because of inconsistent credibility (7).

Search Strategy and Study Selection

We performed systematic literature search using PubMed, Scopus, and Embase databases were performed with keywords "COVID-19" OR "SARS-CoV-2" OR "2019-nCoV" AND "Vitamin D" on 9 December 2020.

Duplicate records were removed and the title/abstract was screened by two independent authors. The full-texts of potentially eligible studies were assessed based on the inclusion and exclusion criteria.

Data Extraction

Two authors independently performed data extraction of first author, publication year, design, age, male (gender), hypertension, diabetes, serum 25-OHD status, the outcome of interest and its effect estimates.

Low serum 25-OHD refers to participants with serum 25-OHD below a cut-off point ranging from 20 to 30 ng/mL. Other cut-off values were excluded to reduce heterogeneity.

The main outcome was mortality defined as non-survivor/death. The secondary outcome was susceptibility and severe COVID-19. Susceptibility was calculated by comparing the COVID-19 positive cohort with the COVID-19 negative cohort. Severe COVID-19 was defined according to the criteria for severe CAP, including the need for intensive unit care or mechanical ventilation (8). The effect estimates of the main and secondary outcome were reported as odds ratios (ORs). Several parameters, including the sensitivity and specificity of low serum 25-OHD, the positive likelihood ratio (PLR) and negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) of studies; and generate hierarchical summary receiver operating characteristic (HSROC) for mortality and severity.

Risk of bias assessment was performed by two independent authors using the Newcastle-Ottawa Scale (NOS). Arising discrepancies were resolved by discussion.

Statistical Analysis

DerSimonian and Laird random-effects model were used to generate pooled ORs and its 95% CI. $P \leq 0.05$ was considered as statistically significant. Assessment of heterogeneity was performed by using I-squared (I^2) and Cochrane Q test, in which an $I^2 > 50\%$ or $p < 0.10$ indicates significant heterogeneity. We performed the qualitative funnel plot analysis and the quantitative Egger's test in order to assess the possibility for publication bias and small-study effects. Sensitivity and specificity, PLR and NLR, and DOR were pooled; HSROC was generated. Restricted-maximum likelihood (REML) random-effects meta-regression was performed using age, male (gender), diabetes, and hypertension as covariates. To perform these analyses, STATA 16 (StataCorp LLC, Texas, US) was used.

RESULTS

There were 14 studies comprising of 999,179 participants in the qualitative and quantitative synthesis (9–22) (**Figure 1**). The baseline characteristics and risk of bias assessment based on NOS is displayed in **Table 1**. Severity occurs in 42% (22–62%). Mortality occurs in 24% (6–41%) of patients in the pooled analysis.

Meta-analysis showed that low serum 25-OHD was associated with higher rate of COVID-19 infection compared to the control group (OR = 2.71 [1.72, 4.29], $p < 0.001$; I^2: 92.6%, $p < 0.001$) (**Figure 2**).

Higher rate of severe COVID-19 was observed in patients with low serum 25-OHD (OR = 2.19 [1.17, 4.10], $p = 0.013$; I^2: 64.3%, $p = 0.025$) (**Figure 3A**), with a sensitivity of 0.86 [0.79, 0.91], specificity of 0.39 [0.23, 0.57], PLR of 1.4 [1.0, 2.0], NLR of 0.36 [0.16, 0.83], and DOR of 4 [1, 12] (**Figure 3B**). Fagan's nomogram indicate that a low serum 25-OHD was associated with 50% post-test probability and normal serum 25-OHD was associated with 21% post-test probability for mortality, in a sample with 42% pre-test probability (**Figure 3C**).

Low serum 25-OHD was associated with higher mortality (OR = 3.08 [1.35, 7.00], $p = 0.011$; I^2: 80.3%, $p < 0.001$) (**Figure 4A**), with a sensitivity of 0.85 [0.68, 0.93], specificity of 0.35 [0.26, 0.45], PLR of 1.3 [1.0, 1.6], NLR of 0.44 [0.18, 1.08], and DOR of 3 [1, 9] (**Figure 4B**). Fagan's nomogram indicate that a low serum 25-OHD

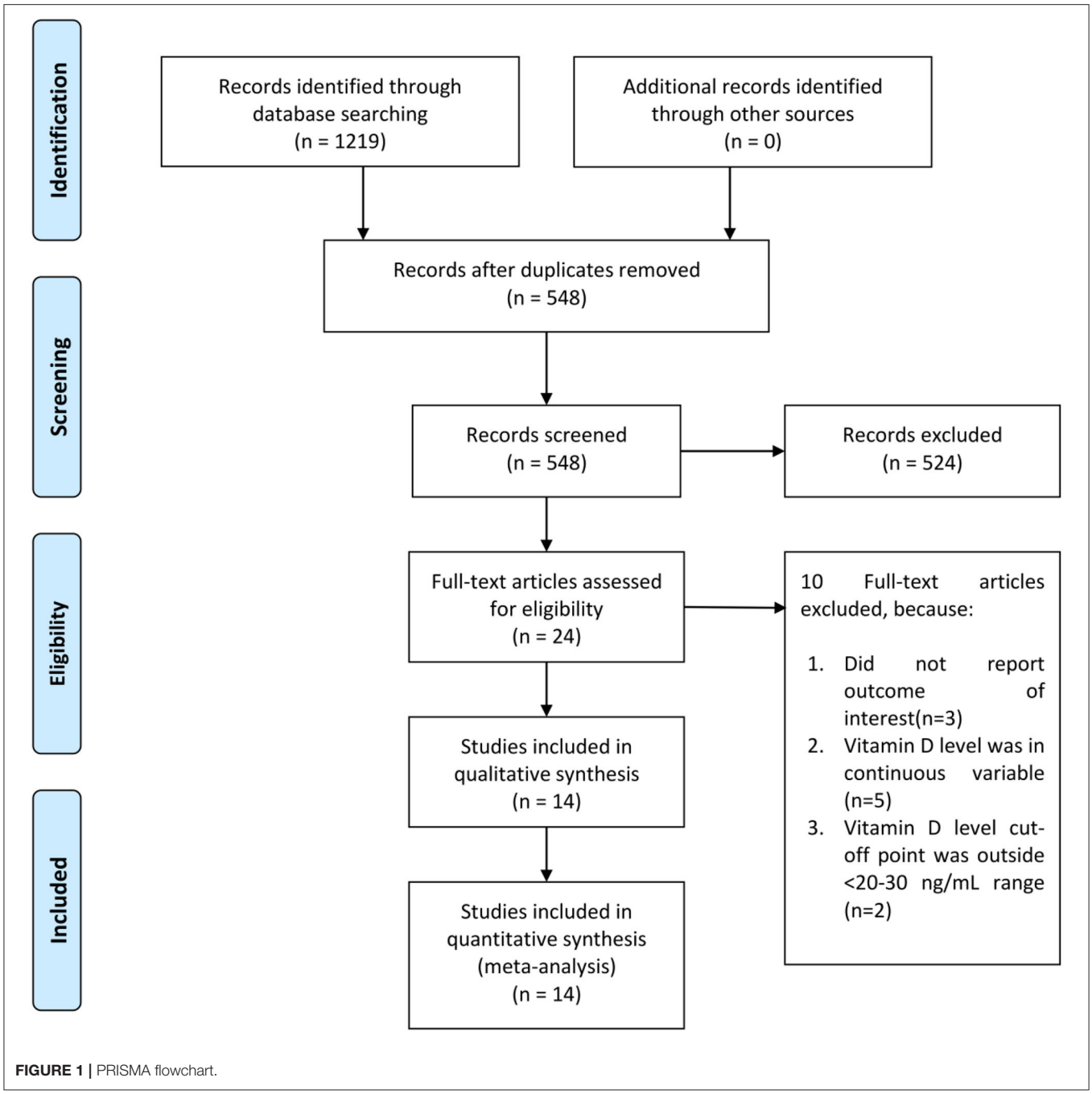


FIGURE 1 | PRISMA flowchart.

was associated with 29% post-test probability and normal serum 25-OHD was associated with 12% post-test probability for mortality, in a sample with 24% pre-test probability (**Figure 4C**).

Funnel plot was asymmetrical for mortality, severity, and susceptibility. Egger's test indicates significant small-study effects for severity ($p = 0.047$) and mortality ($p = 0.046$). There was no indication of small-study effects for susceptibility ($p = 0.615$).

Meta-regression analysis showed that the association between low serum 25-OHD and COVID-19 infection was affected by age (OR = 1.06 [1.01, 1.12], $p = 0.020$) and male gender (OR = 1.04 [1.00, 1.72], $p = 0.039$).

Meta-regression analysis showed that the association between low serum 25-OHD and mortality was affected by male gender (OR = 1.22 [1.08, 1.39], $p = 0.002$) (**Figure 5A**), diabetes (OR = 0.88 [0.79, 0.98], $p = 0.019$) (**Figure 5B**); borderline significant for age (OR = 0.93 [0.87, 1.00], $p = 0.061$) and hypertension (OR = 0.93 [0.87, 1.00], $p = 0.052$).

TABLE 1 | Baseline characteristics of the included studies.

Authors	Design	Sample size	Vitamin D cut-off (ng/mL)	Outcome of interest	Age (years)	Male (%)	Diabetes (%)	Hypertension (%)	NOS
Abrishami et al. (10)	Retrospective observational	73	<25	Mortality	55.2	64.4	15.1	24.7	8
Baktash et al. (9)	Retrospective Observational	105	≤30	Mortality IMV	81	54.3	32.3	51.4	6
Cereda et al. (22)	Prospective Observational	129	<20	Mortality Severity	77	54.3	30.7	70.1	8
Hastie et al. (21)	Observational	656	<25	Mortality	–	–	–	–	7
Hernández et al. (20)	Restrospective Observational (case-control)	216	<20	Mortality Severity Susceptibility	61	62.4	16.5	40	6
Im et al. (18)	Retrospective Observational	200	<20	Susceptibility	57.5 (COVID-19)	–	–	–	5
Jain et al. (12)	Prospective Observational	154	<20	Mortality	46.1	61.7	–	–	5
Katz et al. (11)	Cross-Sectional	987,849 971 (COVID-19)	Deficiency	Susceptibility	Stratified	48.4	–	–	6
Luo et al. (14)	Cross-Sectional	895	<30	Mortality Severity Susceptibility	55.5	45.3	–	–	6
Maghbooli et al. (19)	Retrospective Observational	235	<30	Severity	58.7	61.3	36.6	44.4	6
Meltzer et al. (17)	Retrospective Observational	489	<20	Susceptibility	49.2	25	28	53	8
Merzon et al. (16)	Retrospective Observational	7,807 782 (COVID-19)	<20	Susceptibility	44	41.4	–	–	7
adujkovic et al. (15)	Retrospective Observational	185	<20	Mortality Severity	60	51	10	–	8
De Smet et al. (13)	Retrospective Observational	186	<20	Mortality	69	58.6	14	–	8

IMV, Invasive Mechanical Ventilation; NOS, Newcastle-Ottawa Scale.

DISCUSSION

This meta-analysis indicates that low serum 25-OHD levels was associated with higher infection, severe COVID-19, and mortality rate.

Pooled analysis showed that the susceptibility to COVID-19 was higher in patients with low serum 25-OHD. Meta-regression analysis showed that age and gender (male) significantly increase the association. Thus, elderly patients with low serum 25-OHD are more susceptible to COVID-19 compared to non-elderly patients with low serum 25-OHD. Older adults may experience immunosenescence and inflammaging which may affect immune responses against infection, thus contributing to susceptibility (23, 24). Elderly population produces 75% less cutaneous vitamin D3 than young adults and is thus more prone to lower serum 25-OHD level (25). These, in combination, may explain the increased susceptibility to COVID-19 in older adults. Meta-regression showed that male gender affects the association between low serum 25-OHD and susceptibility to COVID-19 and mortality. Vitamin D affects androgen synthesis in testicular cells, and endogenous testosterone may account for differences in properties of low 25-OHD in males and females (26, 27). This may be of special importance in elderly patients. The exact mechanism on why gender plays a role requires further investigation.

The current analysis showed that low serum 25-OHD was associated with higher mortality and severe COVID-19. However, it should be noted that some of studies were excluded because they only provide a comparison of serum 25-OHD levels in a continuous variable, several of these studies showed a no significant difference between mean serum 25-OHD and mortality and severity of COVID-19. A prospective multicenter observational CovILD study of 109 patients by Pizzini et al. (28) indicates that low serum 25-OHD levels at the onset or 8-week follow-up were not associated with persistent symptom burden, lung function impairment, ongoing inflammation, or more severe CT abnormalities. Our study also indicates that the susceptibility to COVID-19 might be higher in patients with low

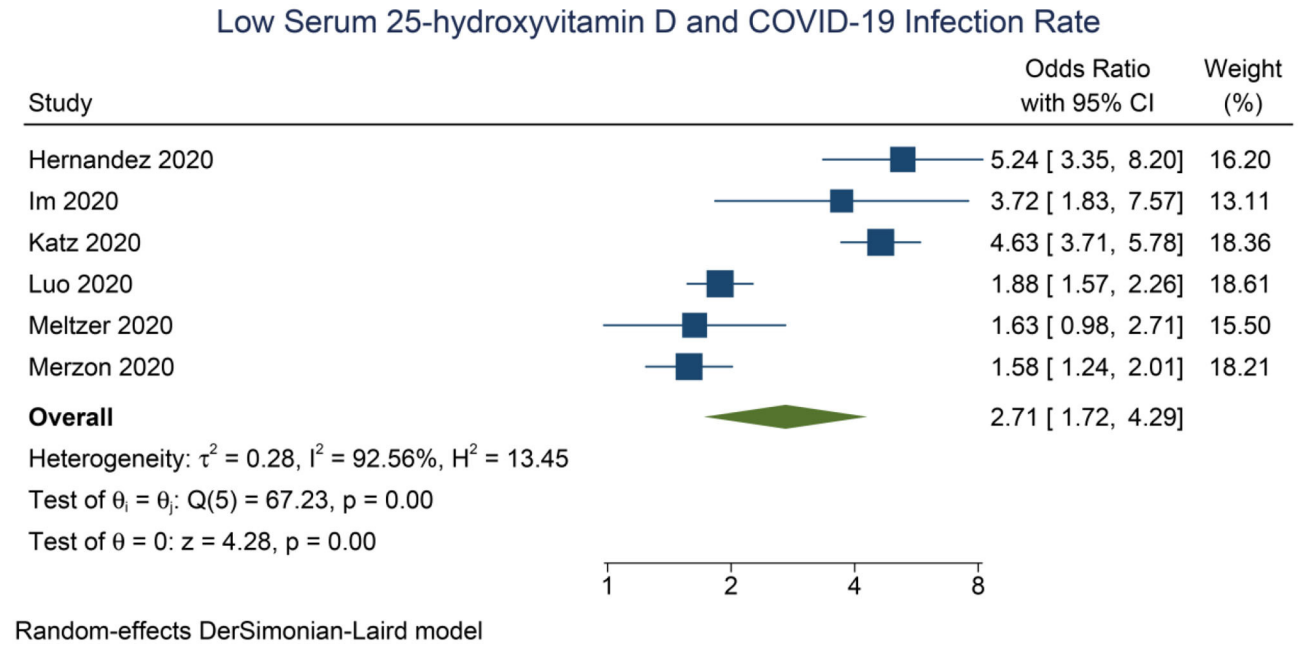


FIGURE 2 | Low Serum 25-hydroxyvitamin D and COVID-19 infection rate.

serum 25-OHD, however, a data of 1,326 patients from the UK biobank cohort indicates that Vitamin D level, adjusted with gender, age, and ethnicity was not significant for susceptibility to COVID-19.

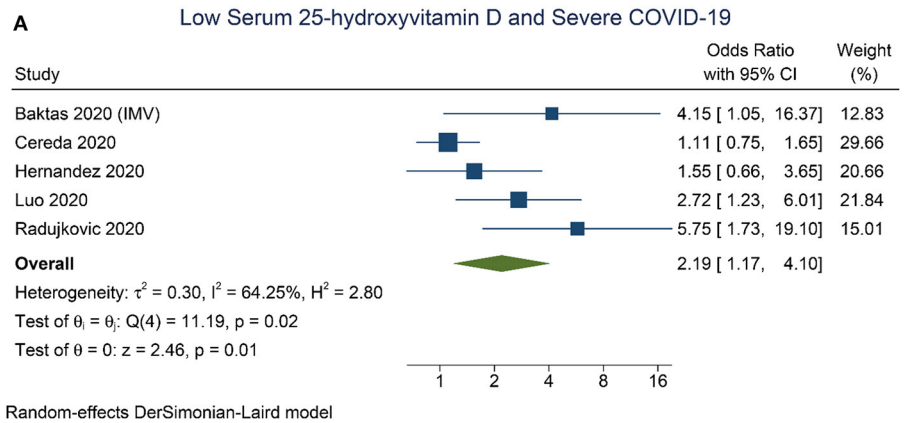
Studies evaluating the serum 25-OHD level often have different cut-off points, we included those with 20–30 ng/mL cut-off points. There was a study showed that 10 ng/mL cut-off point was associated with tenfold risk of mortality (29).

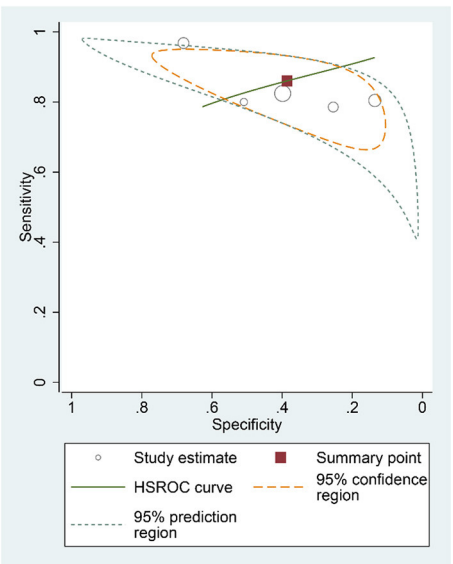
Another possible explanation for the association between low serum 25-OHD and poor outcome is that patients with severe illness are often bedridden and have low intake, leading to low serum 25-OHD level (30). Thus subsequent studies need to be able to demonstrate a causal relationship in order to solidify the evidence. Patients with older age and comorbidities such as obesity, diabetes, cardiovascular disease, hypertension, heart failure are also associated with higher mortality in patients with COVID-19 (31–38). Obesity itself was shown to be associated with low serum 25-OHD level (32). The heterogeneity of the pooled effect estimate was high, meta-regression analysis indicates that gender and comorbidities affect the association between low serum 25-OHD and mortality; thus, one of the potential causes of heterogeneity is the variation in the proportion of comorbidities. Thus a well-designed prospective large cohort studies with rigorous statistical analysis and adequate adjustment to covariates are required to obtain the "true" effect of low serum 25-OHD on mortality in COVID-19.

Previously, a meta-analysis showed that Vitamin D supplementation was associated with reduced risk for acute respiratory tract infection (6). Subgroup analysis in the study indicate that vitamin D3 benefit was observed in <800 international units (IU), a statistically non-significant trend in 800–2,000 IU, and no benefit in ≥2,000 IU supplementation. The potential risk of bias should be noted, the observation was made based on five, nine, and eleven studies respectively; with the statistical significance noted in pooling of five studies. Rastogi et al. (39) conducted a randomized controlled study with short term administration of high-dose vitamin D in asymptomatic or mildly symptomatic COVID-19 patients with vitamin D deficiency and noted that a greater proportion of patients turned negative in the vitamin D supplementation group. Additionally, there was a significant decrease in fibrinogen in patients receiving vitamin D. Nonetheless, the study has a small sample size and lacks adjustment for potential confounders on their analysis. These analyses also did not hard endpoints such as mortality or requirement for a more intensive care. Cohort studies have shown potential benefit of vitamin D supplementation in terms of COVID-19 severity (40). A quasi-experimental study indicate possible benefit of vitamin D bolus on survival of COVID-19 patients in a nursing home (41), however, the control arm has only nine patients compared to 57 in the interventional arm. It is unclear why the control group did not receive vitamin D bolus, this might be a potentially important confounder.

Clinical Implications

Low serum 25-OHD levels was shown to be associated with higher infection rate, severity, and mortality. Whether the relationship is causal remains to be investigated. Vitamin D supplementation is economical and potentially beneficial. Thus,





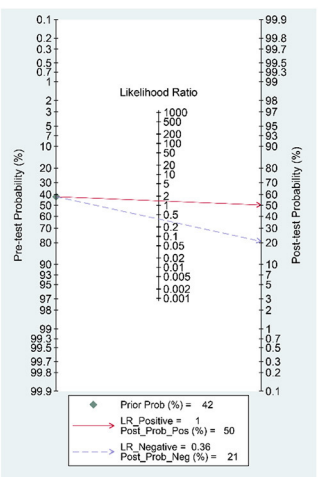


FIGURE 3 | Low Serum 25-hydroxyvitamin D and Severe COVID-19. Forest-plot **(A)**, HSROC curve **(B)**, and Fagan's Nomogram **(C)**. HSROC, hierarchical summary receiver operating characteristic.

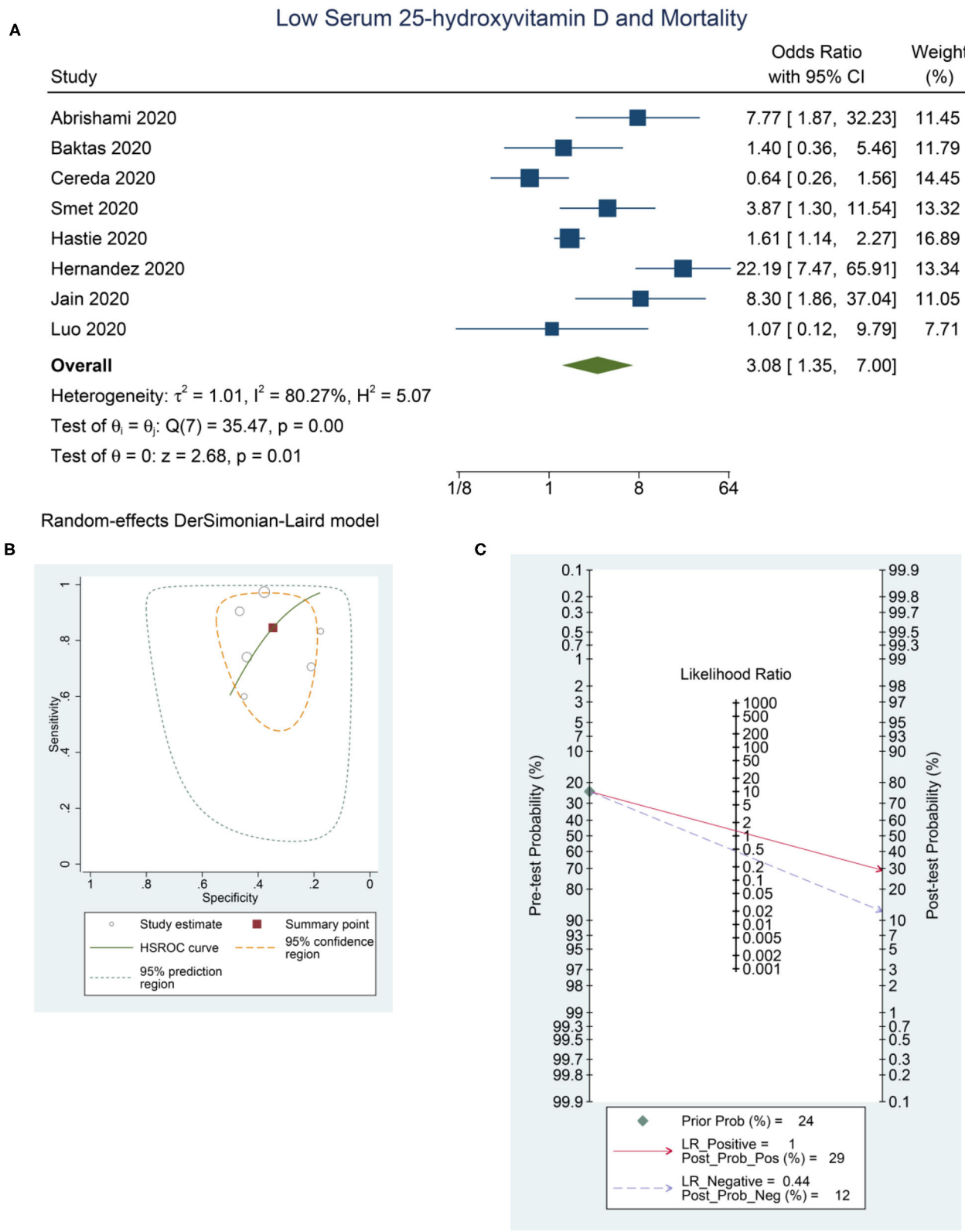


FIGURE 4 | Low Serum 25-hydroxyvitamin D and Mortality. Forest-plot **(A)**, HSROC curve **(B)**, and Fagan's Nomogram **(C)**. HSROC, hierarchical summary receiver operating characteristic.

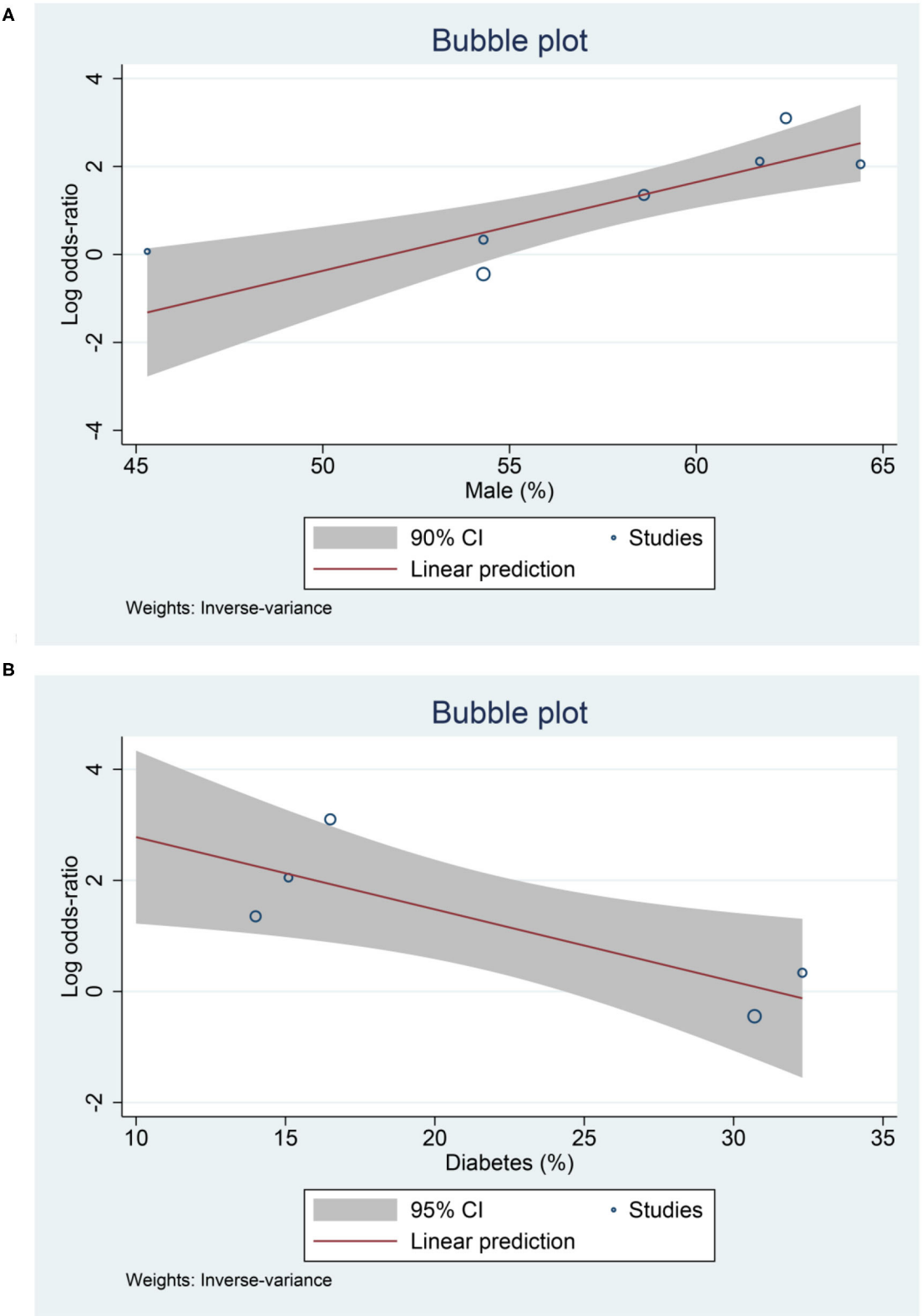


FIGURE 5 | Meta-regression Analysis. Male **(A)** and diabetes **(B)**.

it is recommended to provide supplementation to the Vitamin D deficient patients. Nevertheless, high quality randomized controlled trials are required to determine whether routine Vitamin D supplementation will be useful.

Limitation

The risk of publication bias, which is caused by positive studies are more likely to be published compared to the negative studies, cannot be ruled out. The retrospective design of the studies presents as a potential source of bias. The cut-off point slightly varies among the pooled analysis. Potential unaccounted confounders may cause bias in the studies, for example, bedridden patients often have low serum 25-OHD compared to the healthy counterpart.

One of the study by Katz et al. (11) has the largest sample size of 987,849. However, we only take Katz et al. study for susceptibility, thus it did not affect the pooled mortality and severity. For the susceptibility analysis, Katz et al. contributes to 18.36% of the weight for susceptibility. Largest contributor is Luo et al. which contributes to 18.61% and the smallest was Im et al. to 13.11%. Thus despite the large sample size compared to the others, it was not expected to change the direction of effect estimate. However, small-study effects in the pooled effect estimate for susceptibility might be caused by the studies with minimal sample because they are weighted almost equally with the larger studies (42). Finally, meta-regression analysis was also based on small number of studies.

In conclusion, low serum 25-OHD level was associated with higher rate of COVID-19 infection, severe presentation, and mortality.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MA: conceptualization, investigation, writing — original draft, writing — review and editing, and supervision. AW: data curation, investigation, and writing — original draft. RP: conceptualization, methodology, software, data curation, formal analysis, statistical analysis, investigation, validation, and writing — original draft. BS: investigation, and writing — review and editing. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Fahmi I. *#Covid19 Coronavirus Disease 2019 Situation Report - 31*. (2020). Available online at: https://pers.droneemprit.id/covid19/
2. Lim MA, Pranata R, Huang I, Yonas E, Soeroto AY, Supriyadi R. Multiorgan failure with emphasis on acute kidney injury and severity of COVID-19: systematic review and meta-Analysis. *Can J Kidney Heal Dis.* (2020) 7:205435812093857. doi: 10.1177/20543581209 38573
3. Santoso A, Pranata R, Wibowo A, Al-Farabi MJ, Huang I, Antariksa B. Cardiac injury is associated with mortality and critically ill pneumonia in COVID-19: a meta-analysis. *Am J Emerg Med*. (2020). doi: 10.1016/j.ajem.2020.04.052. [Epub ahead of print].
4. Almerighi C, Sinistro A, Cavazza A, Ciaprini C, Rocchi G, Bergamini A. 1α,25-Dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in Human Monocytes. *Cytokine.* (2009) 45:190–7. doi: 10.1016/j.cyto.2008.12.009
5. Park S, Lee MG, Hong SB, Lim CM, Koh Y, Huh JW. Effect of vitamin D deficiency in korean patients with acute respiratory distress syndrome. *Korean J Intern Med.* (2018) 33:1129–36. doi: 10.3904/kjim.2017.380
6. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ.* (2017) 356:i6583. doi: 10.1136/bmj.i6583
7. Henrina J, Lim MA, Pranata R. COVID-19 and misinformation: how an infodemic fueled the prominence of vitamin D. *Br J Nutr.* (2020) 125:359–60. doi: 10.1017/S0007114520002950
8. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia. *Am J Respir Crit Care Med.* (2019) 200:E45–E67. doi: 10.1164/rccm.201908-1581ST
9. Baktash V, Hosack T, Patel N, Shah S, Kandiah P, Van Den Abbeele K, et al. Vitamin D status and outcomes for hospitalised older patients with COVID-19. *Postgrad Med J.* (2020) 2:1–6. doi: 10.1136/postgradmedj-2020-138712
10. Abrishami A, Dalili N, Mohammadi Torbati P, Asgari R, Arab-Ahmadi M, Behnam B, et al. Possible association of vitamin D status with lung involvement and outcome in patients with COVID-19: a retrospective study. *Eur J Nutr*. (2020). doi: 10.1007/s00394-020-02411-0. [Epub ahead of print].
11. Katz J, Yue S, Xue W. Increased risk for COVID-19 in patients with vitamin D deficiency. *Nutrition.* (2021) 84:111106. doi: 10.1016/j.nut.2020.111106
12. Jain A, Chaurasia R, Sengar NS, Singh M, Mahor S, Narain S. Analysis of vitamin D level among asymptomatic and critically ill COVID-19 patients and its correlation with inflammatory markers. *Sci Rep.* (2020) 10:1–8. doi: 10.1038/s41598-020-77093-z
13. De Smet D, De Smet K, Herroelen P, Gryspeerdt S, Martens GA. Serum 25(OH)D level on hospital admission associated with COVID-19 stage and mortality. *Am J Clin Pathol.* (2020) 25:1–8. doi: 10.1093/ajcp/aqaa252
14. Luo X, Liao Q, Shen Y, Li H, Cheng L. Vitamin D deficiency is inversely associated with COVID-19 incidence and disease severity in Chinese people. *J Nutr.* (2021) 151:98–103. doi: 10.1093/jn/nxaa332
15. Radujkovic A, Hippchen T, Tiwari-Heckler S, Dreher S, Boxberger M, Merle U. Vitamin D deficiency and outcome of COVID-19 patients. *Nutrients.* (2020) 12:1–13. doi: 10.3390/nu12092757
16. Merzon E, Tworowski D, Gorohovski A, Vinker S, Golan Cohen A, Green I, et al. Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. *FEBS J.* (2020) 287:3693–702. doi: 10.1111/febs.15495
17. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of vitamin D status and other clinical characteristics with COVID-19 test results. *JAMA Netw open.* (2020) 3:e2019722. doi: 10.1001/jamanetworkopen.2020.19722
18. Im JH, Je YS, Baek J, Chung MH, Kwon HY, Lee JS. Nutritional status of patients with COVID-19. *Int J Infect Dis.* (2020) 100:390–3. doi: 10.1016/j.ijid.2020.08.018
19. Maghbooli Z, Sahraian MA, Ebrahimi M, Pazoki M, Kafan S, Tabriz HM, et al. Vitamin D sufficiency, a serum 25-hydroxyvitamin D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection. *PLoS One.* (2020) 15:1–13. doi: 10.1371/journal.pone.0239799
20. Hernández JL, Nan D, Fernandez-Ayala M, García-Unzueta M, Hernández-Hernández MA, López-Hoyos M, et al. Vitamin D status in hospitalized patients with SARS-CoV-2 infection. *J Clin Endocrinol Metab.* (2020) 106:1343–53. doi: 10.1210/clinem/dgaa733

21. Hastie CE, Pell JP, Sattar N. Vitamin D and COVID-19 infection and mortality in UK Biobank. *Eur J Nutr.* (2020) 60:545–48. doi: 10.1101/2020.06.26.20140921
22. Cereda E, Bogliolo L, Klersy C, Lobascio F, Masi S, Crotti S, et al. Vitamin D 25OH deficiency in COVID-19 patients admitted to a tertiary referral hospital. *Clin Nutr*. (2020). doi: 10.1016/j.clnu.2020.10.055. [Epub ahead of print].
23. Shea PM, Griffin T s. P, Brennan M, Mulkerrin EC. COVID-19: the older adult and the importance of vitamin D sufficiency. *J Nutr Sci.* (2020) 9:e40. doi: 10.1017/jns.2020.36
24. Huang I, Pranata R. Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis. *J Intensive Care.* (2020) 8:36. doi: 10.1186/s40560-020-00453-4
25. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc.* (2010) 85:752–8. doi: 10.4065/mcp.2010.0138
26. Hofer D, Münzker J, Schwetz V, Ulbing M, Hutz K, Stiegler P, et al. Testicular synthesis and vitamin D action. *J Clin Endocrinol Metab.* (2014) 99:3766–73. doi: 10.1210/jc.2014-1690
27. Sanghera DK, Sapkota BR, Aston CE, Blackett PR. Vitamin D status, gender differences, and cardiometabolic health disparities. *Ann Nutr Metab.* (2017) 70:79–87. doi: 10.1159/000458765
28. Pizzini A, Aichner M, Sahanic S, Böhm A, Egger A, Hoermann G, et al. Impact of vitamin d deficiency on covid-19-a prospective analysis from the covild registry. *Nutrients.* (2020) 12:1–9. doi: 10.3390/nu12092775
29. Carpagnano GE, Di Lecce V, Quaranta VN, Zito A, Buonamico E, Capozza E, et al. Vitamin D deficiency as a predictor of poor prognosis in patients with acute respiratory failure due to COVID-19. *J Endocrinol Invest.* (2020) 44:765–71. doi: 10.1007/s40618-020-01370-x
30. Egsmose C, Lund B, Mcnair P, Lund B, Storm T, Sørensen OH. Low serum levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in institutionalized old people: influence of solar exposure and vitamin D supplementation. *Age Ageing.* (1987) 16:35–40. doi: 10.1093/ageing/16.1.35
31. Pranata R, Huang I, Lim MA, Wahjoepramono EJ, July J. Impact of cerebrovascular and cardiovascular diseases on mortality and severity of COVID-19-systematic review, meta-analysis, and meta-regression. *J Stroke Cerebrovasc Dis.* (2020) 29:104949. doi: 10.1016/j.jstrokecerebrovasdis.2020.104949
32. Pranata R, Lim MA, Yonas E, Vania R, Lukito AA, Siswanto BB, et al. Body mass index and outcome in patients with COVID-19: a dose-response meta-analysis. *Diabetes Metab*. (2020) 47:101178. doi: 10.1016/j.diabet.2020.07.005
33. Pranata R, Supriyadi R, Huang I, Permana H, Lim MA, Yonas E, et al. The association between chronic kidney disease and new onset renal replacement therapy on the outcome of COVID-19 patients: a meta-analysis. *Clin Med Insights Circ Respir Pulm Med.* (2020) 14:1179548420959165. doi: 10.1177/1179548420959165
34. Pranata R, Lim MA, Huang I, Raharjo SB, Lukito AA. Hypertension is associated with increased mortality and severity of disease in COVID-19 pneumonia: A systematic review, meta-analysis and meta-regression. *J Renin Angiotensin Aldosterone Syst.* (2020) 21:1470320320926899. doi: 10.1177/1470320320926899
35. Huang I, Lim MA, Pranata R. Diabetes mellitus is associated with increased mortality and severity of disease in COVID-19 pneumonia - a systematic review, meta-analysis, and meta-regression: diabetes and COVID-19. *Diabetes Metab Syndr Clin Res Rev.* (2020) 14:395–403. doi: 10.1016/j.dsx.2020.04.018
36. Yonas E, Alwi I, Pranata R, Huang I, Lim MA, Gutierrez EJ, et al. Effect of heart failure on the outcome of COVID-19 - a meta analysis and systematic review. *Am J Emerg Med.* (2020). doi: 10.1016/j.ajem.2020.07.009. [Epub ahead of print].
37. Pranata R, Henrina J, Lim MA, Lawrensia S, Yonas E, Vania R, et al. Clinical frailty scale and mortality in COVID-19: a systematic review and dose-response meta-analysis: Clinical Frailty Scale in COVID-19. *Arch Gerontol Geriatr.* (2021) 93:104324. doi: 10.1016/j.archger.2020.104324
38. July J, Pranata R. Prevalence of dementia and its impact on mortality in patients with coronavirus disease 2019: a systematic review and meta-analysis. *Geriatr Gerontol Int*. (2020) 21:172–77. doi: 10.1111/ggi.14107
39. Rastogi A, Bhansali A, Khare N, Suri V, Yaddanapudi N, Sachdeva N, et al. Short term, high-dose vitamin D supplementation for COVID-19 disease: a randomised, placebo-controlled, study (SHADE study). *Postgrad Med J*. (2020). doi: 10.1136/postgradmedj-2020-139065. [Epub ahead of print].
40. Tan CW, Ho LP, Kalimuddin S, Cherng BPZ, Teh YE, Thien SY, et al. Cohort study to evaluate effect of vitamin D, magnesium, and vitamin B12 in combination on severe outcome progression in older patients with coronavirus (COVID-19). *Nutrition.* (2020) 79–80:111017. doi: 10.1016/j.nut.2020.111017
41. Annweiler C, Hanotte B, Grandin de l'Eprevier C, Sabatier JM, Lafaie L, Célarier T. Vitamin D and survival in COVID-19 patients: a quasi-experimental study. *J Steroid Biochem Mol Biol.* (2020) 204:105771. doi: 10.1016/j.jsbmb.2020.105771
42. Zhang Z, Xu X, Ni H. Small studies may overestimate the effect sizes in critical care meta-analyses: a meta-epidemiological study. *Crit Care.* (2013) 17:R2. doi: 10.1186/cc11919

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Akbar, Wibowo, Pranata and Setiabudiawan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

CLINICAL TRIAL
published: 31 March 2021
doi: 10.3389/fnut.2021.617721

Cooking at Home and Adherence to the Mediterranean Diet During the COVID-19 Confinement: The Experience From the Croatian COVIDiet Study

Danijela Pfeifer[1], Josip Rešetar[1,2], Jasenka Gajdoš Kljusurić[1], Ines Panjkota Krbavčić[1], Darija Vranešić Bender[1,3], Celia Rodríguez-Pérez[4,5,6], María Dolores Ruíz-López[5,7] and Zvonimir Šatalić[1]*

[1] Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia, [2] Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia, [3] Clinical Unit of Clinical Nutrition, Department of Internal Medicine, University Hospital Centre Zagreb, Zagreb, Croatia, [4] Department of Nutrition and Food Science, University of Granada, Melilla, Spain, [5] Biomedical Research Centre, Institute of Nutrition and Food Technology (INYTA) 'JoséMataix', University of Granada, Granada, Spain, [6] Biosanitary Research Institute of Granada, Granada, Spain, [7] Department of Nutrition and Food Science, University of Granada, Granada, Spain

OPEN ACCESS

Edited by:
Ioannis Zabetakis,
University of Limerick, Ireland

Reviewed by:
Cinzia Ferraris,
University of Pavia, Italy
Livia Silvia Augustin,
Istituto Nazionale Tumori Fondazione G. Pascale (IRCCS), Italy

***Correspondence:**
Josip Rešetar
jresetar@student.pharma.hr

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: 15 October 2020
Accepted: 22 February 2021
Published: 31 March 2021

Citation:
Pfeifer D, Rešetar J, Gajdoš Kljusurić J, Panjkota Krbavčić I, Vranešić Bender D, Rodríguez-Pérez C, Ruíz-López MD and Šatalić Z (2021) Cooking at Home and Adherence to the Mediterranean Diet During the COVID-19 Confinement: The Experience From the Croatian COVIDiet Study. Front. Nutr. 8:617721. doi: 10.3389/fnut.2021.617721

Introduction: The primary aims of this study were to evaluate the changes in dietary behavior among the Croatian adult population during the COVID-19 outbreak and to explore the impact of confinement on cooking habits.

Methods: The study was based on results from COVIDiet_Int cross-sectional study—a part of COVIDiet project (NCT04449731). A self-administered online questionnaire was used to assess the frequency of food consumption, eating habits, and sociodemographic information. A total number of 4,281 participants (80.5% females and 19.4% males) completed the questionnaire.

Results: The Mediterranean Diet Adherence Screener (MEDAS) score before the confinement was 5.02 ± 1.97, while during the confinement, the MEDAS score increased to 5.85 ± 2.04. Participants who had higher adherence to the Mediterranean diet (MedDiet) during the confinement were mostly females (88.8%), aged between 20 and 50 years, with the highest level of education (66.3%) and normal BMI (70.6%). The majority of participants maintained their dietary behavior as it was before COVID-19 confinement, while 36.9% decreased their physical activity. Participants with higher MEDAS score were more eager to increase their physical activity. Additionally, higher median values of MEDAS score were noted for participants with body mass index values below 24.9 kg/m^2 (6.0 vs. 5.0 for participants with BMI above 25 kg/m^2). Participants in all residence places increased their cooking frequency during the confinement (53.8%), which was associated with an increase in vegetables, legumes, as well as fish and seafood consumption.

Conclusions: According to our findings, Croatian adults exhibited medium adherence to the MedDiet during the COVID-19 confinement. The results suggest that cooking frequency could be positively associated with overall dietary quality, which is of utmost importance in these demanding times.

Keywords: body mass index, cooking, COVID-19 confinement, mediterranean diet, questionnaire, SARS-CoV-2

INTRODUCTION

The novel coronavirus disease, COVID-19, caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), emerged in late December 2019, in China, and since then, it has been one of the greatest public health crises the world has ever faced. As a result of its rapid spread, high infectivity, and relatively high mortality, the World Health Organization (WHO) has declared COVID-19 a pandemic on March 12, 2020 (1). To date, on January 29, more than 100 million cases of COVID-19 have been confirmed and 2,176,159 deaths reported globally (2). In Croatia, up until January 29, the number of COVID-19-positive people was 231,539, while 4,972 people passed away (3).

Although vaccination against SARS-CoV-2 started at the end of the year 2020, the main strategy to attenuate the rate of infection includes community-based non-pharmaceutical interventions (NPIs) such as social distancing, restricting public events, case-based isolation, and confinement (4). Croatia follows a similar strategy pattern—a national confinement policy was implemented; public events and many economic activities were stopped, and schools and universities were closed on March 19. After almost 5 weeks of confinement, due to a beneficial epidemiological situation, Croatia started to reactivate its economic and social life in three phases—the first phase, when some business entities were allowed to work, began on April 27; the second phase, when playgrounds and outdoor sports facilities were opened, began on May 4; the third phase, when shopping centers, schools, and kindergartens were reopened, began on May 11 (5).

Even though NPIs, like confinement and isolation, can mitigate and control the rate of COVID-19 spread (6), they can also lead to boredom, mental distress, emotional imbalance, change in eating behavior, weight gain, and higher risk of cardiovascular disease (7). Staying at home and working from home can result in boredom, which is positively associated with higher energy intake (8). However, more free time during confinement could also bring about positive eating behavior and habits—more time spent cooking, family meals, and better meal planning. Additionally, closure of gyms, recreation facilities, and prohibition of sports activities could result in negative well-being, low-energy expenditure, and cardiometabolic health impairment (9). Maintaining adequate energy balance is essential in the time of COVID-19 pandemic due to a positive link found between severe COVID-19 cases and obesity (10, 11).

Optimal nutritional status has a pivotal role in immune system functioning and could help mitigate the impact of seasonal and emerging viral infections (12). There are already several papers supporting nutraceuticals, probiotics, and supplements alongside a well-balanced diet as a method of managing COVID-19 (13, 14). Bousquet et al. (15) suggest that certain foods may reduce angiotensin-converting enzyme 2 (ACE2) activity, which is, beside multiple physiological roles, a SARS-CoV-2 receptor. Considering the importance of nutrition during COVID-19 pandemic, the European Federation of the Associations of Dietitians (16) as well as the WHO (17) have made public health nutrition advice and guidelines for maintaining an optimal nutrition status. Furthermore, Muscogiuri et al. (18) published a perspective on nutritional recommendations during the COVID-19 confinement. All the recommendations promote nutrients, foods, and dietary patterns that are included in a Mediterranean diet (MedDiet), which is considered healthy and anti-inflammatory (19).

In contrast to other popular diets, the MedDiet has suggestive evidence of an improvement in weight, BMI, total cholesterol, glucose, and blood pressure (20). Obesity, in terms of COVID-19, is associated with a higher risk for hospitalization and higher death rates (21), which also highlights the current necessity for proper nutrition. The MedDiet favorable effects, both therapeutic and preventive, are proposed in detail elsewhere (22, 23). Moreover, a higher adherence to the MedDiet during the COVID-19 crisis is suggested not only to combat the COVID-19-related complications but also to lower the risk of chronic diseases (24). However, due to shortages of some foods in grocery stores during confinement and financial concerns, it is not very certain if people could fully follow the MedDiet guidelines.

The aims of this study were (i) to evaluate the changes in dietary behavior among the Croatian adult population, (ii) to investigate adherence to the MedDiet, (iii) to examine the availability of certain foods, (iv) to determine differences in adherence to MedDiet among two BMI groups (<25 and >25 kg/m^2), and (v) to explore the cooking habits during the COVID-19 outbreak.

MATERIALS AND METHODS

Study Design and Participants

COVIDiet_Int is a cross-sectional study and a part of a COVIDiet project, which included Croatian adults and had no exclusion criteria other than age. A study has been conducted in accordance with the World Medical Association's Declaration of Helsinki, approved by the Research Ethics Committee of the University of Granada (1526/CEIH/2020), and registered as a clinical trial in a public trials registry (NCT04449731). Since the data were collected anonymously by the use of an online questionnaire, without including personal data, no informed consent was

required. Despite that, all the participants were informed about the study objectives and asked for a permission before filling out a questionnaire. The questionnaire was opened from April 7 until May 4. Data were collected through snowball sampling.

Data Collecting—Questionnaire

A self-administered online questionnaire, created using the Google Forms tool, was used to assess the frequency of food consumption, eating habits, and sociodemographic information. Sociodemographic characteristics included age, gender, place of residence, country region, children in care, and education and training qualifications. The assessment of food consumption frequency included selected foods related to the MedDiet pattern through MEDAS-validated questionnaire (24, 25). For the purpose of this study, respondents were asked to estimate the consumed quantity of (i) olive oil (0–1.9, 2–3.9, or >4 tablespoons (1 tablespoon = 13.5 g)/day), (ii) vegetables (0–0.9, 1–1.9, or >2 servings (1 serving = 200 g)/day), (iii) fruit (0–0.9, 1–2.9, or >3 pieces/day), (iv) red meat (0–0.9 or >1 serving (1 serving = 100–150 g)/day), (v) butter, margarine, or cream (0–0.9 or >1 serving (1 serving = 12 g)/day), (vi) carbonated and/or sugary beverages (0–0.9 or >1 time/day), (vii) wine (0–2.9, 3–6.9, or >7 cups (1 cup = 100 ml)/week or never), (viii) legumes (0–0.9, 1–2.9, or >3 servings (1 serving = 150 g)/week), (ix) fish and seafood (0–0.9, 1–2.9, or >3 servings (1 serving = 100–150 g for fish or 200 g for seafood)/week), (x) commercial pastries (0–1.9 or >2 times/week), (xi) nuts (0–0.9, 1–2.9, or >3 servings (1 serving = 30 g)/week), and (xii) cooked vegetables, pasta, rice, or dishes seasoned with tomato, garlic, onion, or leek sauce made over low heat with olive oil (0–0.9, 1–1.9, or >2 times/week). After that, eating habits assessment contained 21 questions aimed at investigating the change in dietary habits during COVID-19 confinement, for example, frequency of snacking, consuming fried food, type of oil used when frying, difficulties in finding certain food, among others. Therefore, participants provided the information whether they had perceived any change related to the nutrition during the COVID-19 confinement in comparison with the situation before that. Additionally, they were also asked to provide information about the change in physical activity as well as in body weight during the confinement. Based on the values that the respondents self-assessed, the body mass index (BMI) was calculated as the ratio of the body weight [in (kg)] and the square of the body height [in (m)]. A more comprehensive insight into the questionnaire description as well as the full form of questionnaire is available in a paper published by Rodríguez-Pérez et al. (24).

Adherence Based on Mediterranean Diet Adherence Screener

The MedDiet adherence was measured by the Mediterranean Diet Adherence Screener, the MEDAS (25). The MEDAS consists of 14 questions where 12 aforementioned questions are related to food consumption frequency and two questions to food intake habits considered characteristic of the MedDiet. Each answer to those questions was coded as 0 (when the MEDAS condition was not met) or 1 (when the MEDAS condition was met).

Score one was associated with the following answers: consuming: (i) four or more tablespoons of olive oil/day; (ii) two or more servings of vegetables/day; (iii) three or more pieces of fruit/day; (iv) less than one serving of red meat or sausages/day; (v) less than one serving of animal fat/day; (vi) less than one cup of sugar-sweetened beverages/day; (vii) seven or more servings of red wine/week; (viii) three or more servings of legumes/week; (ix) three or more servings of fish/week; (x) fewer than two commercial pastries/week; (xi) three or more servings of nuts/week; (xii) two or more servings/week of a dish with a traditional sauce of tomatoes, garlic, onion, or leeks sautéed in olive oil and in the last group of two answers, the following two were preferred: (xiii) olive oil prime source of fat for cooking and (xiv) white meat over red meat. If those conditions were not met, 0 points were recorded. The final score ranged from 0 to 14. Adherence to the MedDiet was assessed by using two scales (24), where the first scale was a continuous one and ranged from 0 to 14 (MEDAS), while the other scale was categorical, allowing the classification of the respondents into low (MEDAS <5), medium (MEDAS: 5–9) and high (MEDAS >9) adherence levels. The MEDAS score before the confinement was calculated as proposed by Rodríguez-Pérez et al. (24).

Diet quality was evaluated through comparison with the MEDAS, since Croatia as a Mediterranean country encourages Mediterranean dietary pattern as a referent, ever since the Ancel Key's Seven Countries Study (Croatia included) (26).

Statistical Analyses

All collected answers were coded as qualitative or quantitative data. Based on the data type, different tests were applied in the analysis. First, the descriptive statistics was conducted for presentation of adherence level to the MedDiet (gender, age, place of residence, regions, children in care, education levels, and the body mass index). From the test, which allows evaluating differences in means or proportions by observed variables across the strata, we used Students' *t*-test (for continuous normal distributed data); Kruskal–Wallis test (for non-normal distributed data), and Chi-squared test (for categorical data). Box-plots were also used to evaluate further the distribution of the variable on adherence to the MedDiet by the aforementioned subgroups.

Logistic regression model was used to explore variable changes with the change in cooking pattern during the confinement (cooking more vs. cooking as usual or less, as reference). Crude regression model (URM) included only the absence or presence of increased cooking habits, while the bivariate-adjusted model included age, gender, and region (Model 1). To remove their influence on the cooking habit, the next bivariate-adjusted model (Model 2) included Model 1 observations and physical activity, education level, and residence. Odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were estimated for all models (Crude, Model 1, and Model 2). The OR >1 indicates greater odds of association with the exposure and outcome or increased occurrence of the examined event. In the squared brackets, the 95% confidence interval is listed for the OR, and if it includes 1, the result is not statistically significant. Software in data analyses SPSS v. 17 and XLStat for Excel 2013 were used.

TABLE 1 | Outlines of questionnaire responses by the level of adherence to the Mediterranean diet, based on the Mediterranean diet adherence screener (MEDAS) of Croatian respondents during COVID-19 confinement.

Respondents' characteristics	MEDAS groups								*P*-value[c]
	Low		Medium		High		All		
	N_L = 1,315	%	N_M = 2,806	%	N_H = 160	%	*N* = 4,281	%	
Gender[a]									<0.001
Female	937	71.3	2,365	84.3	142	88.8	3,444	80.5	
Male	376	28.6	435	15.5	18	11.2	829	19.4	
Age									0.252
<20 years	61	4.6	124	4.4	1	0.6	186	4.3	
20–35 years	554	42.1	1,267	45.2	71	44.4	1,892	44.2	
36–50 years	510	38.8	1,049	37.4	63	39.4	1,622	37.9	
51–65 years	179	13.6	339	12.1	23	14.4	541	12.6	
>65 years	11	0.8	27	1.0	2	1.3	40	0.9	
Place of Residence									0.360
Alone	127	9.7	328	11.7	12	7.5	467	10.9	
Family home	1,098	83.5	2,298	81.9	136	85.0	3,532	82.5	
Shared flat	79	6.0	163	5,8	11	6.9	253	5.9	
Student's residence	11	0.8	17	0.6	1	0.6	29	0,7	
Region by Areas[b]									<0.001
Continental part	1,148	87.3	2,238	79,8	119	74.4	3,505	81.9	
Coastal part	167	12.7	568	20.2	41	25.6	776	18.1	
Children in Care									0.284
No	775	58.9	1,690	60.2	87	54.4	2,552	59.4	
Yes	540	41.1	1,116	39.8	73	45.6	1,729	40.4	
Educational Level									<0.001
University	661	50.3	1,597	56.9	106	66.3	2,364	55.2	
Postgraduate	247	18.8	536	19.1	33	20.6	816	19.1	
Professional	393	29.9	652	23.2	21	13.1	1,066	24.9	
Primary	14	1.1	21	0.7	0	0.0	35	0.8	
BMI									<0.001
Underweight	39	3.0	88	3.1	5	3.1	132	3.1	
Normal	726	55.2	1,901	67.7	113	70.6	2,740	64.0	
Overweight	387	29.4	609	21.7	36	22.5	1,032	24.1	
Obesity	163	12.4	208	7.4	6	3.8	377	8.8	

[a] *Numbers do not add up due to eight respondents who stated that by gender they belong to the category of "Other" (data not shown).*

[b] *Official EU division areas for Croatia—NUTS 2 (27).*

[c] *Differences between the three Mediterranean diet adherence groups were evaluated by the Chi-squared test.*

RESULTS

Respondents' Sociodemographic Characteristics by the Level of Adherence to Mediterranean Diet During the COVID-19 Confinement

The study sample consisted of 4,281 Croatian adults who completed the questionnaire. The main sociodemographic characteristics of respondents by levels of adherence to the Mediterranean diet (low, medium, and high) during the COVID-19 confinement in Croatia are shown in **Table 1**.

Out of all the respondents, 80.5% were females. The majority resided in the continental part of Croatia (81.9%) in family homes (82.5%). A small number of respondents were in the youngest (4.3%) and the oldest age group (0.9%), while 44.2 and 37.9% were aged 20–35 and 35–50, respectively. The highest number of respondents (55.2%) was registered as having university level of education. Moreover, 59.6% did not have children in care and were self-assessed as normal category of body mass index (64.0%).

Respondents who had higher adherence to the MedDiet were mostly females (88.8%), aged between 20 and 50 years old, with the highest level of education (66.3%), and normal BMI (70.6%), who lived in family homes (85.0%) in the continental part of Croatia (74.4%) (**Table 1**). However, observing the subset data for region in box plot structure, higher values of the MEDAS were indicated for respondents residing in the coastal part of Croatia, as for those respondents who lived alone (**Figure 1**).

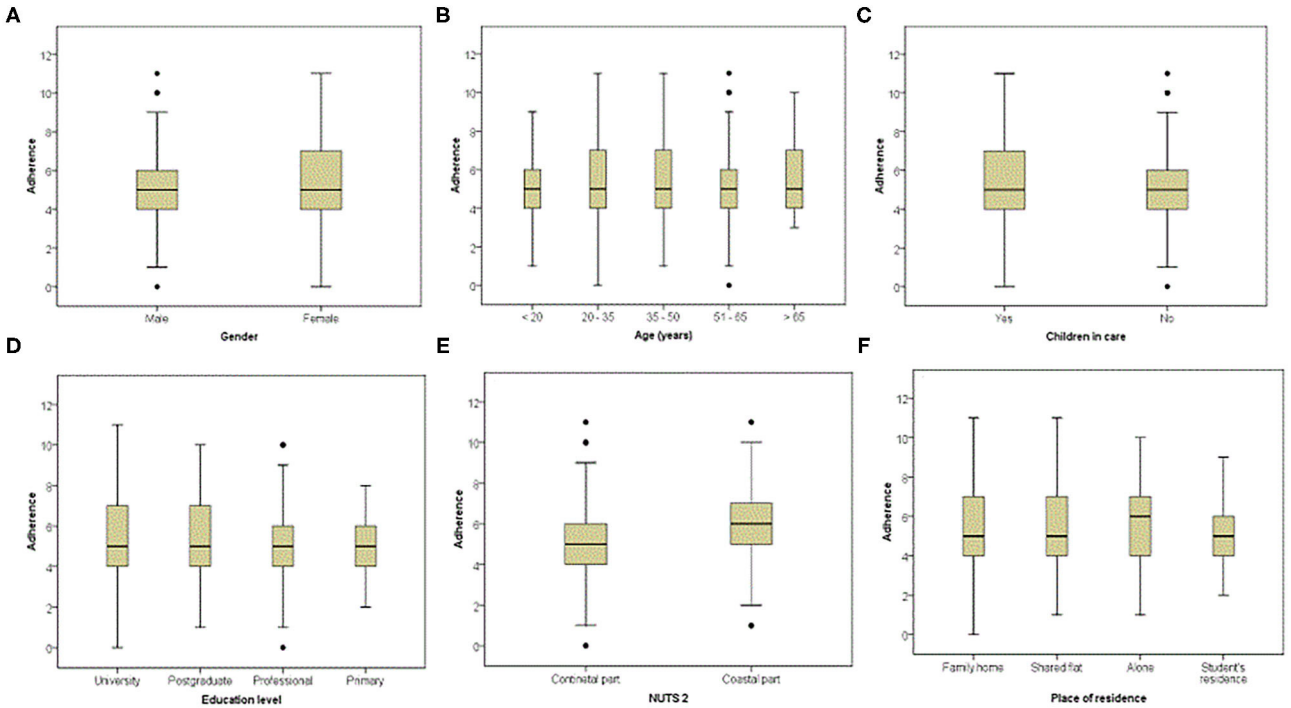


FIGURE 1 | Adherence to the Mediterranean diet based on the Mediterranean diet adherence screener (MEDAS), during the COVID-19 confinement by subgroups, presented as box plots. The interquartile range (IQR) or box boundaries are the 25th percentile, Q1 (box closest to x-axis) and the 75th percentile, Q3 (farthest from x-axis). The line within the box presents the median, Q2 (50th percentile). Lines below or above the box indicate the minimum (Q1–1.5*IQR) or maximum (Q3 + 1.5*IQR), while the dots above and below those lines present outliers.

Respondents' Dietary Behavior by the Level of Adherence to the Mediterranean Diet During the COVID-19 Confinement

Dietary behaviors related to the adherence to the MedDiet are described in **Table 2**. During the COVID-19 confinement, 20.3, 22.1, and 16.3% respondents met the MEDAS condition for olive oil, vegetable, and fruit consumption, respectively. The lowest group of olive oil consumption (0–1.9 tablespoons/day) was noted in 45.6% of respondents, while for vegetable and fruit consumption, most respondents had medium consumption (60.2 and 56.8%). Interestingly, a great number of respondents have met the MEDAS criterion for consumption of red meat (69.8%), as well as for consumption of animal fat (82.8%) and sugar-sweetened beverages (91.8%). The lowest degree of satisfaction was noted for MEDAS conditions of wine (3.0%), legumes (10.7%), and fish and seafood consumption (5.1%). Commercial pastry consumption criterion was met in 61.2% of the respondents, consumption of a dish with a traditional sauce of tomatoes, garlic, onion, or leeks sautéed in olive oil in 59.4%, while the MEDAS condition for nuts consumption was satisfied in 25.3% of the respondents. The majority (70.1%) of the respondents preferred white over red meat, while olive oil was not preferred in 52.7% of the respondents.

As expected, the majority of respondents within the high MEDAS group had high consumption of olive oil (73.1%), vegetables (78.1%), and fruit (58.8%), as well as high weekly consumption of legumes (53.8%), and medium fish and seafood consumption (43.8%). Low red meat, animal fat, and sugar-sweetened beverage consumption was recorded in 94.4, 97.5, and 100.0% of respondents, respectively. Nut consumption and meals with tomatoes, garlic, and onion were noted in the highest rate of consumption in the high MEDAS group. Moreover, 92.5% of high MEDAS respondents preferred the use of olive oil in cooking, and 93.1% preferred white over red meat (**Table 2**).

Although significant differences across the three MEDAS groups were found for all 14 MEDAS conditions (**Table 2**), low consumption of animal fats, sugar-sweetened beverages, and commercial pastries were noted in all groups. Furthermore, wine consumption MEDAS criterion was indicated as the least satisfied one in all the MEDAS groups. However, respondents in the high MEDAS group showed the highest rate of satisfying this criterion (12.5%).

Changes in Dietary Behavior and Styles by the Level of Adherence to the Mediterranean Diet During the COVID-19 Confinement

Changes in eating behavior and lifestyles by the level of adherence to the MedDiet during COVID-19 confinement in Croatia are described in **Tables 3**, **4**. The majority of respondents maintained their dietary behavior as before COVID-19 confinement (**Table 3**). In all MedDiet adherence groups, a

TABLE 2 | Eating behavior by the level of adherence to the Mediterranean diet, based on the Mediterranean diet adherence screener (MEDAS) of Croatian respondents during COVID-19 confinement.

Observed eating behavior	MEDAS groups								*P*-value[a]
	Low		Medium		High		All		
	N_L = 1,315	%	N_M = 2,806	%	N_H = 160	%	N = 4,281	%	
Olive oil consumed (tablespoons (13.5 g)/day)									<0.001
0–1.9	915	69.9	1,032	36.8	6	3.8	1,953	45.6	
2–3.9	330	25.1	1,093	39.0	37	23.1	1,460	34.1	
>4	70	5.3	681	24.3	117	73.1	868	20.3	
Vegetables consumed (servings (200 g)/day)									<0.001
0–0.9	370	28.1	384	13.7	4	2.5	758	17.7	
1–1.9	870	66.2	1,675	59.7	31	19.4	2,576	60.2	
>2	75	5.7	747	26.6	125	78.1	947	22.1	
Fruit consumed (pieces/day)									<0.001
0–0.9	515	39.2	620	22.1	17	10.6	1,152	26.9	
1–2.9	738	56.1	1,645	58.6	49	30.6	2,432	56.8	
>3	62	4.7	541	19.3	94	58.8	697	16.3	
Red meat consumed (servings (100–150 g)/day)									<0.001
0–0.9	553	42.1	2,286	81.5	151	94.4	2,990	69.8	
>1	762	57.9	520	18.5	9	5.6	1,291	30.2	
Butter, margarine or cream consumed (servings (12 g)/day)									<0.001
0–0.9	909	69.1	2,478	88.3	156	97.5	3,543	82.8	
>1	406	30.9	328	11.7	4	2.5	738	17.2	
Carbonated and/or sugary beverages consumed (times/day)									<0.001
0–0.9	1,094	83.2	2,678	95.4	160	100.0	3,932	91.8	
>1	221	16.8	128	4.6	0	0.0	349	8.2	
Wine consumed (cups (100 ml)/week)									<0.001
0–2.9	643	48.9	1,362	48.5	68	42.5	2,073	48.4	
3–6.9	96	7.3	223	7.9	13	8.1	332	7.8	
>7	22	1.7	85	3.0	20	12.5	127	3.0	
I never drink wine	554	42.1	1,136	40.5	59	36.9	1,749	40.9	
Legumes consumed (servings (150 g)/week)									<0.001
0–0.9	570	48.3	848	30.2	16	10.0	1,434	33.5	
1–2.9	721	54.8	1,612	57.4	58	36.3	2,391	55.9	
>3	24	1.8	346	12.3	86	53.8	456	10.7	
Fish-seafood consumed (servings (100–150 g for fish or 200 g for seafood)/week)									<0.001
0–0.9	791	60.2	1,219	43.4	32	20.0	2,042	47.7	
1–2.9	517	39.3	1,432	51.0	70	43.8	2,019	47.2	
>3	7	0.5	155	5.5	58	36.3	220	5.1	
Commercial pastries consumed (times/week)									<0.001
0–1.9	901	68.5	1,637	58.3	81	50.6	2,619	61.2	
>2	414	31.7	1,169	41.7	79	49.4	1,662	38.8	
Nuts consumed (servings (30 g)/week)									<0.001
0–0.9	733	55.7	1,020	36.4	24	15.0	1,777	41.5	
1–2.9	424	32.2	959	34.2	39	24.4	1,422	33.2	
>3	158	12.1	827	29.4	97	60.6	1,082	25.3	
Vegetables, pasta, rice cooked in olive oil (times/week)									<0.001
0–0.9	305	23.2	1,74	6.2	5	3.1	484	11.3	
1–1.9	622	47.3	626	22.3	4	2.5	1,252	29.2	
>2	388	29.5	2,006	71.5	151	94.4	2,545	59.4	
Preferred Olive Oil for Cooking									<0.001
No	1,100	83.7	1,145	40.8	12	7.5	2,257	52.7	
Yes	215	16.3	1,661	59.2	148	92.5	2,024	47.3	

(Continued)

TABLE 2 | Continued

Observed eating behavior	MEDAS groups								*P*-value[a]
	Low		Medium		High		All		
	N_L = 1,315	%	N_M = 2,806	%	N_H = 160	%	N = 4,281	%	
Preferred white meat instead of red meat									<0.001
No	712	54.1	555	19.8	11	6.9	1,278	29.9	
Yes	603	45.9	2,251	80.2	149	93.1	3,003	70.1	

[a]*Differences between the three Mediterranean diet adherence groups were evaluated by the Chi-squared test.*

lower intake of carbonated and/or sugar-sweetened drinks (23.9–27.5% of respondents) as well as alcoholic beverage intake (26.3–27.5%) was noted. Also, a higher intake of vegetables, fruits, and legumes during the confinement was observed in all MEDAS groups. Respondents with a high adherence to MedDiet showed the highest rate of positive changes in these three groups of food (**Table 3**).

However, differences regarding changes in eating behavior were also detected among all the MEDAS groups. Medium and high adherence to MedDiet groups showed similar changes regarding higher olive oil and lower red meat consumption during the confinement. Those changes were more prominent in the high MEDAS group than in the medium one. In contrast, the group with low adherence to MedDiet showed opposite changes, with an interesting result where 14.8% of the participants in this group showed higher red meat consumption during confinement. However, fish and seafood consumption did not show the same trend of change as the other dietary intake groups. Higher consumption of a previously mentioned food group during the confinement was noted only for the group with high adherence to the MedDiet, while respondents in low and medium MEDAS groups described their intake of fish and seafoods as lower than before COVID-19 confinement.

Overall, most respondents employed stewing as the main type of cooking technique during the COVID-19 confinement (78.3%). Fried food consumption remained as usual in 69.3% of respondents, which means that 53.1% of them consumed fried food one to three times a week, while 36.4% ate fried food less than once a week (**Table 4**). As expected, the respondents in high adherence to the MedDiet group showed lower tendency to fried food consumption, namely, 49.4% of them consumed this type of food less than once a week. Regarding the type of oil used for frying, the majority of respondents (63.9%) used sunflower oil, while the highest preference for the use of olive oil (50.0%) was found in respondents with high adherence to MedDiet. Out of all respondents, 41.9% of them consumed one meal per day (out of home), while 21.5% did not consume any meal out of home before the confinement.

All groups agreed that they did not have trouble finding certain types of food, while 56.9% of the respondents stated that their amount of food eaten was the same as before the confinement. Also, 57.5% did not perceive any change in body mass. Interestingly, the highest rate of gaining weight (22.1%) was noted in the low MEDAS group (**Table 4**). Furthermore, modification in physical activity was observed in 36.9% of the respondents who decreased, while 28.7% of them increased their physical activity. Respondents in the high MEDAS group dominantly increased their physical activity (38.1%) level during COVID-19 confinement, while the other two groups showed higher rate of decrease than increase in physical activity.

Moreover, 29.4–35.1% of the respondents, depending on the MEDAS group, had higher frequency of snacking. In 54.2% of the respondents, a lower intake of fast food was found during the confinement, while 53.8% of the overall respondents cooked more often (**Table 4**) and based on the presented share of students and young working people (aged 20–35 years) and those who live in family homes, it seems that the cooking was a shared responsibility in the household. Still, a difference in behavior change among different MEDAS groups was noted ($p < 0.001$), where the respondents in the low MEDAS group were almost equally divided in the cooking as usual group (48.6%) and the cooking more group (48.7%).

While the majority of respondents had no difficulty finding certain types of food during the confinement, 20.1% of the respondents described which type of food was troubling to purchase (**Table 4** and **Figure 2**). The respondents who scored medium MEDAS were the most eager to describe problematic types of food. They had difficulty finding a wide range of different foods, especially yeast, dairy products, and eggs, as well as spices and olive oil (**Figure 2**). The group with high adherence to MedDiet found food for special needs the most difficult to purchase during the confinement, mainly organic, vegan, and gluten-free food. They also had problems finding fish and seafood, as well as canned food. Interestingly, the respondents in the low MEDAS group were the most worried about food delivery, as well as difficulty to consume fast food and food from restaurants (**Figure 2**).

Adherence to Mediterranean Diet and Changes in Dietary Behavior During the COVID-19 Confinement Regarding Body Mass Index of Respondents

Higher median values of MEDAS score were noted for respondents with BMI values between 18.5 and 24.9 kg/m^2. An interesting result, indicating a higher median value of MEDAS score for respondents with a BMI <25 kg/m^2 (6.0) than those with a BMI value above 25 kg/m^2 (5.0) was registered

TABLE 3 | Changes in eating behavior by the level of adherence to the Mediterranean diet, based on the Mediterranean diet adherence screener (MEDAS) of Croatian respondents during the COVID-19 confinement.

	MEDAS groups								*P*-value[a]
	Low		Medium		High		All		
	N_L = 1,315	%	N_M = 2,806	%	N_H = 160	%	N = 4,281	%	
Olive oil consumed (tablespoons/day)									<0.001
As usual	1,087	82.7	2,259	80.5	120	81.3	3,476	81.2	
Lower	118	9.0	152	5.4	3	1.9	273	6.4	
Higher	110	8.4	395	14.1	27	16.9	532	12.4	
Vegetable consumed (servings/day)									<0.001
As usual	950	72.2	1,931	68.8	109	68.1	2,990	69.8	
Lower	15	11.5	228	8.1	6	3.8	385	9.0	
Higher	214	16.3	647	23.1	45	28.1	906	21.2	
Fruit consumed (servings/day)									0.001
As usual	917	69.7	1,920	68.4	103	64.4	2,940	68.7	
Lower	153	11.6	248	8.8	13	8.1	414	9.7	
Higher	245	18.6	638	22.7	44	27.5	927	21.7	
Red meat consumed (servings/day)									<0.001
As usual	934	71.0	1,917	68.3	101	63.1	2,952	69.0	
Lower	187	14.2	695	24.8	54	33.8	936	21.9	
Higher	194	14.8	194	6.9	5	3.1	363	9.2	
Carbonated and/or sugary beverages consumed (cups/day)									<0.001
As usual	900	69.4	1,937	69.0	113	70.6	2,950	69.9	
Lower	314	23.9	738	26.3	44	27.5	1,096	25.6	
Higher	101	7.7	131	4.7	3	1.9	235	5.5	
Legumes consumed (servings/week)									<0.001
As usual	1,103	83.9	2,332	83.1	119	74.4	3,554	83.0	
Lower	90	6.8	167	6.0	4	2.5	261	6.1	
Higher	122	9.3	307	10.9	37	23.1	466	10.9	
Fish-seafood consumed (servings/week)									<0.001
As usual	1,018	77.4	1,974	70.3	102	63.8	3,094	72.3	
Lower	189	14.4	443	15.8	20	12.5	652	15.2	
Higher	108	8.2	389	13.9	38	23.8	535	12.5	
Commercial pastries consumed (units/week)									0.001
As usual	852	64.8	1,592	56.7	95	59.4	2,539	59.3	
Lower	197	15.0	593	21.1	30	18.8	820	19.2	
Higher	266	20.2	621	22.1	35	21.9	922	21.5	
Homemade pastries consumed (units/week)									0.147
As usual	744	56.6	1,469	52.4	89	55.6	2,302	53.8	
Lower	154	11.7	371	13.2	19	11.9	544	12.7	
Higher	417	31.7	966	34.4	52	32.5	1,435	33.5	
Alcoholic beverage consumed									0.760
As usual	832	63.3	1,729	61.6	102	63.7	2,663	62.2	
Lower	361	27.5	784	27,9	42	26.3	1,187	27.7	
Higher	122	9.3	293	10.4	16	10.0	431	10.1	

[a] *Differences between the three Mediterranean diet adherence groups were evaluated by the Chi-squared test.*

(**Figure 3**). Statistically significant differences between those two groups were noticed ($p < 0.001$) regarding the frequency of consuming fried food and increase in snacking during the COVID-19 confinement, where respondents with higher BMI values showed higher intake of fried food, but lower frequency of snacking during the confinement in comparison with the number of snacks before the confinement. Even though the difference between the two groups was not registered for eating more during the confinement ($p = 0.401$), a higher probability of weight gain was observed for respondents with BMI above 25 kg/m^2. Also, respondents with a BMI <25 kg/m^2 were more prone to increasing their physical activity during

TABLE 4 | Cooking preparation practice, changes in eating behavior and life styles by the level of adherence to the Mediterranean diet, based on the Mediterranean diet adherence screener (MEDAS) of Croatian respondents during the COVID-19 confinement.

	MEDAS groups								*P*-value[a]
	Low		Medium		High		All		
	N_L = 1,315	%	N_M = 2,806	%	N_H = 160	%	N = 4,281	%	
Difficult to find certain types of food									0.053
No	1,084	82.4	2,215	78.9	122	76.3	3,421	79.9	
Yes	231	17.6	591	21.1	38	23.8	860	20.1	
Type of cooking									<0.001
Fried	132	10.0	108	3.8	3	1.9	243	5.7	
Griddle	26	2.0	31	1.1	3	1.9	60	1.4	
Microwave	7	0.5	5	0.2	0	0.0	12	0.3	
Oven	242	18.4	354	12.6	18	11.3	614	14.3	
Stew	908	69.0	2,308	82.3	136	85.0	3,352	78.3	
Cooking more often									<0.001
As usual	639	48.6	1,172	41.8	72	45.0	1,883	44.0	
Less	35	2.7	61	2.2	0	0.0	96	2.2	
More	641	48.7	1,573	56.1	88	55.0	2,302	53.8	
Fried foods consumed									<0.001
As usual	937	71.3	1,913	68.2	116	72.5	2,966	69.3	
Lower	248	18.9	739	26.3	44	27.5	1,031	24.1	
Higher	130	9.9	154	5.5	0	0.0	284	6.6	
Frequency of fried food consumed (times/week)									<0.001
1–3	836	63.6	1,380	49.2	58	36.3	2,274	53.1	
4–6	87	6.6	80	2.9	2	1.3	169	3.9	
>7	5	0.4	8	0.3	0	0.0	13	0.2	
<1	341	25.9	1,138	40.6	79	49.4	1,558	36.4	
Never	46	3.5	200	7.1	21	13.1	267	6.2	
Type of oil used for frying									<0.001
Olive oil	134	10.2	764	27.2	80	50.0	978	22.8	
Other	166	12.6	384	13.7	19	11.9	569	13.3	
Sunflower oil	1,015	77.2	1,658	59.1	61	38.1	2,734	63.9	
Frequency of snacking									0.017
As usual	748	56.9	1,504	53.6	84	52.5	2,336	54.5	
Lower	148	11.3	316	11.3	29	18.1	493	11.5	
Higher	419	31.9	986	35.1	47	29.4	1,452	33.9	
Frequency of fast food consumed									0.001
As usual	575	43.7	1,201	42.8	85	53.1	1,861	43.5	
Lower	694	52.8	1,553	55.3	72	45.0	2,319	54.2	
Higher	46	3.5	52	1.9	3	1.9	101	2.4	
Ate more									0.066
No	736	56.0	1,593	56.8	105	65.6	2,434	56.9	
Yes	579	44.0	1,213	43.2	55	34.4	1,847	43.1	
Modification of physical activity									<0.001
No physical activity	142	10.8	157	5.6	5	3.1	304	7.1	
It has decreased	483	36.7	1,047	37.3	51	31.9	1,581	36.9	
It has increased	329	25.0	840	29.9	61	38.1	1,230	28.7	
It remains as usual	361	27.5	762	27.2	43	26.9	1,166	27.2	
Weight gain									0.013
I do not know	308	23.4	616	22.0	23	14.4	947	22.1	
No	716	54.4	1,643	58.6	104	65.0	2,463	57.5	
Yes	291	22.1	547	19.5	33	20.6	871	20.3	

(Continued)

TABLE 4 | Continued

	MEDAS groups								*P*-value[a]
	Low		Medium		High		All		
	N_L = 1,315	%	N_M = 2,806	%	N_H = 160	%	N = 4,281	%	
Meals consumed (number/day)[b]									<0.001
1	572	43.5	1,153	41.1	67	41.9	1,792	41.9	
2	298	22.7	596	21.2	13	8.1	907	21.2	
3	209	15.9	422	15.0	30	18.8	661	15.4	
None	236	17.9	635	22.6	50	31.3	921	21.5	

[a] *Differences between the three Mediterranean diet adherence groups were evaluated by the Chi-squared test.*
[b] *Referred as the usual number of meals out of home before the confinement.*

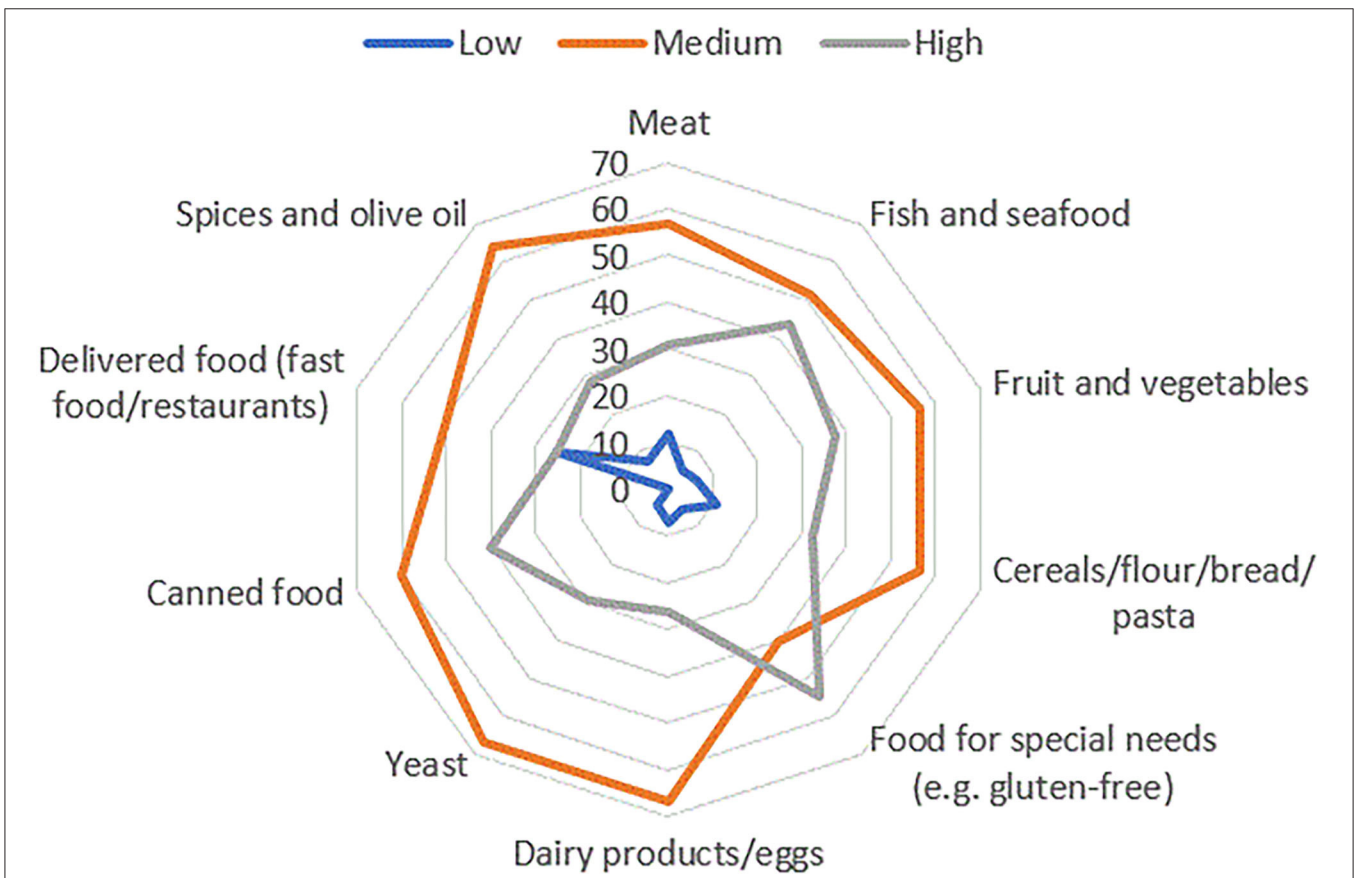


FIGURE 2 | Graphical representation of types of food, which respondents had difficulty purchasing during COVID-19 confinement in Croatia. Respondents' answers were divided in accordance with the level of adherence to the Mediterranean diet (low, medium, and high).

the confinement than respondents with higher BMI values (**Supplementary Table 1**).

The models presenting the OR are presented in **Supplementary Table 2**. Odds ratio is defined as the ratio of changes in dietary behavior of respondents with BMI above 25 kg/m^2 to the changes in dietary behavior of respondents with a BMI below 25 kg/m^2, as a measure of association between the "exposure" and the outcome. Here, the outcomes were responses of respondents with a BMI >25 kg/m^2, while the changes in dietary behavior were used as the exposures. The used models confirm previous results that the respondents with higher BMI had 37% higher likelihood to decrease their frequency of snacking during the confinement. While models do not indicate higher intake of fried food for the respondents with BMI above 25 kg/m^2, a lower consumption of sugar-sweetened beverages, and commercial and homemade pastries was noted for those respondents. In spite of the results showing beneficial changes, models, in accordance with previous results, show that the odds

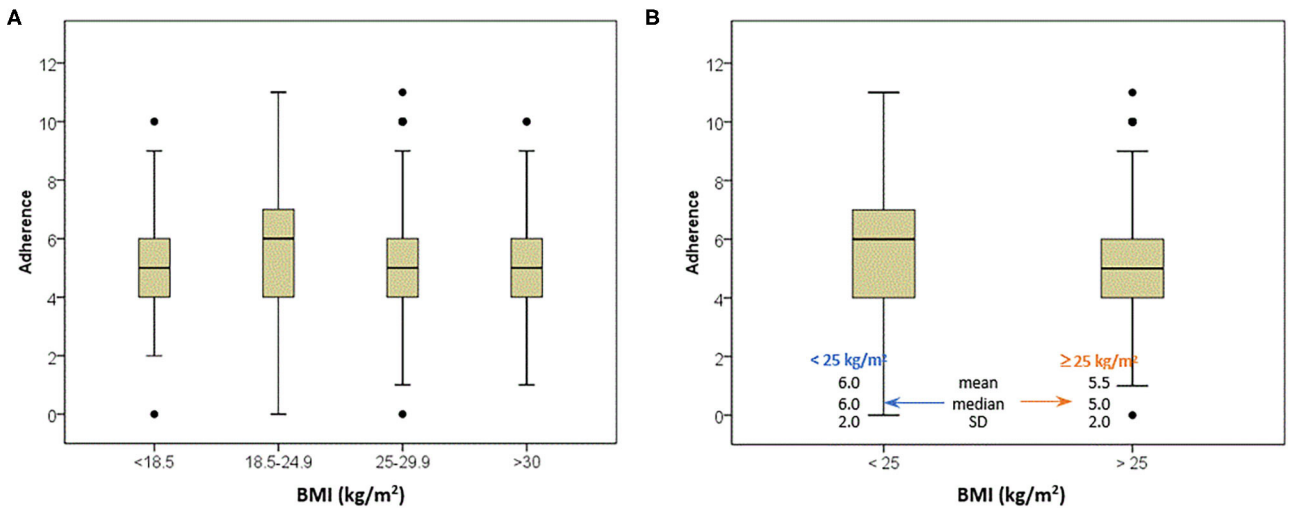


FIGURE 3 | Box plots of the body mass index of respondents according to adherence with the Mediterranean diet pattern.

are 1.76 higher for weight gained by respondents with higher BMI values (**Supplementary Table 2**).

The Change in Cooking Frequency During the COVID-19 Confinement

Considering gender (**Supplementary Table 3**), 2.1% of female respondents cooked less, while 56.1% of them increased the cooking frequency, as well as 44.3% of the male respondents, respectively. Respondents in all residence places increased the cooking frequency during the confinement as well as those who had children in care (57.0%), had higher education levels (university degree: 59.2%; postgraduate level: 52.9%), aged 20–50 years, and with medium or high MEDAS (56.1 and 55.0%, respectively).

The models presenting the OR are presented in **Supplementary Table 5**. In our study, the outcome was the cooking practice, while the factors which were scored in the MEDAS were used as the exposures. We can conclude that greater consumption of wine was noted (OR = 1.79 for 3–6.9 cups/week) for the respondents who cooked more during the confinement, while preferring olive oil for cooking and white meat consumption did not show a correlation within the group cooking more (**Supplementary Table 5**). The results of multivariate analysis did not show significant association of consumption of MedDiet-related food groups in the group that cooked more during the confinement, so the cross table observing 14 parameters for the MEDAS evaluation by the change in cooking frequency was conducted (**Supplementary Table 4**). For those respondents who increased the cooking frequency—significant changes are in the MEDAS i, vii, x, xi, xii, and xiii. Respondents who decreased their cooking frequency drank three or more cups of wine per week, but also 45.8% of them never drank wine. The respondents who cooked more during the confinement had higher preference for the use of olive oil as main source of added fat (51.2%) in comparison with the respondents who cooked as usual (43.1%) or less (35.4%). However, for the preference of white over red meat, no statistically significant changes were noted (p = 0.263). Those who cooked more and as usual exhibited similar results (71.0 vs. 69.4%, respectively), while the respondents who cooked less showed lower preference for white meat (**Supplementary Table 4**). However, the respondents with increased cooking frequency during the confinement also had higher olive oil and nut intake as well as higher weekly consumption of vegetables, pasta, or rice cooked in olive oil (**Supplementary Table 4**), which was not confirmed by multivariate analysis (**Supplementary Table 5**).

To elucidate the association between changes in food frequency consumption and cooking frequency during the COVID-19 confinement, models regarding the cooking practice as outcome and the changes in dietary behavior as the exposures were made (**Table 5**). Following the results from the previously presented models where those respondents who cooked more had higher wine intake, these models also show higher alcoholic beverage consumption. Furthermore, the respondents who increased their cooking frequency showed 2.24 higher odds of higher vegetable intake during the confinement. Along with the increased vegetable consumption, respondents who cooked more consumed more legumes (OR = 1.38), fish and seafood (OR = 1.33), as well as homemade pastries. However, they also exhibited higher frequency of snacking and consumption of fried food, but lower intake of fast food (**Table 5**).

DISCUSSION

This research is aimed at investigating the effect of the COVID-19 confinement on dietary behaviors of adult population in Croatia. During the confinement, the major part of Croatian respondents presented medium adherence to the Mediterranean diet (5.85 ± 2.04). Our findings suggest that the respondents who increased

TABLE 5 | Associations between the change in cooking practice and the change in dietary behavior of Croatian respondents during the COVID-19 confinement.

	As usual or less		Cooking more		All		*P*-value[a]	Crude[b]		Model 1[c]		Model 2[d]	
	***N* = 1,979**	**%**	***N* = 2,302**	**%**	***N* = 4,281**	**%**		**OR**	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**
Vegetable consumed							<0.001						
Higher	239	12.0	667	29.0	906	21.2		**2.24**	**[1.86;2.71]**	**2.17**	**[1.79;2.63]**	**2.18**	**[1.79;2.65]**
Lower	145	7.3	240	10.4	385	9.0		**1.37**	**[1.05;1.79]**	**1.41**	**[1.08;1.85]**	**1.35**	**[1.02;1.77]**
As usual	1,595	79.9	1,395	60.6	2,990	69.8		Ref.		Ref.		Ref.	
Fruit consumed							0.512						
Higher	315	15.9	612	26.6	927	21.7		1.08	[0.90;1.29]	1.14	[0.95;1.37]	1.14	[0.95;1.37]
Lower	174	8.8	240	10.4	414	9.7		0.92	[0.71;1.18]	0.87	[0.67;1.12]	0.90	[0.69;1.17]
As usual	1,490	75.3	1,450	63.0	2,940	68.7		Ref.		Ref.		Ref.	
Red meat consumed							0.131						
Higher	121	6.1	272	11.8	393	9.2		1.22	[0.94;1.58]	1.27	[0.98;1.66]	1.27	[0.97;1.66]
Lower	358	18.1	578	25.1	936	21.9		1.16	[0.97;1.39]	1.18	[0.98;1.42]	1.14	[0.95;1.38]
As usual	1,500	75.8	1,452	63.1	2,952	69.0		Ref.		Ref.		Ref.	
Carbonated and/or sugary beverages consumed							0.200						
Higher	79	4.0	156	6.8	235	5.5		0.98	[0.71;1.35]	1.02	[0.74;1.40]	1.09	[0.78;1.51]
Lower	456	23.0	640	27.8	1.096	25.6		0.85	[0.72;1.02]	0.86	[0.72;1.02]	0.90	[0.75;1.07]
As usual	1,444	73.0	1,506	65.4	2.950	68.9		Ref.		Ref.		Ref.	
Legumes consumed							0.025						
Higher	130	6.6	336	14.6	466	10.9		**1.38**	**[1.09;1.76]**	**1.37**	**[1.08;1.74]**	**1.32**	**[1.03;1.68]**
Lower	99	5.0	162	7.0	261	6.1		0.98	[0.73;1.32]	0.98	[0.72;1.33]	1.02	[0.75;1.38]
As usual	1,750	88.4	1,804	78.4	3,554	83.0		Ref.		Ref.		Ref.	
Fish-seafood consumed							0.035						
Higher	168	8.5	367	15.9	535	12.5		**1.33**	**[1.07;1.66]**	**1.34**	**[1.08;1.68]**	1.26	[1.00;1.58]
Lower	260	13.1	392	17.0	652	15.2		1.09	[0.89;1.33]	1.07	[0.87;1.31]	1.00	[0.81;1.24]
As usual	1,551	78.4	1,543	67.0	3,094	72.3		Ref.		Ref.		Ref.	
Commercial pastries consumed							0.077						
Higher	322	16.3	600	26.1	922	21.5		0.93	[0.76;1.13]	0.91	[0.75;1.11]	0.88	[0.72;1.07]
Lower	313	15.8	507	22.0	820	19.2		1.23	[0.99;1.51]	1.23	[0.99;1.52]	1.19	[0.96;1.48]
As usual	1,344	67.9	1,195	51.9	2,539	59.3		Ref.		Ref.		Ref.	
Homemade pastries consumed							<0.001						
Higher	435	22.0	1,000	43.4	1,435	33.5		**2.01**	**[1.70;2.36]**	**1.98**	**[1.68;2.34]**	**2.09**	**[1.76;2.48]**
Lower	251	12.7	293	12.7	544	12.7		0.89	[0.70;1.13]	0.87	[0.68;1.11]	0.86	[0.67;1.10]
As usual	1,293	65.3	1,009	43.8	2,302	53.8		Ref.		Ref.		Ref.	
Alcoholic beverage consumed							<0.001						
Higher	119	6.0	312	13.6	431	10.1		**1.93**	**[1.51;2.46]**	**1.75**	**[1.37;2.25]**	**1.58**	**[1.23;2.03]**

(Continued)

TABLE 5 | Continued

	As usual or less		Cooking more		All		*P*-value[a]	Crude[b]		Model 1[c]		Model 2[d]	
	***N* = 1,979**	**%**	***N* = 2,302**	**%**	***N* = 4,281**	**%**		**OR**	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**
Lower	508	25.7	679	29.5	1,187	27.7		0.86	[0.73;1.01]	0.90	[0.76;1.06]	0.89	[0.75;1.05]
As usual	1,352	68.3	1,311	57.0	2,663	62.2		Ref.		Ref.		Ref.	
Fried foods consumed							<0.001						
Higher	66	3.3	218	9.5	284	6.6		**2.37**	**[1.73;3.26]**	**2.52**	**[1.82;3.48]**	**2.73**	**[1.96;3.80]**
Lower	340	17.2	691	30.0	1,031	24.1		**1.62**	**[1.36;1.93]**	**1.64**	**[1.38;1.96]**	**1.63**	**[1.36;1.95]**
As usual	1,573	79.5	1,393	60.5	2,966	69.3		Ref.		Ref.		Ref.	
Frequency of snacking							<0.001						
Higher	437	22.1	1,015	44.1	1,452	33.9		**1.97**	**[1.67;2.31]**	**2.00**	**[1.69;2.36]**	**1.95**	**[1.65;2.31]**
Lower	235	11.9	258	11.2	493	11.5		0.95	[0.77;1.18]	0.96	[0.77;1.19]	0.92	[0.74;1.16]
As usual	1,307	66.0	1,029	44.7	2,336	54.6		Ref.		Ref.		Ref.	
Frequency of fast-food consumed							<0.001						
Higher	36	1.8	65	2.8	101	2.4		1.21	[0.75;1.96]	1.25	[0.77;2.04]	1.18	[0.72;1.93]
Lower	870	44.0	1,449	63.0	2,319	54.2		**1.68**	**[1.44;1.94]**	**1.68**	**[1.44;1.95]**	**1.74**	**[1.49;2.03]**
As usual	1,073	54.2	788	34.2	1,861	43.5		Ref.		Ref.		Ref.	
Weight gain							0.885						
Yes	322	16.3	549	23.9	871	20.4		1.05	[0.86;1.30]	1.07	[0.87;1.32]	1.07	[0.86;1.33]
No	1,238	62.6	1,225	53.2	2,463	57.5		1.02	[0.86;1.20]	1.02	[0.86;1.21]	1.00	[0.84;1.19]
I do not know	419	21.2	528	22.9	947	22.1		Ref.		Ref.		Ref.	

[a] *Differences between the groups were evaluated by the Chi-squared test.*

[b] *Crude model: unadjusted for any variable.*

[c] *Model 1: multivariate adjusted model for gender (women, men, and other), age groups (<20, 20–35 years, 36–50 years, 51–65 years, and >65 years), and regions (Continental and Coastal).*

[d] *Model 2: multivariate adjusted model for gender, age groups, regions, residence (alone, family home, shared flat, and student's residence), education level (university, postgraduate, professional, and primary), and physical activity (higher, lower, similar, never). Odds ratios (ORs) and corresponding 95% confidence intervals were estimated for all models. In addition, statistically significant ORs are highlighted in bold.*

their cooking frequency during the confinement also exhibited beneficial dietary behavior changes toward greater adherence to the MedDiet. Those respondents showed higher preference to the use of olive oil for cooking, 2.24 higher odds of increased vegetable consumption, as well as increased legumes and fish intake during the confinement.

Consumption of home-cooked meals on a regular basis was associated with an overall healthier diet (28), as well as with higher adherence to the MedDiet (29). The MedDiet may have a beneficial effect against viral infections and is therefore the advised eating approach during confinement (1, 18, 19). Adherence to this healthy and balanced nutritional pattern is important for achieving optimal function of the immune system and coping with stress and anxiety caused by an abnormal and disturbing situation worldwide. Owing to high fruit and vegetable intake, the MedDiet is abundant in antioxidants, especially vitamin E, vitamin C, and carotene, which play a crucial role in supporting the immune system. Also, vitamin D deficiency may cause a greater tendency for respiratory illnesses; therefore, the important role of this vitamin is emphasized. Due to the COVID-19 confinement policies, sun exposure was limited, which indicates a greater need for the intake of vitamin D from food and/or supplements (1, 18). Furthermore, adequate intake of omega-3 fatty acids supports the immune system, due to anti-inflammatory properties of their metabolites (12). Besides previously mentioned micronutrients, zinc is a trace element that is known for its role in maintaining the immune system (18). Te Velthuis et al. (30) have reported that increasing the intracellular level of zinc impairs the replication of RNA viruses, such as SARS-coronavirus in cell culture. All previously described nutrients are abundant in the MedDiet, so adherence to this healthy diet could be of great importance during the COVID-19 confinement.

Our study showed medium adherence to the MedDiet during the COVID-19 confinement. To be precise, the mean of the MEDAS score was 5.85 ± 2.04, and the mean of the MEDAS score before the confinement was 5.02 ± 1.97, which is significantly lower than the MEDAS score noted in Spain (24), but similar to that of the Danish population (31). This result might be due to the fact that the majority of respondents resided in the continental part of Croatia, whereas only 18.1% of the overall respondents were from the coastal part where higher adherence to the MedDiet is more common. Relative results suggest that the respondents in the coastal part of Croatia had higher MEDAS values than the respondents residing in the continental part.

High adherence to the MedDiet was noted in only 160 respondents (3.7% of all participants), while females showed higher adherence than male respondents, as seen in the Danish population (31). Similar to our results, female medical school students were associated with better adherence to the MedDiet (32, 33). Moreover, Papadaki et al. (34) reported a higher adherence in employed females. The same result was noted in Croatia, where employed females showed a higher MEDAS score than male participants (35). Furthermore, the importance of mothers in determining a better family diet quality has been reported by Schnettler et al. (36). As for female respondents, those respondents who lived in family homes also presented higher adherence to the MedDiet. A similar result was also noted in the Spanish population (24).

Considering the age of the respondents, those who were 20–50 years old presented the highest adherence to the MedDiet, which differs from the Spanish and Danish populations during the COVID-19 confinement, where respondents aged 51 years and older showed higher adherence to the MedDiet in comparison with the youngest ones (24, 31). The positive change in diet quality with increasing age was also suggested by Thiele et al. (37). Moreover, expected correlation between higher educational level and higher adherence to the MedDiet was found, as reported by Rodríguez-Pérez et al. (24).

While the majority of respondents rated their eating behavior as being the same as before the COVID-19 confinement, changes were noted in the remaining respondents. Lower consumption was noted for sugar-sweetened/carbonated beverages and alcohol. This result might be due to movement restriction, resulting in a reduction of time spent in local coffee shops and grocery stores. Moreover, Gray-Phillip et al. (38) reported that the usual on-premise (e.g., night bars) alcohol consumption was between midnight and 2 am, while for take-away alcohol (bought in stores), common purchase was made from 8 to 10 pm. On-premise consumption was consequently reduced because night clubs were not allowed to open during the confinement. Furthermore, grocery stores were closed by 5 pm, which may also result in less frequent purchase of take-away alcohol beverages. These findings were a probable reason for documented decrease in alcohol intake on an international level (39).

Respondents with medium and high adherence to the MedDiet also described higher olive oil and lower red meat consumption. These two MEDAS groups cover 69.3% of the overall respondents, so these results might suggest that the Croatian population increased their adherence to the MedDiet, as seen in the Spanish population (24).

Furthermore, the majority of the respondents consumed fried food during the COVID-19 confinement the same as before, that is, this type of food was consumed one to three times a week. As for the Croatian population, moderate intake of fried food was observed by Rodríguez-Pérez et al. (24). However, in contrast to the Spanish population, the Croatian population showed higher preference to frying in sunflower than in olive oil. The correlation between fried food consumption and overall diet quality is not well-addressed. Grosso et al. (40) documented that lower adherence to the MedDiet was associated with higher intake of sweets, sweetened beverages, fast food, and fried food, as well as with lower fruit, vegetable, pasta, cheese, and fish consumption in Italian adolescents. Furthermore, the correlation between fried food consumption and developing chronic diseases is inconsistent. The EPIC cohort study in Spain (41) showed that the consumption of fried food was not correlated with chronic diseases. Inconsistence in results regarding previously mentioned association was also stated by Gadiraju et al. (42). Our results suggest that 5.7% of the respondents reported frying as a type of cooking. This may suggest that they occasionally ordered food, which may be associated with higher intake of saturated fats and sweets, as well as lower consumption of nutrient-dense food such as fruits and vegetables (43, 44).

It is important to point out that confinement induces negative feelings such as stress and anxiety. Those feelings are usually accompanied by eating more of energy-dense food, especially in respondents who are overweight and obese (1). Sidor and Rzymski (45) have reported that eating and snacking more during the COVID-19 confinement were behaviors most frequently noticed in obese respondents. Apart from eating and snacking more, those respondents showed a tendency for higher intake of salty foods, meat, and dairy, while they consumed vegetables, fruits, and legumes less frequently. In overall responses, a higher frequency of snacking was noted (29.4–35.1% depending on MEDAS group), while snacking more during the confinement was also reported in the Danish and Lithuanian population (31, 46). Interestingly, respondents with BMI below 25 kg/m^2 were more prone to increase the number of snacks during the confinement, which is not in accordance with the results presented by Sidor and Rzymski (45). This result may be due to boredom caused by staying at home. Along with higher frequency of snacking, respondents with BMI $<$ 25 kg/m^2 were inclined to increase their physical activity, which may enable maintenance of energy balance. Out of all the respondents, 36.9% decreased their physical activity, as a consequence of the confinement, which is also reported by Ammar et al. (39). However, a recent study conducted by Lesser and Nienhuis (47) showed that modulation in physical activity during the COVID-19 confinement depends on the usual physical activity of respondents, whether they are usually active or inactive. In that study, active respondents had a higher level of physical activity, while inactive respondents described a lower level of physical activity during the confinement. Furthermore, the majority of respondents did not change the amount of eaten food, nor was there a change in body mass during the confinement perceived. Although the amount of consumed food was the same as before the confinement, the respondents with BMI above 25 kg/m^2, as well as the respondents with lower adherence to the MedDiet, noticed an increase in body mass more frequently than the respondents with lower BMI values and higher adherence to the MedDiet. Although the results show beneficial changes in dietary behavior (lower snacking frequency, lower sugar-sweetened beverages, commercial and homemade pastry consumption) in respondents with higher BMI values, the odds are 1.76 higher for weight gain in those respondents.

Another expected result was that the respondents who had BMI values below 25 kg/m^2 presented higher adherence to the MedDiet in comparison with the respondents with higher BMI values. It has been reported that high adherence to the MedDiet shows inverse association with BMI and obesity (48), as well as a beneficial role of the MedDiet in the prevention of weight gain, abdominal obesity (49), and protection from onset of chronic inflammation, type 2 diabetes, and metabolic syndrome (50).

Out of all the respondents who had difficulty finding certain types of food during the confinement, the respondents with medium and high adherence to the MedDiet found fruit and vegetables, as well as fish and seafood difficult to purchase. This might be explained by the usual purchase of these groceries on the market from local manufacturers, which were not allowed to work during the confinement. Moreover, along with markets being shut down, the Google Trends tool shows increased search for delivery of groceries by local manufacturers.

In order to limit the dissemination of viral infection, the recommendation was to stay home. This general shutdown policy resulted in several changes in lifestyle behavior. While the majority of respondents maintained almost all their behaviors the same as before the COVID-19 confinement, almost 54% of all the respondents stated that they cooked more frequently during the confinement, while their intake of fast food was lower. Generally speaking, there are some determinants involved in cooking frequency. Females are more prone to cooking, as well as people who live with a partner or have children in care (51). The results from our study are consistent with these determinants where 56.1% of female and 44.3% of male respondents cooked more during the confinement. Those respondents with children in care were also more prone to increasing their cooking frequency. Another determinant for cooking is leisure time, that is, people working longer hours indulge less in cooking (51). Due to the general confinement, people might be more prone to increase their cooking frequency because they were unable to go to restaurants and were not working or had a home office. An increase in cooking frequency and staying at home may have a beneficial impact on children and adolescents with a result of increasing their cooking skills (52). Moreover, during the confinement, people were advised not to go to the grocery store every day, which may have a positive influence resulting in healthier diet and lower odds of being obese due to imposed meal planning (53). While the motivation for introducing new lifestyle behaviors, such as cooking more and meal planning, resides in the fear of getting infected, this might be a trigger for habit formation (54, 55).

It is important to emphasize how cooking more frequently influences other dietary behaviors. It is suggested that eating more of home-cooked meals positively influences dietary behaviors, such as having greater adherence to the MedDiet, as well as a higher probability of having normal BMI value and body fat percentage (29). Furthermore, people with greater cooking skills also had higher weekly vegetable consumption and lower consumption of "unhealthier" food groups (56, 57). Our results indicate that the respondents who increased their cooking frequency during the COVID-19 confinement preferred olive oil as the main source of added fat. With an increase in cooking, respondents also significantly increased their vegetable intake, that is, those who cooked more had 2.24 higher odds of an increase in vegetable consumption during the confinement in comparison with the respondents who cooked the same as before or less. An increase in legume, fish and seafood, as well as homemade pastry consumption was also noted in the respondents who increased their cooking frequency during the confinement. These beneficial changes in dietary behavior reported by the respondents who cooked more during the confinement indicate that the higher cooking frequency might result in an overall greater diet quality. This result is confirmed by Wolfson et al. (58), whereas cooking more frequently was associated with a higher Healthy Eating Index.

This study has many strengths in providing insight into dietary behaviors under a new and challenging situation during

the COVID-19 confinement policy in Croatia. The use of an online questionnaire proved itself as a useful tool in providing a relatively large number of respondents, which would be demanding to obtain by face-to-face interviews due to regulations for social distancing and general confinement. The COVIDiet questionnaire provided a great amount of information about dietary and lifestyle behaviors during the confinement.

In spite of the numerous strengths, some limitations must be mentioned. Due to web-based voluntary sampling, the responses resulted in some selection bias. Although an online questionnaire provided a large number of respondents, they were predominantly female (80.5%), 20–50 years old (82.1%), living in family homes (82.5%) in the continental part of Croatia (81.9%). It should be highlighted that the disproportion between continental and coastal parts of Croatia is more pronounced due to a large number of respondents residing in Zagreb, the capital city of Croatia (42.9%). The same selection bias was found for the respondents in the oldest age group, those living in a student's residence, as well as those with primary education level, where the proportion of these respondents overall was <1%. This might be because of the lower ability to use smart technologies for older people and those with primary education. Moreover, it should be emphasized that the respondents were untrained and were not able to ask for explanations for any doubts regarding questions. This might have resulted in under-/overestimation of actual food proportion intakes. Also, BMI was declared by participants and, therefore, should be treated only as a rough estimate of the exact number.

CONCLUSION

COVID-19 is one of the greatest public health threats the world has been faced with, while nutritional status may have an important role in disease severity and clinical outcome. The most prevalently advised dietary pattern during this challenging time is the Mediterranean diet, well-known for its anti-inflammatory and immunomodulatory properties. According to our findings, Croatian adults exhibited medium adherence to the MedDiet during the COVID-19 confinement. Furthermore, higher adherence and higher eagerness to increase physical activity was associated with lower BMI values (<25 kg/m^2). Regarding the dietary and lifestyle behavior aspects during the crisis, no change among the majority of respondents, in almost all of them, was found. An exception was the cooking frequency, where 53.8% of the respondents increased cooking incidence during the confinement. Those who cooked more also displayed an increase in vegetables, legumes, as well as fish and seafood consumption. These results suggest that cooking frequency could be positively associated with an overall dietary quality, which is of utmost importance in these demanding times.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because data has not been uploaded to publicly accessible repository. Requests to access the datasets should be directed to JR, jresetar@student.pharma.hr.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of the University of Granada (1526/CEIH/2020). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CR-P and MR-L conceptualized and designed the study. IP, DV, and ZS were responsible for the conduction of the study. JK analyzed the data. DP and JG interpreted the data. DP, JR, and JG prepared the draft of the manuscript. All authors conducted critical analyses and then finally approved the manuscript. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

We thank all the subjects for their contribution and our friends and colleges for the dissemination of the survey questionnaire. Additionally, we thank Prof. Ana Kovačić for English editing and academic writing revision.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.617721/full#supplementary-material

REFERENCES

1. Mattioli AV, Sciomer S, Cocchi C, Maffei S, Gallina S. Quarantine during COVID-19 outbreak: Changes in diet and physical activity increase the risk of cardiovascular disease. *Nutr Metab Cardiovasc Dis.* (2020) 30:1409–17. doi: 10.1016/j.numecd.2020.05.020
2. World Health Organization. Coronavirus (COVID-19) (2020). Available online at: https://covid19.who.int (accessed January 29, 2020).
3. Croatian Institute of Public Health. Koronavirus – najnoviji podatci (2020). Available online at: https://www.hzjz.hr/priopcenja-mediji/koronavirus-najnoviji-podatci (accessed January 29, 2020).
4. Chowdhury R, Heng K, Shawon MSR, Goh G, Okonofua D, Ochoa-Rosales C, et al. Dynamic interventions to control COVID-19 pandemic: a multivariate prediction modelling study comparing 16 worldwide countries. *Eur J Epidemiol.* (2020) 35:389–99. doi: 10.1007/s10654-020-00649-w
5. COVID-19 Health System Response Monitor. Policy responses for Croatia - Measures in other sectors (2020). Available online at: https://www.covid19healthsystem.org/countries/croatia/livinghit.aspx?Section=6.1%20Measures%20in%20other%20sectors&Type=Section (accessed September 3, 2020).
6. Mattioli AV, Puviani MB, Nasi M, Farinetti A. COVID-19 pandemic: the effects of quarantine on cardiovascular risk. *Eur J Clin Nutr.* (2020) 74:852–55. doi: 10.1038/s41430-020-0646-z

7. Di Renzo L, Gualtieri P, Pivari F, Soldati L, Attinà A, Cinelli G, et al. Eating habits and lifestyle changes during COVID-19 lockdown: an Italian survey. *J Transl Med.* (2020) 18:229. doi: 10.1186/s12967-020-02399-5
8. Moynihan AB, van Tilburg WAP, Igou ER, Wisman A, Donnelly AE, Mulcaire JB. Eaten up by boredom: consuming food to escape awareness of the bored self. *Front Psychol.* (2015) 6:369. doi: 10.3389/fpsyg.2015.00369
9. King AJ, Burke LM, Halson SL, Hawley JA. The challenge of maintaining metabolic health during a global pandemic. *Sports Med.* (2020) 50:1233–41. doi: 10.1007/s40279-020-01295-8
10. Dugail I, Amri EZ, Vitale N. High prevalence for obesity in severe COVID-19: Possible links and perspectives towards patient stratification. *Biochimie.* (2020) 179:257–65. doi: 10.1016/j.biochi.2020.07.001
11. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. *Obesity.* (Silver Spring) (2020) 28:1195–99. doi: 10.1002/oby.22831
12. Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients.* (2020) 12:1181. doi: 10.3390/nu12041181
13. Infusino F, Marazzato M, Mancone M, Fedele F, Mastroianni CM, Severino P, et al. Diet supplementation, probiotics, and nutraceuticals in sars-cov-2 infection: a scoping review. *Nutrients.* (2020) 12:1718. doi: 10.3390/nu12061718
14. Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, et al. Evidence that vitamin D supplementation could reduce risk of influenza and covid-19 infections and deaths. *Nutrients.* (2020) 12:988. doi: 10.3390/nu12040988
15. Bousquet J, Anto JM, Iaccarino G, Czarlewski W, Haahtela T, Anto A, et al. Is diet partly responsible for differences in COVID-19 death rates between and within countries? *Clin Transl Allergy.* (2020) 10:16. doi: 10.1186/s13601-020-00323-0
16. European Federation of the Association of Dietitians (EFAD). Covid-19 Information on Nutritional Support (2020). Available online at: http://www.efad.org/en-us/covid-19 (accessed September 3, 2020).
17. World Health Organization. Food and Nutrition Tips during Self-Quarantine (2020). Available online at: https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/technical-guidance/food-and-nutrition-tips-during-self-quarantine (accessed September 3, 2020).
18. Muscogiuri G, Barrea L, Savastano S, Colao A. Nutritional recommendations for CoVID-19 quarantine. *Eur J Clin Nutr.* (2020) 74:850–51. doi: 10.1038/s41430-020-0635-2
19. Zabetakis I, Lordan R, Norton C, Tsoupras A. COVID-19: the inflammation link and the role of nutrition in potential mitigation. *Nutrients.* (2020) 12:1466. doi: 10.3390/nu12051466
20. Dinu M, Pagliai G, Angelino D, Rosi A, Dall'Asta M, Bresciani L, et al. Effects of popular diets on anthropometric and cardiometabolic parameters: an umbrella review of meta-analyses of randomized controlled trials. *Adv Nutr.* (2020) 11:815–33. doi: 10.1093/advances/nmaa006
21. Huang Y, Lu Y, Huang YM, Wang M, Ling W, Sui Y, et al. Obesity in patients with COVID-19: a systematic review and meta-analysis. *Metabolism.* (2020) 113:154378. doi: 10.1016/j.metabol.2020.154378
22. Angelidi AM, Kokkinos A, Katechaki E, Ros E, Mantzoros CS. Mediterranean diet as a nutritional approach for COVID-19. *Metabolism.* (2021) 114:154407. doi: 10.1016/j.metabol.2020.154407
23. Maiorino MI, Bellastella G, Longo M, Caruso P, Esposito K. Mediterranean diet and covid-19: hypothesizing potential benefits in people with diabetes. *Front Endocrinol.* (2020) 11:574315. doi: 10.3389/fendo.2020.574315
24. Rodríguez-Pérez C, Molina-Montes E, Verardo V, Artacho R, García-Villanova B, Guetta-Hernández EJ, et al. Changes in dietary behaviours during the covid-19 outbreak confinement in the Spanish COVIDiet study. *Nutrients.* (2020) 12:1730. doi: 10.3390/nu12061730
25. Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A short screener is valid for assessing mediterranean diet adherence among older Spanish men and women. *J Nutr.* (2011) 141:1140–45, doi: 10.3945/jn.110.135566
26. Keys A. Coronary heart disease in seven countries. *Nutrition.* (1997) 13:249–53. doi: 10.1016/s0899-9007(96)00410-8
27. *National Classification of NUTS 2 Regions, No. 96*. Croatia: Official Journal (2012).
28. Wolfson JA, Bleich SN. Is cooking at home associated with better diet quality or weight-loss intention? *Public Health Nutr.* (2015) 18:1397–406. doi: 10.1017/S1368980014001943
29. Mills S, Brown H, Wrieden W, White M, Adams J. Frequency of eating home cooked meals and potential benefits for diet and health: cross-sectional analysis of a population-based cohort study. *Int J Behav Nutr Phys Act.* (2017) 14:109. doi: 10.1186/s12966-017-0567-y
30. Te Velthuis AJW, van den Worm SHE, Sims AC, Baric RS, Snijder EJ, van Hemert MJ. Zn2+ inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLoS Pathog.* (2010) 6:e1001176. doi: 10.1371/journal.ppat.1001176
31. Giacalone D, Bom Frøst M, Rodríguez-Pérez C. Reported changes in dietary habits during the covid-19 lockdown in the danish population: the danish COVIDiet study. *Front Nutr.* (2020) 7:592112. doi: 10.3389/fnut.2020.592112
32. Fiore M, Ledda C, Rapisarda V, Sentina E, Mauceri C, D'Agati P, et al. Medical school fails to improve Mediterranean diet adherence among medical students. *Eur J Public Health.* (2015) 25:1019–23. doi: 10.1093/eurpub/ckv127
33. Baydemir C, Ozgur EG, Balci S. Evaluation of adherence to Mediterranean diet in medical students at Kocaeli University, Turkey. *J Int Med Res.* (2018) 46:1585–94. doi: 10.1177/0300060518757158
34. Papadaki A, Wood L, Sebire SJ, Jago R. Adherence to the Mediterranean diet among employees in South West England: Formative research to inform a web-based, work-place nutrition intervention. *Prev Med Rep.* (2015) 2:223–8. doi: 10.1016/j.pmedr.2015.03.009
35. Genc A-M. *Assessment of dietary habits among workers with the MEDAS questionnaire*. (bachelor's thesis). University of Zagreb, Zagreb, Croatia (2018).
36. Schnettler B, Lobos G, Miranda-Zapata E, Denegri M, Ares G, Hueche C. Diet quality and satisfaction with life, family life, and food-related life across families: a cross-sectional pilot study with mother-father-adolescent triads. *Int J Environ Res Public Health.* (2017) 14:1313. doi: 10.3390/ijerph14111313
37. Thiele S, Mensink GBM, Beitz R. Determinants of diet quality. *Public Health Nutr.* (2004) 7:29–37. doi: 10.1079/PHN2003516
38. Gray-Phillip G, Huckle T, Callinan S, Parry CDH, Chaiyasong S, Coung PV, et al. Availability of alcohol: location, time and ease of purchase in high- and middle-income countries: data from the international alcohol control study. *Drug Alcohol Rev.* (2018) 37:S36–S44. doi: 10.1111/dar.12693
39. Ammar A, Brach M, Trabelsi K, Chtourou H, Boukhris O, Masmoudi L, et al. Effects of COVID-19 home confinement on eating behaviour and physical activity: results of the ECLB-COVID19 international online survey. *Nutrients.* (2020) 12:1583. doi: 10.3390/nu12061583
40. Grosso G, Marventano S, Buscemi S, Scuderi A, Matalone M, Platania A, et al. Factors associated with adherence to the mediterranean diet among adolescents living in Sicily, Southern Italy. *Nutrients.* (2013) 5:4908–23. doi: 10.3390/nu5124908
41. Guallar-Castillón P, Rodríguez-Artalejo F, Lopez-Garcia E, León-Muñoz LM, Amiano P, Ardanaz E, et al. Consumption of fried foods and risk of coronary heart disease: Spanish cohort of the European Prospective Investigation into Cancer and Nutrition study. *BMJ.* (2012) 344:e363. doi: 10.1136/bmj.e363
42. Gadiraju TV, Patel Y, Gaziano JM, Djoussé, L. Fried food consumption and cardiovascular health: a review of current evidence. *Nutrients.* (2015) 7:8424–30. doi: 10.3390/nu7105404
43. Lachat C, Nago E, Verstraeten R, Roberfroid D, Van Camp J, Kolsteren P. Eating out of home and its association with dietary intake: a systematic review of the evidence. *Obes Rev.* (2012) 13:329–46. doi: 10.1111/j.1467-789X.2011.00953.x
44. Llanaj E, Ádány R, Lachat C, D'Haese M. Examining food intake and eating out of home patterns among university students. *PLoS ONE.* (2018) 13:e0197874. doi: 10.1371/journal.pone.0197874
45. Sidor A, Rzymski P. Dietary choices and habits during COVID-19 lockdown: experience from Poland. *Nutrients.* (2020) 12:1657. doi: 10.3390/nu12061657
46. Kriaucioniene V, Bagdonaviciene L, Rodríguez-Pérez C, Petkeviciene J. Associations between changes in health behaviours and body weight during the COVID-19 Quarantine in Lithuania: The Lithuanian COVIDiet Study. *Nutrients.* (2020) 12:3119. doi: 10.3390/nu12103119

47. Lesser IA, Nienhuis CP. The impact of COVID-19 on physical activity behavior and well-being of Canadians. *Int J Environ Res Public Health.* (2020) 17:3899. doi: 10.3390/ijerph17113899
48. Schröder H, Marrugat J, Vila J, Covas MI, Elosua R. Adherence to the traditional mediterranean diet is inversely associated with body mass index and obesity in a Spanish population. *J Nutr.* (2004) 134:3355–61. doi: 10.1093/jn/134.12.3355
49. Agnoli C, Sieri S, Ricceri F, Giraudo MT, Masala G, Assedi M, et al. Adherence to a Mediterranean diet and long-term changes in weight and waist circumference in the EPIC-Italy cohort. *Nutr Diabetes.* (2018) 8:22. doi: 10.1038/s41387-018-0023-3
50. Giuliano D, Esposito K. Mediterranean diet and metabolic diseases. *Curr Opin Lipidol.* (2008) 19:63–8. doi: 10.1097/MOL.0b013e3282f2fa4d
51. Mills S, White M, Brown H, Wrieden W, Kwasnicka D, Halligan J, et al. Health and social determinants and outcomes of home cooking: a systematic review of observational studies. *Appetite.* (2017) 111:116–34. doi: 10.1016/j.appet.2016.12.022
52. Hagmann D, Siegrist M, Hartmann C. Acquisition of cooking skills and associations with healthy eating in Swiss adults. *J Nutr Educ Behav.* (2020) 52:483–91. doi: 10.1016/j.jneb.2019.12.016
53. Ducrot P, Méjean C, Aroumougame V, Ibanez G, Allés B, Kesse-Guyot E, et al. Meal planning is associated with food variety, diet quality and body weight status in a large sample of French adults. *Int J Behav Nutr Phys Act.* (2017) 14:12. doi: 10.1186/s12966-017-0461-7
54. Gardner B, Lally P, Wardle J. Making health habitual: the psychology of 'habit-formation' and general practice. *Br J Gen Pract.* (2012) 62:664–6. doi: 10.3399/bjgp12X659466
55. Carden L, Wood W. Habit formation and change. *Curr Opin Behav Sci.* (2018) 20:117–22. doi: 10.1016/j.cobeha.2017.12.009
56. Hartmann C, Dohle S, Siegrist M. Importance of cooking skills for balanced food choices. *Appetite.* (2013) 65:125–31. doi: 10.1016/j.appet.2013.01.016
57. Framer N, Lee LJ, Powell-Wiley TM, Wallen GR. Cooking frequency and perception of diet among US adults are associated with us healthy and healthy mediterranean-style dietary related classes: a latent class profile analysis. *Nutrients.* (2020) 12:3268. doi: 10.3390/nu12113268
58. Wolfson JA, Leung CW, Richardson CR. More frequent cooking at home is associated with higher Healthy Eating Index-2015 score. *Public Health Nutr.* (2020) 23:2384–94. doi: 10.1017/S1368980019003549

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Pfeifer, Rešetar, Gajdoš Kljusurić, Panjkota Krbavčić, Vranešić Bender, Rodríguez-Pérez, Ruíz-López and Šatalić. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

MINI REVIEW
published: 07 April 2021
doi: 10.3389/fimmu.2021.661806

Breastfeeding and COVID-19: From Nutrition to Immunity

Emilia Vassilopoulou[1], *Gavriela Feketea*[2,3], *Lemonica Koumbi*[1], *Christina Mesiari*[1], *Elena Camelia Berghea*[4,5*] and *George N. Konstantinou*[6]

[1] Department of Nutritional Sciences and Dietetics, International Hellenic University, Thessaloniki, Greece, [2] PhD School, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, [3] Department of Pediatrics, Pediatric Allergy Outpatient Clinic, "Karamandaneio", Children Hospital, Patras, Greece, [4] Department of Pediatrics, Allergology and Clinical Immunology Outpatient Clinic, Clinical Hospital of Emergency for Children MS Curie, Bucharest, Romania, [5] Department of Pediatrics, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, [6] Department of Allergy and Clinical Immunology, 424 General Military Training Hospital, Thessaloniki, Greece

Breastfeeding not only provides the optimum source of nutrients for the neonate and its first strong shield against infection but also lays the foundation for somatic and psychological bonding between the mother and child. During the current COVID-19 pandemic, although the guidelines of the relevant international and national agencies recommend breastfeeding by SARS-CoV-2–infected mothers, considerable insecurity persists in daily clinical practice regarding the safety of the infants and the perceived advantages and disadvantages of discontinuation of breastfeeding. This is a systematic review of the currently available information regarding the transmissibility of SARS-CoV-2 through or while breastfeeding and the protection against infection that breast milk might provide. The accumulated body of knowledge regarding the role of breast milk in the development of the neonatal immune system and protection against infection by other respiratory viruses is discussed, with a focus on the anti-inflammatory role of the antibodies, microbes, and viruses provided to the infant in breast milk and its relevance to the case of SARS-CoV-2.

Keywords: antibodies, anti-inflammation, breastfeeding, COVID-19, SARS-CoV-2, microbiome, virome, respiratory infection

OPEN ACCESS

Edited by:
Ioannis Zabetakis,
University of Limerick, Ireland

Reviewed by:
Paola Roggero,
IRCCS Ca 'Granda Foundation Maggiore Policlinico Hospital, Italy
Markus Xie,
Genentech, United States

****Correspondence:***
Elena Camelia Berghea
bcamelia@gmail.com

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: *31 January 2021*
Accepted: *10 March 2021*
Published: *07 April 2021*

Citation:
Vassilopoulou E, Feketea G, Koumbi L, Mesiari C, Berghea EC and Konstantinou GN (2021) Breastfeeding and COVID-19: From Nutrition to Immunity. Front. Immunol. 12:661806. doi: 10.3389/fimmu.2021.661806

HIGHLIGHTS

- Nature has designed the mother's breast milk to nurture the neonate and to protect of the dyad in "psyche and soma", ensuring the transfer of antibodies, microbes, and viruses, but also emotional stimulation.
- Since SARS-CoV-2 is highly transmissible, breastfeeding should be encouraged, but observing all appropriate safety measures, for the mother and close contacts
- With the vaccination schedule in progress, the pregnant mothers-to-be should probably be prioritized.

GRAPHICAL ABSTRACT |

BRIEF HISTORICAL PERSPECTIVE

Since the beginning of 2020, when the World Health Organization (WHO) announced a new strain of coronavirus, the SARS-CoV-2, which provokes coronavirus disease 19 (COVID-19) (1), the whole world has been dominated by the COVID-19 pandemic. In January 2021, only 1 year later, approximately 85 million cases had been confirmed, resulting in more than 1.8 million deaths (2).

During the same period, approximately 140 million births have been registered (3) and a great dilemma arose regarding the possible need to discontinue the breastfeeding of infants of infected mothers (4). Although, to date, evidence on the risk of vertical transmission, *via* the respiratory tract or through the breast milk itself, is limited, breastfeeding has generally been accepted as the preferred nutrition for the infant of the infected mother. In breast milk from infected mothers, IgA antibodies against SARS-CoV-2 have been detected, which may account for the reduced clinical impact of the disease in breastfed infants upon future viral exposure (5).

This mini-review summarizes the current evidence, collected in a systematic literature search and narrative review of original articles related to breastfeeding by SARS-CoV-2–infected mothers, and deploying knowledge gained from other respiratory virus-transmitted diseases about the protective anti-inflammatory effects of breastfeeding. The specific questions addressed in the review were whether or not the infant should be breastfed when (1): the mother is diagnosed with SARS-CoV-2 before or immediately after delivery; (2) the lactating mother is positive for SARS-CoV-2 but the infant is negative; (3) both mother and infant are positive for SARS-CoV-2; (4) or the mother is negative but the infant is positive for SARS-CoV-2, based on evidence from the literature and knowledge gained from other respiratory virus infections.

METHODS

The review followed the Preferred Reporting of Systematic Reviews and Meta-Analysis (PRISMA) guidelines to collect articles on breastfeeding by SARS-CoV-2–infected mothers (6). This review was not registered with PROSPERO, which does not currently accept registration of reviews.

We searched the PubMed, Scopus, Web of Science, and MedRxiv electronic databases up to December 31st 2020 to identify original published studies describing lactating women with a confirmed diagnosis of COVID-19, using the following search keywords and phrases: ("COVID-19" OR "2019-nCoV" OR "novel coronavirus" OR "SARS-CoV-2" OR "coronavirus 2") AND breastfeeding. Reference tracking was carried out to identify other studies eligible for inclusion.

Each reference retrieved was screened independently by two researchers, following predefined criteria to determine eligibility for the systematic review. Studies were excluded if: 1. they did not involve humans (e.g., *in vitro* or animal research), and 2. they were non-original articles (e.g., book chapters, review articles, metanalysis, guidelines). There were no date or language restrictions on the search.

All the original observational studies, case reports and case series of breastfeeding women diagnosed with COVID-19 identified in the search were included. In view of the heterogeneity observed across the studies, it was decided to perform a narrative synthesis, using the Synthesis Without Meta-analysis (SWiM) reporting guidelines (intended to complement the PRISMA guidelines) (7). Descriptive statistics were presented (frequency and proportions) based on the total cases with available information. In addition, the current guidelines issued by international and national health organizations were retrieved and summarized. Documentation on other maternal respiratory viral infections and breastfeeding was gathered and the evidence on the potential protective effect of breastfeeding for the infants is presented and discussed.

SUMMARY OF THE ESTABLISHED PRINCIPLES

Current Recommendations About Breastfeeding and COVID-19

Human breastfeeding enhances both maternal and infant health, with a dynamic, bidirectional exchange between the mother and the infant, which constitutes the cornerstone of infant and child well-being.

Despite concerns of transmission from the infected mother to the infant, global and national health stakeholders have so far univocally encouraged breastfeeding during the COVID-19 pandemic. The WHO (8), the United Nations International Children's Emergency Fund (UNICEF) (9), the Union of European Neonatal & Perinatal Societies (UENPS) (10), and the US Centers for Disease Control and Prevention (CDC) (11), all highlight the well-established overall short- and long-term immunological and psychosomatic benefits of breastfeeding for the dyad. The current recommendations point out that there is, at present, insufficient evidence about the transmission of COVID-19 through breastfeeding. For this reason, strict measures of mother-infant separation and discontinuation of breastfeeding are to be avoided, regardless of a positive diagnosis and the intensity of symptoms, unless the severity is of such a level that the mother cannot take care of the infant, in which case, expressed, fresh, unpasteurized breast milk should be provided for the baby (8).

In spite of these guidelines, there has considerable scepticism during the pandemic among the front-line healthcare professionals, gynecologists, midwives, and pediatricians, on whether they should encourage the infected mother to breast feed her baby (12).

STUDIES RELATED TO BREASTFEEDING OF SARS-COV-2–INFECTED MOTHERS

From the search in the four electronic databases, 537 articles were retrieved, and after removal of duplicates, 383 were screened. Among 46 articles reaching the final assessment for eligibility, 18 were excluded, as they did not meet all inclusion criteria, i.e., they did not provide data on whether the mother and/or infant was infected, whether the infant was breastfeeding and what practices were used. Finally, 21 case reports and 7 original articles, published up until December 31st 2020 were included, originating mainly from China (11 case reports; 1original article) and Italy (2 case reports; 2 original articles). **Figure 1** presents the process of inclusion of studies in the review.

For purposes of this review the studies were sub-grouped into four categories according to the infected member of the dyad, and the measures undertaken are discussed accordingly. A summary of the 28 studies is presented in **Table 1**.

SARS-CoV-2–Positive Mother Before or on Delivery

Some mothers were prohibited from holding the neonates, in order to minimize the risk of infection (36, 39) and expressed breast milk was proposed as the optimum feeding solution (38). Mothers in a good clinical condition were encouraged to breastfeed, with all appropriate instructions and precautions (37). In the case of admission of preterm infants to the neonatal intensive care unit (NICU), expressed breast milk was suggested, if available (36). Overall, among the 231 births from SARS-CoV-2–positive mothers, 13 neonates (5.8%) tested positive within the first 48 hours of life (31–34, 36, 38, 39).

SARS-CoV-2–Positive Lactating Mother, With Negative Infant

Generally, breastfeeding was encouraged (13, 14, 17, 23, 24, 26), but in order to reduce the risk of infection (36, 39), instructions for appropriate precautions were given (37). Measures undertaken to minimize the risk of transmission during breastfeeding were mask wearing, handwashing, routine cleaning and disinfection of all surfaces touched, thorough cleaning and sterilization of infant feeding equipment before and after use, breast washing with gauze saturated with soap and water, and avoidance of falling asleep with the baby (13, 14, 17). Alternatively, expression of breast milk and feeding of the infant by a healthy family member or a caretaker was recommended (19). In some cases, when feeding became a controversial issue, the parents were to allowed decide if the infant would be breast fed, due to the insecurity caused by the perceived risk of transmission to the infant (16, 22). Insecurity about the decision was increased when SARS-CoV-2 was traced in the breast milk, in which case, breastfeeding was suspended and could be resumed after a period of isolation, followed by a confirmed negative test on the mother (15, 20). Of a total of 63 women, 37 (58.7%) discontinued breastfeeding and were isolated from their babies. Of over 38 samples of breast milk tested in SARS-CoV-2–positive mothers, only 2 (5.2%) were positive for SARS-CoV-2 (16, 24).

Both Mother and Infant SARS-CoV-2–Positive

Breastfeeding was strongly encouraged where both members of the dyad were positive (27, 30, 31), and further investigation was suggested on the role of IgG antibodies (27) in the possible

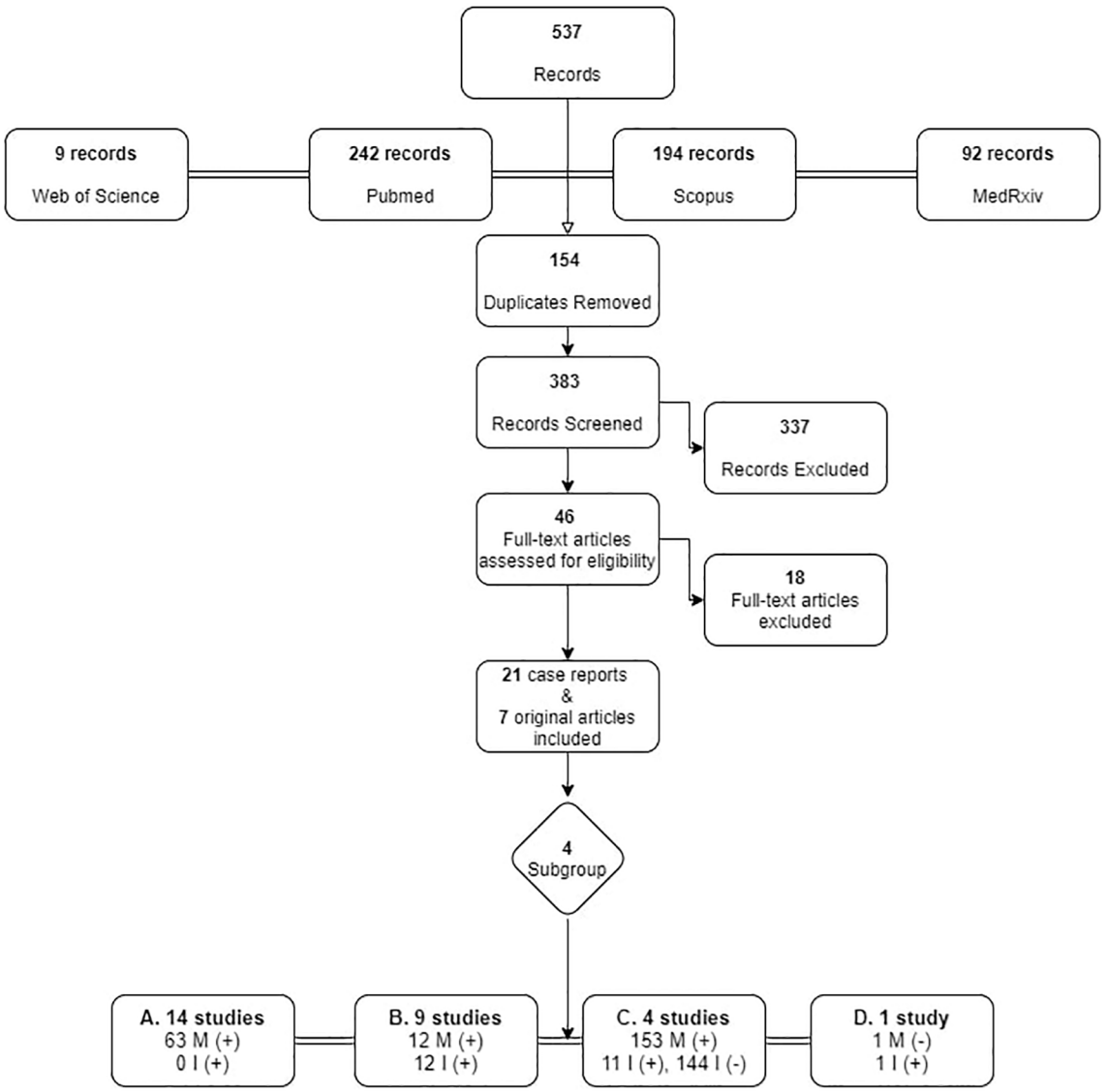


FIGURE 1 | Search strategy flowchart for original articles on breastfeeding and SARS-Cov-2. M, Mother; I, Infant. +: SARS-CoV-2 infected, -: negative for SARS-CoV-2.

protective role of breast milk antibodies on the immunity of the infants (33, 35). Among the breast milk samples tested for SARS-CoV-2, 27% (3/11) were positive (27, 28, 30, 34, 35), but the milk was not considered to be the cause of infection of the infants (29).

HEALTHY MOTHER AND INFANT SARS-COV-2–POSITIVE INFANT

In the one reported case of an infected infant with a healthy mother, the mother was encouraged to remain in quarantine with her baby and to continue breastfeeding. Despite the close contact, the mother remained negative for SARS-CoV-2 (40).

THE CURRENT "STATE OF THE ART"

The Anti-Inflammatory Effects of Breastfeeding: Knowledge Gained From Other Respiratory Viruses

Respiratory infections are a leading cause of morbidity in children. During the first year of life, breastfeeding provides protection from these infections, dependent on its duration (41), mainly against lower respiratory infection. The immaturity of the infant's immune system at birth increases the risk of infection by external agents, including viruses and bacteria (42), which is related to the unpreparedness of the neonatal respiratory and gastrointestinal (GI) tracts to resist invasion.

TABLE 1 | Studies included in the review of breastfeeding (BF) and SARS-CoV-2 infection (N=28).

Authors	Year	Country	Study Type	No of infected mothers	Mother's age (years)	Infant's Age (months)	Mother's Symptoms	Infant's Symptoms/test	Feeding mode	Maternal Separation (Y, yes/N, no)	SARS-CoV-2 in breastmilk	Conclusion
A. Infected mother–non-infected infant												
Lowe and Bopp (13)	2020	Australia	Case report	1	31	<1	Fever & respiratory symptoms	(–)	BF	N	N/A	BF & room-in with precautions
Pereira et al. (14)	2020	Spain	Retrospective case series	22	34 (19–43)	<1	50% symptomatic (6 mild symptoms, 5 pneumonia)	(–)	20/22 BF 2/22 IF	9/11 symptomatic mothers isolated	N/A	BF with precautions, Upon maternal isolation: feeding with pasteurized DHM or IF until BF is resumed
Lang and Zhao (15)	2020	China	Case report	1	30	<1	Dry cough	(–)	BF after isolation	Y	(–)	BF after isolation and negative test, PBM during isolation
Bastug et al. (16)	2020	Turkey	Case report	1	20	<1	Asymptomatic	(–)	PBF, When SARS-CoV-2 detected in BM- BF discontinued	Y	(+)	Decision about BF by parent & doctor
Gabriel et al. (17)	2020	Spain	Observational prospective study	7	33.4 (31–37)	<1	6/7 asymptomatic 1/7 fever, myalgia, malaise, headache	(–)	BF	N/A	(–)	Direct BF safe or hand: expressed BM with precautions
Chu et al. (18)	2020	China	Case report	1	30	<1	Gastrointestinal symptoms, fever	(–)	PBM	Y	(–)	SARS-CoV-2 rarely transmitted through BM- possible induction of passive immunity *via* IgG
Liu et al. (19)	2020	China	Case report	1	37	6	Fever, cough, sore throat, fatigue	(–)	BF discontinued after mother's positive test	Y	N/A	PBM and feeding to infant by a healthy caretaker/family member
Feng et al. (20)	2020	China	Case report	1	33	2	Fever	(–)	Suspended BF after diagnosis	Y	N/A	BF temporal suspension
Perrone et al. (21)	2020	Italy	Case report	1	N/A	<1	fever, anosmia, & malaise	(–)	BF + expressed maternal milk	N	(–)	BF with precautions when virus is not traced in milk
Liu et al. (22)	2020	China	Prospective study	19	31 (27–34)	<1	fever (11/19), 5/19 cough or dyspnea, 2/19 diarrhea or another gastrointestinal symptom	(–)	Term Formula	Y	(–)	Not vertical transmission with BF
Dong et al. (23)	2020	China	Case report	1	33	<1	Cough & chest tightness	(–)	BF	Y	(–)	IgG & IgA of BM might provide immune protection
Zhu et al. (24)	2020	China	Case report	5	32 (27–34)	<1	Main symptoms: fever, chest distress or dyspnea, cough, nasal congestion, rhinorrhea, poor appetite, or diarrhea	(–)	N/A	N/A	1/5 (+)	Generally BF encouragement but when SARS-CoV-2 BM increased risk of transmission
Dong et al. (25)	2020	China	Case report	1	29	<1	Fever, nasal congestion, respiratory difficulties	(–)	N/A	Y	(–)	When increased IgG & IgM levels-infection at delivery cannot ruled out
Li et al. (26)	2020	China	Case Report	1	30	<1	Dry cough, fever	(–)	N/A	Y	(–)	mother-to-child transmission is unlike.
B. Infected mother–Infected infant												
Yu et al. (27)	2020	China	Case report	1	32	13	Nasal congestion	(+) Fever, dry cough, nasal congestion	Directly BF & complementary after 6 months	N	(–)	BF safe
Groß et al. (28)	2020	Germany	Case series	2	N/A	<1	Mild symptoms	(+) Respiratory, breathing symptoms icterus	BF	N	Mother's 1 (–) Mother's 2 (+)	BF with precautions/further investigation on virus transmission potential during BF
Tam et al. (29)	2020	Australia	Case series	1	40	8	Sore throat, myalgia, productive cough, fever	(+) Mild coryzal symptoms, non-productive cough	BF discontinued and PBM when infant was confirmed with COVID-19	N	(+)	In infected infants BF should be continued/nil adverse effects/ SARS-CoV-2 RNA in breast milk sample did not indicate viable virus
Salvatori et al. (30)	2020	Italy	Case report	2	31 (26–36)	<1	50% anosmia & dysgeusia, 50% back & thoracic pain	(+) 50% asymptomatic 50% diarrhea, cough, poor feeding	BF	N	(–)	BF supported, transmission more possibly through respiratory droplets
Phadke et al. (31)	2020	India	Case report	1	33	<1	Asymptomatic	(+) asymptomatic	IF After positive test for infant: direct BF	Y	N/A	BF & room-in with precautions

(Continued)

TABLE 1 | Continued

Authors	Year	Country	Study Type	No of infected mothers	Mother's age (years)	Infant's Age (months)	Mother's Symptoms	Infant's Symptoms/test	Feeding mode	Maternal Separation (Y, yes/N, no)	SARS-CoV-2 in breastmilk	Conclusion
Kirtsman et al. (32)	2020	Canada	Case report	1	40	<1	myalgia, decreased appetite, fatigue, dry cough & fever	(+) Neutropenic, mild hypothermia, feeding difficulties & hypoglycemic episodes	BF	N	(+)	Further studies to neonates are needed to identify transmission routes
Hinojosa-Velasco et al. (33)	2020	Mexico	Case report	1	21	<1	Cough, odynophagia, headache, diarrhea, rhinorrhea, sore throat, fever	(+) newborn jaundice, tachypnea, hypernatremia, central cyanosis, dyspnea, and oxygen saturation of less than 92%, (NICU)	Synthetic milk formula	Y	(+)	BF might be protective
Wang et al. (34)	2020	China	Case report	1	34	<1	Fever, vaginal bleeding, lower abdominal pain	(+)	Infant Formula	Y	(–)	No BF
Fan et al. (35**)**	2020	China	Case series	2	31.5 (29–34)	<1	1: Nasal congestion, fever 2: fever, nasal congestion, sore throat	(+) 1: fever and abdominal distension with lymphopenia 2: mild neonatal pneumonia and lymphopenia	IF	1/2 Y	(–)	Low risk of vertical transmission *via* BF, potential protective role of passive antibodies
C. Infected mother- both infected and non-infected infants												
ElHalik et al. (36)	2020	United Arab Emirates	Retrospective observational study	35	32 (24–42)	<1	Negative	34/36 (–) 2/36 (+) 1 asymptomatic 1 admitted to NICU with respiratory distress	32/36 direct BF or PBM	27/36 roomed-in	N/A	Direct BF or PBM encouraged with precautions
Ronchi et al. (37)	2020	Italy	Prospective multicenter study	61	32 (28–36)	<1	Symptoms at diagnosis: 34/61 asymptomatic 27/61 at least 1 symptom	0/62 on birth 2/62 positive for SARS-CoV-2 at 7 and 20d of life respectively	45/62 Exclusive BF 13/62 BF + IF 3/62 exclusive IF 1/62 BF + PBM	51/52 roomed-in exclusive BF	N/A	BF and room-in encouraged to infected mothers with good clinical condition with precautions
Patil et al. (38)	2020	United States of America	Retrospective cross-sectional study	45	30 (27.5–35.5)	<1	27/45 asymptomatic 16/45 mild symptoms 2/45 ICU	42/45 (+) SARS-CoV-2 3/45 -(+)/admitted to NICU-asymptomatic	31/33	33/45 roomed-in 5/45 separated, 7/45 preterm admitted to NICU	No tested sample	BF and room-in under proper precautions
Bertino et al. (39)	2020	Italy	Prospective Collaborative Observational Study	12	30.5 (24–38)	<1	10/12 symptomatic: fever, myalgia, rhinitis, cough, dyspnea, sore throat, conjunctivitis, diarrhea, chest pain, anosmia, ageusia	4/12 (+) SARS-CoV-2 asymptomatic	11/12 exclusively BF	No	11/12 (–) 1/12 (+)	BF or PBM (in isolation) encouraged irrespective swab test results with proper precautions
D. Non-infected mother-infected infant												
Le et al. (40)	2020	Vietnam	Case report	1 (non-infected)	N/A	3	None (–)	(+) Rhinorrhea, nasal congestion & fever	Exclusively BF	No	No tested sample	Need to further investigate SARS-CoV-2 transmission routes to pediatric population

BF, breastfeeding; IF, infant formula; Y, yes; n, no; N/A, not available; DHA, donor human milk; PBM, pumped breast milk; PBF, pumped breastfeeding; BM, breast milk; (–), negative; (+), positive; NICU, neonatal intensive care unit.

Breast milk changes in synthesis, from colostrum through a transitional stage to mature milk, ensuring appropriate nutrition for the infant (43). Intensive, continuing research has provided an appreciable body of documentation on the health benefits of breastfeeding for both mother (44, 45) and child. Regarding immunity, human milk induces in the infant the regulation and development of the innate (46) and adaptive immune systems (47, 48), with a major long-term role in health and disease (49).

The anti-inflammatory protection conveyed by breast milk is effected by both chemical components and cellular interactions. Colostrum and transitional milk safeguard the infant *via* an abundant glycoprotein, lactoferrin, which has multilevel actions, lymphostimulatory, anti-inflammatory, anti-bacterial, anti-viral and anti-fungal (50). Its protective functions are attributed to its iron-binding properties, inhibition of interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), stimulation of the activity and maturation of lymphocytes, and preservation of an antioxidant environment (51). Lactoferrin, along with other milk peptides protects against bacteria and fungus (52). *In vitro* studies have shown that lactoferrin inhibits the invasion and growth of respiratory syncytial virus (RSV), interacting directly with the F glycoprotein on the surface of the virus, which is essential for viral penetration. Adenovirus infection was also observed to be inhibited *in vitro* due to interference of lactoferrin with the primary receptors present at the cellular level. Milk regurgitation into the nose after breastfeeding has been suggested to increase phage adherence to mucosal surfaces in the respiratory tract, in addition to the gut, eliminating in this way mucosal bacteria and protecting against recurrent respiratory infections in breastfed infants, in the longer term (53).

New knowledge gained during the 2003 SARS-CoV-1 epidemic was that lactoferrin interacts with heparin sulphate glycosaminoglycan (HSPG) cell receptors, interfering with the first anchoring sites of the virus on the cell, and thus preventing the initial contact between the SARS-CoV and host cells. Lactoferrin has also been shown to block the interaction between spike viral protein and HSPC in an angiotensin-converting enzyme 2 (ACE2) receptor, which otherwise results in the full infection (54).

Other dominant oligosaccharides in human milk serve both as a direct barrier to pathogens and as a prebiotic, i.e., aliment for probiotics, which promote synthesis of a healthy microbiota (42). The binding capacity of the oligosaccharides has proven protective against viruses with high morbidity and mortality, including human immunodeficiency virus (HIV) (55) and rotavirus (56), and breast milk mucin is able to aggregate poxviruses prior to their entry into host cells (57).

Additional protective properties of breast milk are provided by the transfer of maternal immune cells to the infant, including macrophages, neutrophils and lymphocytes (58). The concentration of these cells in the human breast milk vary according to the age of the infant, taking into account that in the early stage of lactation, the neonatal immune system is completely immature (59). Thus, the proportion of the different leukocytes vary between colostrum (macrophages 40–50%, neutrophils 40–50% and lymphocytes 5–10%) and more mature milk (macrophages 85%, lymphocytes 15%).

The immune properties of the mother are also transferred to the breastfed infant in the form of secretory IgG (60) and IgA in maternal milk (61). Breast milk attains the highest concentration in IgG antibodies in the colostrum, and their concentration drops after the first month of life (62) and stops abruptly with weaning (63). IgG antibodies, transferred to the fetus through the placenta during intrauterine life and to the infant after birth in breast milk, constitute the infant's first defense system. In mothers immunized against RSV, s-IgG antibodies were detected in breastmilk (64), providing protection to the infant against the main cause of respiratory infection during the first year of life (65). IgA antibodies coat the GI and respiratory mucosa and block the entrance of foreign antigens (50) and viruses (63). In premature infants, the IgA levels are higher, for enhanced protection (66). In the event of an infection, in either the mother or the child (67), breast milk conveys a plethora of antipathogenic and anti-inflammatory bioactive factors (68) to protect the infant. In attacks by respiratory virus infections, such as RSV, protection is mediated by polymeric IgA antibodies to a protein of the RSV surface membrane, inhibiting virus replication (50).

In addition to the transfer of antibodies, human milk triggers immune-protective responses by the host. In influenza, type I interferons (IFN), cytokines with strong simultaneous anti-viral, pro-apoptotic and pro-inflammatory properties, are produced significantly more often in breastfed infants, transforming viral attacks to *formes frustes* (69).

Breastfeeding has been documented to exert more effective protection against a spectrum of pneumonia-causing viruses, including influenza, RSV and parainfluenza, in girls than in boys (70); these findings were interpreted by the researchers as a "nature"-provided advantage for survival of females, in order to preserve the species.

Protection against viral invasion appears to be enhanced by the regurgitation of breast milk into the upper respiratory tract, conveying viable commensal, mutualistic, and probiotic bacteria (71) and viruses (53) that colonize the upper respiratory tract, contributing to the maturation of the infant's immune system.

Bacteria in the human milk are one of the earliest sources of prokaryotic microorganisms transferred to the infant, following the maternal microbial colonization through the amniotic fluid, placenta and umbilical fluid (72), and a more substantial transmission of vaginal and gut microorganisms to the newborn through the birthing canal (73). The human milk microbiota (HMM) originates from the maternal GI tract and skin, and from the infant's mouth (71). HMM and other human milk components, such as human milk oligosaccharides (HMO), reflect environmental factors, e.g., viral exposure, weather, and diet, in addition to the immune status of the mother (74).

Human milk transmits a significant load of viruses, eukaryotic and bacteriophages, from the mother to the infant, which enhance the maturation of both innate and adaptive immune systems. Eukaryotic viruses may impact the health status directly, while bacteriophages act *via* the bacterial ecology.

HIGHLIGHTING FUTURE DIRECTIONS IN BREASTFEEDING RESEARCH

In children, COVID-19 infection rates are lower than in adults, while fatality rates are almost zero (71). Children generally have milder symptoms, but there are reports of the development of a novel multisystem inflammatory syndrome in children (MIS-C) similar to Kawasaki disease, predicating continuous vigilance (53).

The current policy is that breastfeeding is contraindicated in only a limited number of viral diseases, i.e., HIV, cytomegalovirus (CMV) in preterm infants, and human T-lymphotropic virus I (72). As it constitutes an incomparable feeding method for babies, international and national health authorities strongly recommend exclusive breastfeeding for at least the first six months of life: "Breastfeeding is one of the most effective ways to ensure child health and survival" - WHO (8); "Low rates and early cessation of breastfeeding have important adverse health and social implications for women, children, the community and the environment, result in greater expenditure on national health care provision, and increase inequalities in health" - European Commission (EC) (75)".

The unsurpassed benefits of breastfeeding include not only irreplaceable nutrition, ensuring healthy growth for the infant, but also prevention of obesity in later life (74). It is a mainstay for promoting the immune development of the infant by both immunological factors transferred from mother to infant through breast milk (76) and microorganisms colonizing the organs and viruses enriching the virome.

SARS-CoV-2 is probably transmitted in multiple ways, including through droplets *via* the respiratory tract and invasion by enterocytes (73). GI symptoms manifest first in infancy, and lactoferrin in breast milk has been suggested to be capable of strengthening junctions between microbes in the gut and thus amplifying the innate defense. Although at present, this is only an assumption for SARS-CoV-2, in other strains of SARS-CoV, lactoferrin was shown to increase mucosal immunity and prevent viral anchoring on cell receptors (77). Cytokines and growth factors in breast milk excite the infant's immune system and balance the anti-inflammatory and pro-inflammatory cytokines, lessening their effect and preventing the "cytokine storm" described in other viral infections, such as H1N1 'swine flu' and H5N1 'bird flu' (78).

The most abundant antibody in breast milk, sIgA, provides adequate specific protection against pathogens, among which also are viruses. The specificity of sIgA is determined by the immune response of the mother to previous infection, probably explaining the low rates of infection or milder symptoms of the infected breastfed infants of SARS-CoV–infected mothers. Concerning this phenomenon, the cases where infection is not protected against by breastfeeding should be considered. For example, in pertussis, the sIgA in the human milk of infected mothers was higher than in that of control subjects, but did not protect against infection of the infant, and therefore vaccination of pregnant women was recommended (79). The evidence to date on pregnant women infected by SARS-CoV-2 does not demonstrate a more severe or complex clinical picture than in the general infected population. Further investigation is needed to accumulate knowledge regarding anti-SARS-CoV-sIgA produced through breastfeeding for the neonate.

In view of the time-dependent protective effect of exclusive breastfeeding against viral infections, and the increased maternal contact of the breastfed infant compared with the infant receiving artificial or mixed feeding (41), and the high transmissibility of SARS-CoV, protective measures should be strictly observed for safeguarding the lactation process.

AUTHOR CONTRIBUTIONS

EV contributed to the conception, designed the review, and wrote the first draft. EV and GF collected the relevant literature and independently reviewed the studies, while GK further evaluated the studies where conflict was presented in the decision. EV, CM, LM, EB, and GK wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. The American Journal of Managed Care*A Timeline of COVID-19 Developments in 2020* (2020). AJMC. Available at: https://www.ajmc.com/view/a-timeline-of-covid19-developments-in-2020 [Accessed 2021, January 2].
2. World Health Organization. *WHO Coronavirus Disease (COVID-19) Dashboard* (2020). Available at: https://covid19.who.int/ [Accessed 2021, January 2].
3. World Mapper. *Births 2020*. (2020). Available at: https://worldmapper.org/maps/births-2020/ [Accessed 2021, January 2].
4. Shuai WS, Zhou X, Guang LX, Yan LY, Li W, Sharifu LM, et al. Experience of Clinical Management for Pregnant Women and Newborns with Novel Coronavirus Pneumonia in Tongji Hospital, China. *Curr Med Sci* (2020) 40 (2):285–9. doi: 10.1007/s11596-020-2174-4
5. Lalaguna Mallada P, Díaz-Gómez NM, Costa Romero M, San Feliciano Martín L, Gabarrell Guiu C. Impacto de la pandemia de Covid-19 en la lactancia y cuidados al nacimiento. Importancia de recuperar las buenas prácticas. *Rev Esp Salud Publica* (2020) 94.
6. PRISMA. [cited 2021 Jan 23]. http://www.prisma-statement.org/Protocols/Registration
7. Campbell M, McKenzie JE, Sowden A, Katikireddi SV, Brennan SE, Ellis S, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: Reporting guideline. *BMJ* (2020) 368:l6890. doi: 10.1136/bmj.l6890
8. *Breastfeeding and COVID-19*. [cited 2021 Jan 23]. Available at: https://www.who.int/news-room/commentaries/detail/breastfeeding-and-covid-19.
9. UNICEF. *Infant and Young Child Feeding in the Context of COVID-19* (2020). Available at: https://www.unicef.org/media/68281/file/IYCF-Programming-COVID19-Brief.pdf. [Accessed 2021, January 2].
10. Davanzo R, Moro G, Sandri F, Agosti M, Moretti C, Mosca F. Breastfeeding and coronavirus disease-2019: Ad interim indications of the Italian Society of Neonatology endorsed by the Union of European Neonatal & Perinatal Societies. *Matern Child Nutr* (2020) 16(3):e13010. doi: 10.1111/mcn.13010
11. Coronavirus Disease (COVID-19) and Breastfeeding | Breastfeeding | CDC.
12. Dimopoulou D, Triantafyllidou P, Daskalaki A, Syridou G, Papaevangelou V. Breastfeeding during the novel coronavirus (COVID-19) pandemic: guidelines and challenges. *J Matern Neonatal Med* (2020) 1–7. doi: 10.1080/14767058.2020.1838481

13. Lowe B, Bopp B. COVID-19 vaginal delivery - A case report. *Aust N Z J Obstet Gynaecol* (2020) 60(3):465–6. doi: 10.1111/ajo.13173
14. Pereira A, Cruz-Melguizo S, Adrien M, Fuentes L, Marin E, Forti A, et al. Breastfeeding mothers with COVID-19 infection: a case series. *Int Breastfeed J* (2020) 15(1):69. doi: 10.1186/s13006-020-00314-8
15. Jing LG, Zhao H. Can SARS-CoV-2-infected women breastfeed after viral clearance? *J Zhejiang Univ Sci B* (2020) 21(5):405–7. doi: 10.1631/jzus.B2000095
16. Bastug A, Hanifehnezhad A, Tayman C, Ozkul A, Ozbay O, Kazancioglu S, et al. Virolactia in an Asymptomatic Mother with COVID-19. *Breastfeed Med Off J Acad Breastfeed Med* (2020) 15(8):488–91. doi: 10.1089/bfm.2020.0161
17. Gabriel MÁM, Martínez AMM, Martínez MEM, Pedroche JA. Negative Transmission of SARS-CoV-2 to Hand-Expressed Colostrum from SARS-CoV-2-Positive Mothers. *Breastfeed Med* (2020) 15(8):492–4. doi: 10.1089/bfm.2020.0183
18. Chu H, Li J, Yan J, Bai T, Schnabl B, Zou L, et al. Persistent SARS-CoV-2 RNA Positive in Feces but Negative in Breastmilk: A Case Report of COVID-19 in a Breastfeeding Patient. *Front Med* (2020) 7(December):1–5. doi: 10.3389/fmed.2020.562700
19. Liu X, Zhou L, Zhu Y. Report on a lactating patient with COVID-19. *Infection* (2020) 100:(0123456789). doi: 10.1007/s15010-020-01532-2
20. Feng Y, Li J, Wu J, Xia M, Xu W. Insight from a COVID-19-Infected Lactating Mother with A Healthy Breastfed Infant. *Front Med Case Rep* (2020) 01 (05):1–6. doi: 10.47746/FMCR.2020.1502
21. Perrone S, Giordano M, Meoli A, Deolmi M, Marinelli F, Messina G, et al. Lack of viral transmission to preterm newborn from a COVID-19 positive breastfeeding mother at 11 days postpartum. *J Med Virol* (2020) 92(11):2346–7. doi: 10.1002/jmv.26037
22. Liu W, Wang J, Li W, Zhou Z, Liu S, Rong Z. Clinical characteristics of 19 neonates born to mothers with COVID-19. *Front Med* (2020) 14(2):193–8. doi: 10.1007/s11684-020-0772-y
23. Dong Y, Chi X, Hai H, Sun L, Zhang M, Xie WF, et al. Antibodies in the breast milk of a maternal woman with COVID-19. *Emerg Microbes Infect* (2020) 9 (1):1467–9. doi: 10.1080/22221751.2020.1780952
24. Zhu C, Liu W, Su H, Li S, Shereen MA, Lv Z, et al. Breastfeeding Risk from Detectable Severe Acute Respiratory Syndrome Coronavirus 2 in Breastmilk. *J Infect* (2020) 81(3):452–82. doi: 10.1016/j.jinf.2020.06.001
25. Dong L, Tian J, He S, Zhu C, Wang J, Liu C, et al. Possible Vertical Transmission of SARS-CoV-2 from an Infected Mother to Her Newborn. *JAMA - J Am Med Assoc* (2020) 323(18):1846–8. doi: 10.1001/jama.2020.4621
26. Li Y, Zhao R, Zheng S, Chen X, Wang J, Sheng X, et al. Lack of vertical transmission of severe acute respiratory syndrome Coronavirus 2, China. *Emerg Infect Dis* (2020) 26(6):1335–6. doi: 10.3201/eid2606.200287
27. Yu Y, Li Y, Hu Y, Li B, Xu J. Breastfed 13 month-old infant of a mother with COVID-19 pneumonia: a case report. *Int Breastfeed J* (2020) 15(1):68. doi: 10.1186/s13006-020-00305-9
28. Groß R, Conzelmann C, Müller JA, Stenger S, Steinhart K, Kirchhoff F, et al. Detection of SARS-CoV-2 in human breastmilk. *Lancet* (2020) 395 (10239):1757–8. doi: 10.1016/S0140-6736(20)31181-8
29. Tam PCK, Ly KM, Kernich ML, Spurrier N, Lawrence D, Gordon DL, et al. Detectable Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Human Breast Milk of a Mildly Symptomatic Patient With Coronavirus Disease 2019 (COVID-19). *Clin Infect Dis* (2020) 60:465–6. doi: 10.1093/cid/ciaa673/5848850
30. Salvatori G, De Rose DU, Concato C, Alario D, Olivini N, Dotta A, et al. Managing COVID-19-Positive Maternal-Infant Dyads: An Italian Experience. *Breastfeed Med* (2020) 15(5):347–8. doi: 10.1089/bfm.2020.0095
31. Phadke AK, Kumble A, Varghese A, Sherigar B, Reddy R. A case report of neonatal coronavirus disease-19 infection in South India. *Indian J Case Rep* (2020) 6(8):461–3. doi: 10.32677/IJCR.2020.v06.i08.014
32. Kirtsman M, Diambomba Y, Poutanen SM, Malinowski AK, Vlachodimitropoulou E, Parks WT, et al. Probable congenital sars-cov-2 infection in a neonate born to a woman with active sars-cov-2 infection. *Cmaj* (2020) 192(24):E647–50. doi: 10.1503/cmaj.200821
33. Hinojosa-Velasco A, de Oca PVBM, García-Sosa LE, Mendoza-Durán JG, Pérez-Méndez MJ, Dávila-González E, et al. A case report of newborn infant with severe COVID-19 in Mexico: Detection of SARS-CoV-2 in human breast milk and stool. *Int J Infect Dis* (2020) 100:21–4. doi: 10.1016/j.ijid.2020.08.055
34. Wang S, Guo L, Chen L, Liu W, Cao Y, Zhang J, et al. A case report of neonatal 2019 coronavirus disease in China. *Clin Infect Dis* (2020) 71(15):853–7. doi: 10.1093/cid/ciaa225
35. Fan C, Lei D, Fang C, Li C, Wang M, Liu Y, et al. Perinatal Transmission of 2019 Coronavirus Disease–Associated Severe Acute Respiratory Syndrome Coronavirus 2: Should We Worry? *Clin Infect Dis* (2020) 395:1757–8. doi: 10.1093/cid/ciaa226/5809260
36. Saleh Elhalik M. Clinical profile of neonates delivered from mothers with confirmed COVID-19 infection: An experience from a Tertiary Perinatal Care Center in Dubai, UAE. *J Pediatr Neonatal Care* (2020) 10(4):128–30. doi: 10.15406/jpnc.2020.10.00427
37. Ronchi A, Pietrasanta C, Zavattoni M, Saruggia M, Schena F, Sinelli MT, et al. Evaluation of Rooming-in Practice for Neonates Born to Mothers with Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Italy. *JAMA Pediatr* (2020) 4:405–6. doi: 10.1001/jamapediatrics.2020.5086
38. Patil UP, Maru S, Krishnan P, Carroll-Bennett R, Sanchez J, Noble L, et al. Newborns of COVID-19 mothers: short-term outcomes of colocating and breastfeeding from the pandemic's epicenter. *J Perinatol* (2020) 40(10):1455–8. doi: 10.1038/s41372-020-0765-3
39. Bertino E, Moro GE, De Renzi G, Viberti G, Cavallo R, Coscia A, et al. SARS-COV-2 IN HUMAN BREAST MILK AND NEONATAL OUTCOME - A. *Lancet* (2020) 61:2–8. doi: 10.2139/ssrn.3611974
40. Le HT, Nguyen LV, Tran DM, Do HT, Tran HT, Le YT, et al. The first infant case of COVID-19 acquired from a secondary transmission in Vietnam. *Lancet Child Adolesc Heal* (2020) 4(5):405–6. doi: 10.1016/S2352-4642(20)30091-2
41. Pandolfi E, Gesualdo F, Rizzo C, Carloni E, Villani A, Concato C, et al. Breastfeeding and respiratory infections in the first 6 months of life: A case control study. *Front Pediatr* (2019) 7(APR):1582–90. doi: 10.3389/fped.2019.00152
42. Newburg DS, Walker WA. Protection of the neonate by the innate immune system of developing gut and of human milk [Internet]. *Pediatr Res* (2007) 61:2–8. doi: 10.1203/01.pdr.0000250274.68571.18
43. Andreas NJ, Kampmann B, Mehring Le-Doare K. Human breast milk: A review on its composition and bioactivity. In: *Early Human Development*, vol. 91. US: Elsevier Ireland Ltd (2015). p. 629–35.
44. Ahn S, Corwin EJ. The association between breastfeeding, the stress response, inflammation, and postpartum depression during the postpartum period: Prospective cohort study. *Int J Nurs Stud* (2015) 52(10):1582–90. doi: 10.1016/j.ijnurstu.2015.05.017
45. Kendall-Tackett K. A new paradigm for depression in new mothers: The central role of inflammation and how breastfeeding and anti-inflammatory treatments protect maternal mental health. *Int Breastfeed J* (2007) 2:419–45. doi: 10.1186/1746-4358-2-6
46. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nat Immunol* (2015) 16:343–53. doi: 10.1038/ni.3123
47. Alexander KL, Targan SR, Elson CO. Microbiota activation and regulation of innate and adaptive immunity. *Immunol Rev* (2014) 260:206–20. doi: 10.1111/imr.12180
48. Pacheco AR, Barile D, Underwood MA, Mills DA. The impact of the milk glycobiome on the neonate gut microbiota. *Annu Rev Anim Biosci* (2015) 3:419–45. doi: 10.1146/annurev-animal-022114-111112
49. Oddy WH, Kendall GE, Li J, Jacoby P, Robinson M, de Klerk NH, et al. The Long-Term Effects of Breastfeeding on Child and Adolescent Mental Health: A Pregnancy Cohort Study Followed for 14 Years. *J Pediatr* (2010) 156 (4):568–74. doi: 10.1016/j.jpeds.2009.10.020
50. Oddy WH. A review of the effects of breastfeeding on respiratory infections, atopy, and childhood asthma. *J Asthma J Asthma* (2004) 41:605–21. doi: 10.1081/JAS-200026402
51. Legrand D. Overview of Lactoferrin as a Natural Immune Modulator. *J Pediatr* (2016) 173:S10–5. doi: 10.1016/j.jpeds.2016.02.071
52. Yin C, Wong JH, Ng TB. Recent Studies on the Antimicrobial Peptides Lactoferricin and Lactoferrampin. *Curr Mol Med* (2014) 14(9):1139–54. doi: 10.2174/1566524014666141015151749
53. Mohandas S, Pannaraj PS. Beyond the Bacterial Microbiome: Virome of Human Milk and Effects on the Developing Infant. *Nestle Nutr Inst Workshop Ser* (2020) 94:86–93. doi: 10.1159/000504997

54. Lang J, Yang N, Deng J, Liu K, Yang P, Zhang G, et al. Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. *PloS One* (2011) 6(8):96–104. doi: 10.1371/journal.pone.0023710
55. Henrick BM, Yao XD, Nasser L, Roozrogousheh A, Rosenthal KL. Breastfeeding behaviors and the innate immune system of human milk: Working together to protect infants against inflammation, HIV-1, and other infections [Internet]. Vol. 8, Frontiers in Immunology. *Front Media SA* (2017) 5:1. doi: 10.3389/fimmu.2017.01631
56. Krawczyk A, Lewis MG, Venkatesh BT, Nair SN. Effect of Exclusive Breastfeeding on Rotavirus Infection among Children. *Indian J Pediatr* (2016) 83(3):220–5. doi: 10.1007/s12098-015-1854-8
57. Habte HH, Kotwal GJ, Lotz ZE, Tyler MG, Abrahams M, Rodriques J, et al. Antiviral activity of purified human breast milk mucin. *Neonatology* (2007) 92 (2):96–104. doi: 10.1159/000100808
58. Bode L, McGuire M, Rodriguez JM, Geddes DT, Hassiotou F, Hartmann PE, et al. It's alive: Microbes and cells in human milk and their potential benefits to mother and infant. *Adv Nutr* (2014) 36:571–3. doi: 10.3945/an.114.006643
59. Hassiotou F, Geddes DT. Immune cell-mediated protection of the mammary gland and the infant during breastfeeding. *Adv Nutr* (2015) 6(3):267–75. doi: 10.3945/an.114.007377
60. Fouda GG, Martinez DR, Swamy GK, Permar SR. The Impact of IgG Transplacental Transfer on Early Life Immunity. *ImmunoHorizons* (2018) 2 (1):14–25. doi: 10.4049/immunohorizons.1700057
61. Hanson L, Hahn-Zoric M, Berndes M, Ashraf R, Herias V, Jalil F, et al. Breast feeding: Overview and breast milk immunology. *Pediatr Int* (1994) 36(5):557–61. doi: 10.1111/j.1442-200X.1994.tb03246.x
62. Czosnykowska-Łukacka M, Lis-Kuberka J, Królak-Olejnik B, Orczyk-Pawiłowicz M. Changes in Human Milk Immunoglobulin Profile During Prolonged Lactation. *Front Pediatr* (2020) 8:428. doi: 10.3389/fped.2020.00428
63. Van De Perre P. Transfer of antibody via mother's milk. In: *Vaccine*. Philadelphia, PA, USA: Elsevier BV (2003). p. 3374–6.
64. Mazur NI, Horsley NM, Englund JA, Nederend M, Magaret A, Kumar A, et al. Breast Milk Prefusion F Immunoglobulin G as a Correlate of Protection Against Respiratory Syncytial Virus Acute Respiratory Illness. *J Infect Dis* (2018) 219(1):59–67. doi: 10.1093/infdis/jiy477/5068768
65. Wetzke M, Schwerk N. Respiratory syncytial virus infections. In: *Pneumologe*, vol. 16. US: Springer Verlag (2019). p. 232–41.
66. Gross SJ, Buckley RH, Wakil SS, McAllister DC, David RJ, Faix RG. Elevated IgA concentration in milk produced by mothers delivered of preterm infants. *J Pediatr* (1981) 99(3):389–93. doi: 10.1016/S0022-3476(81)80323-X
67. Hassiotou F, Hepworth AR, Metzger P, Tat Lai C, Trengove N, Hartmann PE, et al. Maternal and infant infections stimulate a rapid leukocyte response in breastmilk. *Clin Transl Immunol* (2009) 2(4):131–4. doi: 10.1038/cti.2013.1
68. Riskin A, Almog M, Peri R, Halasz K, Srugo I, Kessel A. Changes in immunomodulatory constituents of human milk in response to active infection in the nursing infant. *Pediatr Res* (2012) 71(2):220–5. doi: 10.1038/pr.2011.34
69. Melendi GA, Coviello S, Bhat N, Zea-Hernandez J, Ferolla FM, Polack FP. Breastfeeding is associated with the production of type i interferon in infants infected with influenza virus. *Acta Paediatr Int J Paediatr* (2010) 99(10):1517–21. doi: 10.1111/j.1651-2227.2010.01862.x
70. Libster R, Hortoneda JB, Laham FR, Casellas JM, Israele V, Polack NR, et al. Breastfeeding prevents severe disease in full term female infants with acute respiratory infection. *Pediatr Infect Dis J* (2009) 28(2):131–4. doi: 10.1097/INF.0b013e31818a8a82
71. Ruiz L, García-Carral C, Rodriguez JM. Unfolding the human milk microbiome landscape in the omicsera. *Front Microbiol* (2019) 10 (JUN):644–6. doi: 10.3389/fmicb.2019.01378
72. Zimmermann P, Curtis N. Coronavirus infections in children including COVID-19: An overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. *Pediatr Infect Dis J* (2020) 39:355–68. doi: 10.1097/INF.0000000000002660
73. Toubiana J, Poirault C, Corsia A, Bajolle F, Fourgeaud J, Angoulvant F, et al. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. *BMJ* (2020) 369:m2094. doi: 10.1136/bmj.m2094
74. Wagner CL. A Commentary on the Review Entitled, "A Scoping Review of the Human Milk Microbiome" by Groer et al. *J Hum Lact* (2020) 36(4):644–6. doi: 10.1177/0890334420931835
75. *Protection, promotion and support of breastfeeding in Europe: a blueprint for action Promotion of Breastfeeding in Europe*. Philadelphia, PA, USA (2020).
76. Verd S, Ponce-Taylor J, Ginovart G. Vertical Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 from the Mother to the Infant. *JAMA Pediatr* (2020) 174:1003–4. doi: 10.1001/jamapediatrics.2020.2135
77. Peroni DG, Fanos V. Persistent SARS-CoV-2 RNA Positive in Feces but Negative in Breastmilk: A Case Report of COVID-19 in a Breastfeeding Patient. *Acta Paediatr Int J Paediatr* (2020) 109:2139–40. doi: 10.1111/apa.15417
78. Green J, Petty J, Bromley P, Walker K, Jones L. COVID-19 in babies: Knowledge for neonatal care. *J Neonatal Nurs* (2020) 26:239–46. doi: 10.1016/j.jnn.2020.06.005
79. Pandolfi E, Gesualdo F, Carloni E, Villani A, Midulla F, Carsetti R, et al. Does breastfeeding protect young infants from pertussis? Case-control study and immunologic evaluation. *Pediatr Infect Dis J* (2017) 36(3):e48–53. doi: 10.1097/INF.0000000000001418

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Vassilopoulou, Feketea, Koumbi, Mesiari, Berghea and Konstantinou. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

BRIEF RESEARCH REPORT
published: 02 June 2021
doi: 10.3389/fnut.2021.689296

Clinical Characteristics and Survival Analysis in Frequent Alcohol Consumers With COVID-19

Ricardo Wesley Alberca[1], Paula Ordonhez Rigato[2], Yasmim Álefe Leuzzi Ramos[1], Franciane Mouradian Emidio Teixeira[1,3], Anna Cláudia Calvielli Branco[1,3], Iara Grigoletto Fernandes[1], Anna Julia Pietrobon[1,3], Alberto Jose da Silva Duarte[1], Valeria Aoki[1], Raquel Leão Orfali[1] and Maria Notomi Sato[1]*

[1] Laboratorio de Dermatologia e Imunodeficiencias (LIM-56), Departamento de Dermatologia, Faculdade de Medicina FMUSP, Instituto de Medicina Tropica, Universidade de São Paulo, São Paulo, Brazil, [2] Technical Division of Medical Biology, Immunology Center, Adolfo Lutz Institute, São Paulo, Brazil, [3] Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection can generate a systemic disease named coronavirus disease–2019 (COVID-19). Currently, the COVID-19 pandemic has killed millions worldwide, presenting huge health and economic challenges worldwide. Several risk factors, such as age, co-infections, metabolic syndrome, and smoking have been associated with poor disease progression and outcomes. Alcohol drinking is a common social practice among adults, but frequent and/or excessive consumption can mitigate the anti-viral and anti-bacterial immune responses. Therefore, we investigated if patients with self-reported daily alcohol consumption (DAC) presented alteration in the immune response to SARS-CoV-2. We investigated 122 patients with COVID-19 (101 male and 46 females), in which 23 were patients with DAC (18 men and 5 women) and 99 were non-DAC patients (58 men and 41 women), without other infections, neoplasia, or immunodeficiencies. Although with no difference in age, patients with DAC presented an increase in severity-associated COVID-19 markers such as C-reactive protein (CRP), neutrophil count, and neutrophil-to-lymphocyte ratio. In addition, patients with DAC presented a reduction in the lymphocytes and monocytes counts. Importantly, the DAC group presented an increase in death rate in comparison with the non-DAC group. Our results demonstrated that, in our cohort, DAC enhanced COVID-19-associated inflammation, and increased the number of deaths due to COVID-19.

Keywords: COVID-19, SARS-CoV-2, alcohol, consumption, inflammation

OPEN ACCESS

Edited by:
Ioannis Zabetakis,
University of Limerick, Ireland

Reviewed by:
Pan Liu,
Northwestern University, United States
Ivan Milos Stankovic,
University of Belgrade, Serbia

****Correspondence:***
Ricardo Wesley Alberca
ricardowesley@gmail.com

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Nutrition

Received: *31 March 2021*
Accepted: *29 April 2021*
Published: *02 June 2021*

Citation:
Alberca RW, Rigato PO, Ramos YÁL, Teixeira FME, Branco ACC, Fernandes IG, Pietrobon AJ, Duarte AJdS, Aoki V, Orfali RL and Sato MN (2021) Clinical Characteristics and Survival Analysis in Frequent Alcohol Consumers With COVID-19. Front. Nutr. 8:689296. doi: 10.3389/fnut.2021.689296

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection can generate a severe systemic and multi-organ disease named coronavirus disease-2019 (COVID-19). SARS-CoV-2 has infected and killed millions worldwide (1). Several risk factors, such as co-infections (2), old age, chronic respiratory diseases, and comorbidities (3) have been associated with poor outcomes of COVID-19.

During the pandemic, reports have highlighted the increase in alcohol consumption and the increased risk to trigger or exacerbate depressive and anxious episodes (4). Excessive consumption of alcohol can impair the immune response (5) present a deleterious effect in chronic viral infection (6, 7) and increase the severity and recovery time for respiratory infections (8–11).

The use of substances such as tobacco smoking is an established risk factor for severe COVID-19 (12), but few reports have identified people with alcohol use disorder or daily alcohol consumption (DAC) in their cohorts, with reports identifying no influence on neither drinking alcohol on severity nor death rates in patients with COVID-19 (13, 14). Therefore, we aimed to perform an investigation in our cohort to assess if DAC could influence the disease course and outcome of COVID-19.

METHODS

Patients were recruited fromat a special ward in the university hospital (Hospital das Clinicas da Universidade de São Paulo - HCFMUSP) for moderate and severe patients with COVID-19. Inclusion criteria consisted of a positive diagnosise for COVID-19 by the detection of SARS-CoV-2 RNA in nasopharyngeal swab by reverse-transcriptase PCR (RT-PCR). DAC was determined by self-report, some patients were clinically diagnosed with alcohol-dependency prior to COVID-19, and some reported necessity for DAC of over two drinks.

Patients with and without DAC with type 2 Diabetes Mellitus (DM) and/or systemic arterial hypertension (SAH) were also included. The exclusion criteria were the presence of co-infections, renal or heart diseases, immunodeficiencies, or neoplasia. This investigation was approved by the Ethics Committee of HCFMUSP with approval for the usage of digital data from patients (no. 30800520.7.0000.0068-2020) and was performed in accordance with the 2013 revision of the Declaration of Helsinki. On the first day after hospitalization, EDTA plasma samples were obtained from a single venipuncture, and laboratory analysis was performed at the Central Laboratory of HCFMUSP (Divisão de Laboratório Central—HCFMUSP). Statistical analysis for age and laboratory data was performed using the Mann-Whitney test for comparisons between groups. Survival curve statistical analysis was performed using the Log-rank (Mantel-Cox) test and the Gehan-Breslow-Wilcoxon test with GraphPad Prism 8 software (GraphPad Software, Inc., San Diego, CA).

Were included in this investigation 23 individuals with DAC (18 men and 5 women) and 99 patients with non-DAC (58 men and 41 women) that were hospitalized between June 2020 and 1 December 2020.

RESULTS

From the 23 DAC patients with COVID-19, 11 had moderate level COVID-19 and were admitted into the general ward (GW) (±48%) and 12 were admitted into the intensive care unit (ICU) (±52%) and needed assisted mechanical ventilation. It is important to highlight that among the 23 DAC patients with COVID-19, one patient has DM (±4%), six patients have SAH (±26%), and five patients have both DM and SAH (±21%).

From the total 99 non-DAC patients with COVID-19, 52 were admitted into the GW (±52%) and 47 were admitted into the ICU (±47%). Among the 99 patients with COVID-19: 4 patients have DM (±4%), 23 patients have SAH (±23%), and 24 patients have both DM and SAH (±24%).

The Patients with non-DAC and DAC did not present any age difference or significant statistical differences in the levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, indirect bilirubin, glutamyl transferase gamma, creatinine, lactate dehydrogenase, total protein and fractions, albumin, globulin, urea, and D-dimer, prothrombin time (**Table 1**). Nevertheless, both non-DAC and DAC presented alterations in alanine aminotransferase, aspartate aminotransferase, direct bilirubin, glutamyl transferase gamma, creatinine, lactate dehydrogenase, C-reactive protein (CRP), urea, D-dimer, and the prothrombin time were outside the considered normal range (**Table 1**).

We observed an increase in CRP and a reduction in platelets and activated partial thromboplastin time in patients with DAC in comparison to those with non-DAC (**Table 1**). This indicates an overall increase in COVID-19 biomarkers, such as urea, D-dimer, and creatinine levels in both groups, but a further increase in CRP levels showed a decrease in platelet counts, which was previously associated with COVID-19 severity (15, 16). The CRP level, especially at early hospitalization, has been associated with the extension of COVID-19-induced lung injury and disease progression, and death (16, 17). Models for predicting the disease outcome of COVID-19 are based on inflammatory markers on the first day of hospitalization (18).

Interestingly, although the total leukocyte count was similar among groups (**Figure 1A**), DAC groups presented an increase in the neutrophil and neutrophil to lymphocyte ratio (**Figures 1B,D**). Neutrophil count and the neutrophil-to lymphocyte-ratio are also established hallmarks of COVID-19 and are associated with disease severity (19, 20). An increase in neutrophils, as well as neutrophil migration to the lungs, is associated with lung inflammation and immunothrombosis in patients with COVID-19 (21). The neutrophil- to lymphocyte-ratio is a widely used maker for COVID-19 prognoses (19, 20, 22, 23), but the increase in the DAC group was due to both an increase in the neutrophil count (**Figure 1B**) and a reduction in the lymphocytes count in relation to the non-DAC group (**Figure 1C**).

Lymphopenia, the reduction in the number of lymphocytes, is common in patients with COVID-19, with a significant reduction in T helper cells, T cytotoxic cells, Natural Killer, and B cells, impacting the anti-SARS-CoV-2 and other co-infections (2, 24, 25). In fact, lymphopenia is also associated with the severity and recovery from COVID-19 (26).

In our cohort, the number of monocytes in patients with DAC was reduced in comparison with patients with non-DAC (**Figure 1E**). Currently, reports have identified phenotypical alteration on monocytes of patients with COVID-19 (27), with a reduction in the antigen-presenting potential and an increase in the pro-inflammatory markers (28). Nevertheless, the reduction in monocytes of patients with DAC may also indicate

TABLE 1 | Patients' clinical characteristics and laboratory data.

	Non-DAC ($N = 99$)		DAC ($N = 23$)			
	Mean	**SEM**	**Mean**	**SEM**	**Reference numbers**	***p*-value**
Male/Female	58/41	18/5				
Age (years)	55.08	1.335	56.38	3.135	–	0.2963
Laboratory data						
Alanine aminotransferase (U/L)	**48.43**	**5.797**	**48.13**	**7.436**	**<41**	**0.9238**
Aspartate aminotransferase (U/L)	**43.44**	**4.149**	**51.42**	**9.261**	**<37**	**0.7681**
Alkaline phosphatase (U/L)	115	18.89	107.2	15.78	40–129	0.7865
Total bllirubin (mg/dL)	0.4538	0.06193	0.4533	0.0485	0.2–1.0	0.3681
Direct bilirubin (mg/dL)	**0.3578**	**0.0604**	**0.3183**	**0.03358**	**<0.3**	**0.6342**
Indirect bilirubin (mg/dL)	0.09385	0.01291	0.03358	0.02393	0.1–0.6	0.2229
Glutamyl transferase gamma (U/L)	**164.9**	**30.59**	**156.6**	**19.33**	**8–61**	**0.4998**
Creatinine (mg/dL)	**2.12**	**0.2077**	**2.042**	**0.3794**	**0.7–1.2**	**0.9659**
Lactate dehydrogenase (U/L)	**457.9**	**33.48**	**451.2**	**55.76**	**135–225**	**0.4321**
C-reactive protein (mg/L)	**77.54****	**9.205**	**154.2****	**27.97**	**<5.0**	**0.0089**
Total protein and fractions (g/dL)	5.794	0.2328	6.37	0.2574	6.6–8.7	0.1698
Albumin (g/dL)	2.919	0.1046	3.255	0.1895	3.4–4.8	0.2643
Globulin (g/dL)	2.875	0.2065	3.085	0.1464	1.7–3.5	0.3153
Urea (mg/dL)	**75.13**	**5.447**	**85.17**	**12.7**	**10–50**	**0.5556**
D-dimer (ng/mL)	**1963**	**252.2**	**1497**	**175.5**	**<500**	**0.4949**
Platelets (x 10^3/mm^3)	277.7*	15.51	206.6*	21.89	150–400	0.0474
Prothrombin time (seconds)	**13.99**	**0.3263**	**13.08**	**0.3433**	**9.4–12.5**	**0.1133**
Activated partial thromboplastin time (seconds)	38.87*	2.408	28.84*	1.034	25.1–36.5	0.0169

*P values indicate differences between groups. *p < 0.05 and **p < 0.01 were considered statistically significance. Bold represents values outside reference range. Reference values from Divisão de Laboratorio Central do HC/FMUSP.*

a deficient immune response to SARS-CoV-2, as monocytes have a fundamental role in the immune response to pathogenic microorganisms (29).

We identified a reduction in both eosinophils and basophils in the DAC group in comparison with the non-DAC (**Figures 1F,G**). Low eosinophil and basophil counts have been associated with the worst anti-SARS-CoV-2 response (30).

We failed to identify differences in hospitalization time of the DAC group, which was 29.6 ± 6.1 days and the non-DAC group, which was 29.8 ± 3 days. It is noteworthy that a significant increase in death rate in the DAC group in comparison with the non-DAC group (**Figure 2**) [$p = 0.0417$ using the Log-rank (Mantel-Cox) test and $p = 0.0209$ using the Gehan-Breslow-Wilcoxon test]. During hospitalization, 8 patients from the DAC group and 14 patients from the non-DAC group passed away due to COVID-19 besides the efforts of the medical staff.

DISCUSSION

Excessive alcohol consumption can increase the severity and recovery time for infections (8). Several reports have highlighted the negative impact of excessive alcohol consumption on the bacterial respiratory infection (31) and viral respiratory infections such as respiratory syncytial virus (9) and influenza (10).

The increased risk for respiratory viral infections is due to the immune modulation caused by alcohol consumption (8, 10). A previous report identified an increase in neutrophils and tumor necrosis factor-α (TNF) and monocyte chemoattractant protein-1 in the lungs of mice after alcohol consumption (32). Importantly, neutrophil count and TNF levels are associated with severe COVID-19 infection (33). In our cohort, patients with DAC presented a significant increase in the neutrophil and neutrophil to lymphocyte ratio, indicating a possibility that the frequent or excessive alcohol could further increase the severity of the infection caused by SARS-CoV-2.

Another hallmark alteration in chronic alcohol consumption is the reduction in the number and activity of NK cells, T cells, and B cells, decreasing the overall immune response and antibody production (34). This study verified that patients with DAC suffered from a significant reduction in the lymphocyte count in comparison to non-DAC and, that alcohol consumption was further detrimental to the lymphopenia suffered by patients with COVID-19 (22).

Short-term alcohol consumption can also impair the monocyte immune response, with an increase in the production of inflammatory cytokines but a decrease in the activation of the inflammatory complex by microbial components (35). Monocytes isolated from individuals after alcohol consumption present an increase in the activation of the inflammatory complex (inflammasome) and production of pro-inflammatory cytokine, IL-1β (36).

Importantly, patients with COVID-19 also present phenotypical alteration on monocytes, increased pro-inflammatory response, and reduced antigen presentation

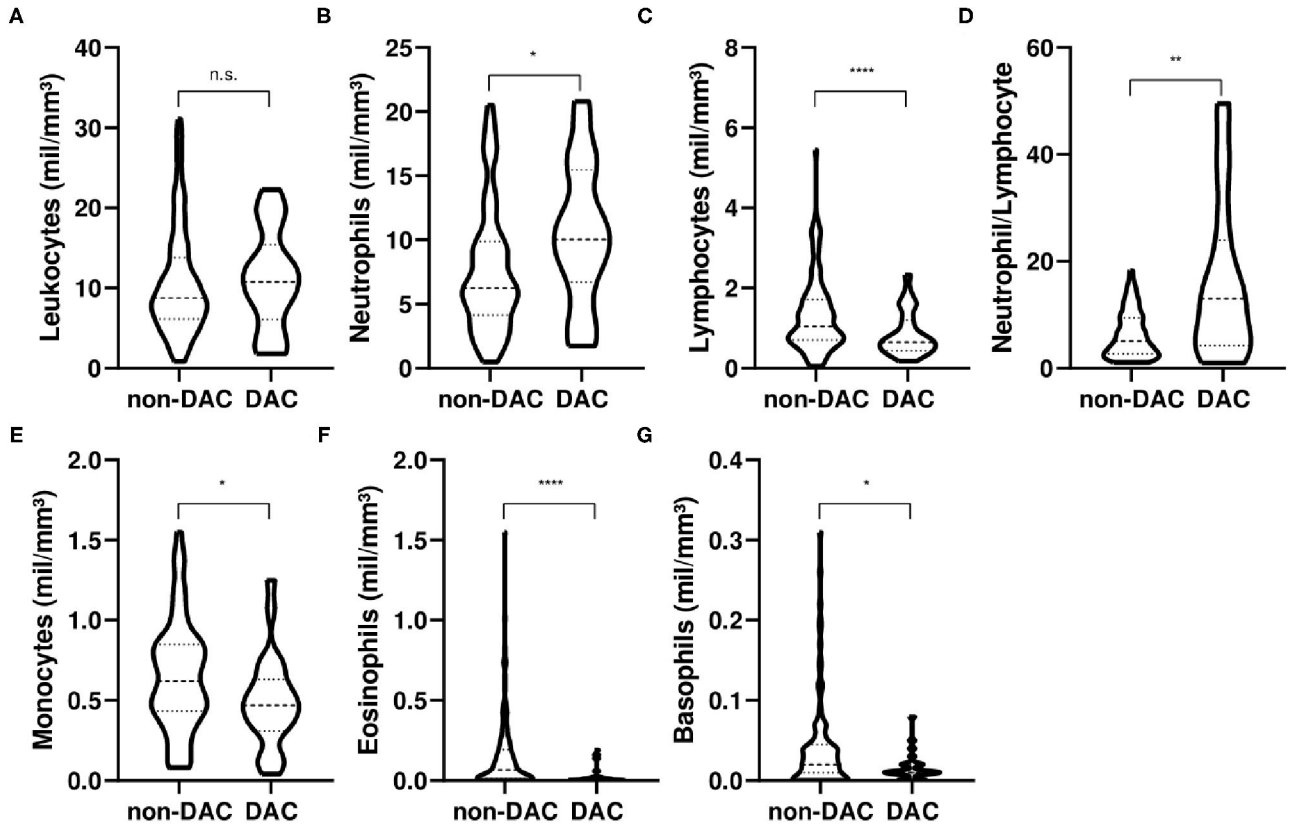


FIGURE 1 | Clinical features of patients with COVID-19. **(A)** Leukocyte counts, **(B)** Neutrophil counts, **(C)** Lymphocyte counts, **(D)** Neutrophil-to-lymphocyte ratio, **(E)** Monocyte counts, **(F)** Eosinophil counts, and **(G)** Basophil counts from the first day of hospitalization due to the SARS-CoV-2 infection. DAC, patients with self-reported daily alcohol consumption (DAC) and COVID-19; Non-DAC, patients without DAC and COVID-19. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, n.s., non-statistically significant difference. Mann-Whitney test used for comparisons. Data collected between June 1, 2020 and December 1, 2020.

potential (27, 28). Although the role of monocytes' on COVID-19 is still under investigation, alcohol consumption may exacerbate the dysregulated profile of monocyte on COVID-19.

The long-term consumption of alcoholic beverages has been associated with longer hospitalization and intensive care usage upon respiratory infections (8). In our cohort, we did not identify any differences in hospitalization time between DAC and non-DAC groups.

Alcohol consumption is also associated with a reduction of anti-inflammatory molecules, such as IL-10 (37), which may further aggravate the COVID-19-associated lung inflammation and established dysregulation in the anti-inflammatory immune response (24). Besides, alcohol consumption modulates the gut microbiome (38), and curbs the viral immune response (39), which may expose the individual to SARS-CoV-2 gastrointestinal infection (40, 41), leading to, increased gastrointestinal dysbiosis (42) and more severe respiratory complication due to COVID-19 (43).

Excessive alcohol consumption also impacts the absorption of nutrients, with a reduction in essential minerals and vitamins (44). Chronic alcohol consumption can impair vitamin A (45) and vitamin D (46) levels. Vitamin A deficiency reduces B-and T-cell immune response to influenza in mice (47, 48), and could further enhance the COVID-19-mediated immune dysregulation (24). Vitamin D deficiency has been associated with an increase in respiratory distress syndrome (49), COVID-19 severity (50), and deaths (51).

Although to our knowledge, no investigation on the angiotensin-converting enzyme (ACE2) expression in the lungs has been performed following alcohol consumption, investigations have identified that chronic alcohol consumption can increase the expression of ACE in the blood (52) and angiotensin II type 2 (AT2) receptor in the lungs (53). This leads to the hypothesis that alcohol consumption might modulate the ACE2 receptor, the entry receptor of SARS-CoV-2, in a similar way to other diseases, either increasing or reducing COVID-19 risk (54–56).

It is noteworthy that we identified an increase in mortality in the DAC group in comparison to the non-DAC group due to COVID-19 respiratory complications, which could indicate that frequent alcohol consumption may increase the death risk by COVID-19.

This study possesses two limitations: (1) the quantity of alcohol ingested by members of the DAC group may vary and

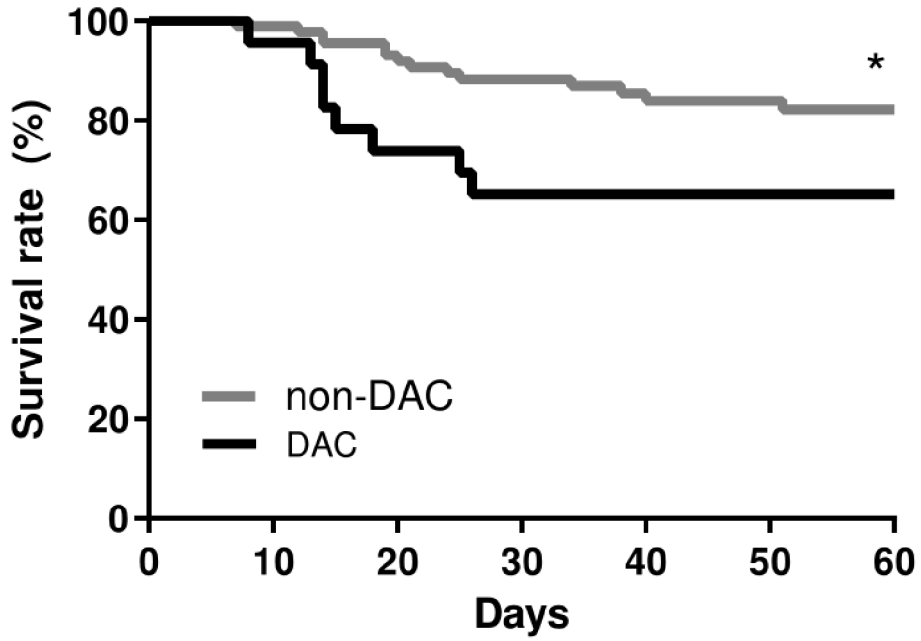


FIGURE 2 | Survival curve of patients with COVID-19. DAC, patients with self-reported DAC and COVID-19; Non-DAC, patients without DAC and COVID-19. *$p <$ 0.05 difference. Comparison using the Log-rank (Mantel-Cox) test and the Gehan-Breslow-Wilcoxon test. Data collected between June 1, 2020 and December 1, 2020.

could be an important variant; (2) it was impossible to isolate DAC from other comorbidities (SAH and/or DM) due to the number of individuals with DAC. Therefore, it is possible that the synergic effect of alcohol consumption with comorbidities influenced the COVID-19 outcome.

CONCLUSION

In summary, in our cohort, frequent alcohol consumption alters the COVID-19 clinical characteristics, further increasing pro-inflammatory markers and increasing the mortality risk due to COVID-19-associated complications. Due to the limited number of patients, further investigations should explore the possible synergic effect of alcohol consumption with other comorbidities on COVID-19 severity and mortality.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo—HCFMUSP with approval for the usage of digital data from patients (no. 30800520.7.0000.0068-2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors contributed to data collection, analysis, write and review of the article, and approved the submitted version.

FUNDING

This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Grant Numbers: 19/02679-7, 17/18199-9. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES: 88887.503842/2020-00.

REFERENCES

1. WHO Coronavirus Disease (COVID-19). *Dashboard*. WHO Coronavirus Disease (COVID-19) Dashboard. Available online at: https://covid19.who.int/ (accessed September 21, 2020).
2. Alberca RW, Yendo TM, Leuzzi Ramos YÁ, Fernandes IG, Oliveira LM, Teixeira FME, et al. Case report: COVID-19 and Chagas disease in two coinfected patients. *Am J Trop Med Hyg.* (2020) 103:2353–6. doi: 10.4269/ajtmh.20-1185
3. Jakhmola S, Indari O, Baral B, Kashyap D, Varshney N, Das A, et al. Comorbidity assessment is essential during COVID-19 treatment. *Front Physiol.* (2020) 11:984. doi: 10.3389/fphys.2020.00984

4. Garcia LP, Sanchez ZM. Alcohol consumption during the COVID-19 pandemic: a necessary reflection for confronting the situation. *Cad Saude Publ.* (2020) 36:e00124520. doi: 10.1590/0102-311X00124520
5. Sarkar D, Jung MK, Wang HJ. Alcohol and the immune system. *Alcohol Res Curr Rev.* (2015) 298:543–4. doi: 10.1136/bmj.298.6673.543
6. Monnig MA. Immune activation and neuroinflammation in alcohol use and HIV infection: evidence for shared mechanisms. *Am J Drug Alcohol Abuse.* (2017) 43:7–23. doi: 10.1080/00952990.2016.1211667
7. Khan KN, Yatsuhashi H. Effect of alcohol consumption on the progression of hepatitis C virus infection and risk of hepatocellular carcinoma in Japanese patients. *Alcohol Alcohol.* (2000) 35:286–95. doi: 10.1093/alcalc/35.3.286
8. Saitz R, Ghali WA, Moskowitz MA. The impact of alcohol-related diagnoses on pneumonia outcomes. *Arch Intern Med.* (1997) 157:1446–52. doi: 10.1001/archinte.157.13.1446
9. Wyatt TA, Bailey KL, Simet SM, Warren KJ, Sweeter JM, DeVasure JM, et al. Alcohol potentiates RSV-mediated injury to ciliated airway epithelium. *Alcohol.* (2019) 80:17–24. doi: 10.1016/j.alcohol.2018.07.010
10. Greenbaum A, Chaves SS, Perez A, Aragon D, Bandyopadhyay A, Bennett N, et al. Heavy alcohol use as a risk factor for severe outcomes among adults hospitalized with laboratory-confirmed influenza, 2005-2012. *Infection.* (2014) 42:165–70. doi: 10.1007/s15010-013-0534-8
11. Happel KI, Nelson S. Alcohol, immunosuppression, and the lung. *Proc Am Thorac Soc.* (2005) 2:428–32. doi: 10.1513/pats.200507-065JS
12. Alberca RW, Lima JC, de Oliveira EA, Gozzi-Silva SC, Ramos YÁL, de Andrade MMS, et al. COVID-19 disease course in former smokers, smokers and COPD patients. *Front Physiol.* (2021) 11:637627. doi: 10.3389/fphys.2020.637627
13. Zhong R, Chen L, Zhang Q, Li B, Qiu Y, Wang W, et al. Which factors, smoking, drinking alcohol, betel quid chewing, or underlying diseases, are more likely to influence the severity of COVID-19? *Front Physiol.* (2020) 11:623498. doi: 10.3389/FPHYS.2020.623498
14. Dai M, Tao L, Chen Z, Tian Z, Guo X, Allen-Gipson DS, et al. Influence of cigarettes and alcohol on the severity and death of COVID-19: a multicenter retrospective study in Wuhan, China. *Front Physiol.* (2020) 11:588553. doi: 10.3389/FPHYS.2020.588553
15. Bongiovanni D, Klug M, Lazareva O, Weidlich S, Biasi M, Ursu S, et al. SARS-CoV-2 infection is associated with a pro-thrombotic platelet phenotype. *Cell Death Dis.* (2021) 12:1–10. doi: 10.1038/s41419-020-03333-9
16. Wang L. C-reactive protein levels in the early stage of COVID-19. *Med Mal Infect.* (2020) 50:332–4. doi: 10.1016/j.medmal.2020.03.007
17. Sharifpour M, Rangaraju S, Liu M, Alabyad D, Nahab FB, Creel-Bulos CM, et al. C-Reactive protein as a prognostic indicator in hospitalized patients with COVID-19. *PLoS ONE.* (2020) 15:e0242400. doi: 10.1371/journal.pone.0242400
18. Yildiz H, Yombi JC, Castanares-Zapatero D. Validation of a risk score to predict patients at risk of critical illness with COVID-19. *Infect Dis.* (2020) 53:137–8. doi: 10.1080/23744235.2020.1823469
19. Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, et al. Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. *J Transl Med.* (2020) 18:206. doi: 10.1186/s12967-020-02374-0
20. Lian J, Jin C, Hao S, Zhang X, Yang M, Jin X, et al. High neutrophil-to-lymphocyte ratio associated with progression to critical illness in older patients with COVID-19: a multicenter retrospective study. *Aging.* (2020) 12:13849–59. doi: 10.18632/aging.103582
21. Middleton EA, He X-Y, Denorme F, Campbell RA, Ng D, Salvatore SP, et al. Neutrophil extracellular traps (NETs) contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. *Blood.* (2020) 136:1169–79. doi: 10.1182/blood.2020007008
22. Alberca RW, de Andrade MMS, Castelo Branco ACC, Pietrobon AJ, Pereira NZ, Fernandes IG, et al. Frequencies of CD33+ CD11b+ HLA-DR- CD14- CD66b+ and CD33+ CD11b+ HLA-DR- CD14+ CD66b- cells in peripheral blood as severity immune biomarkers in COVID-19. *Front Med.* (2020) 7:580677. doi: 10.3389/FMED.2020.580677
23. Zhang B, Zhou X, Zhu C, Song Y, Feng F, Qiu Y, et al. Immune phenotyping based on the neutrophil-to-lymphocyte ratio and IgG level predicts disease severity and outcome for patients with COVID-19. *Front Mol Biosci.* (2020) 7:157. doi: 10.3389/fmolb.2020.00157
24. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis.* (2020) 71:762–8. doi: 10.1093/cid/ciaa248
25. Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. *Clin Microbiol Infect.* (2020) 26:1622–9. doi: 10.1016/j.cmi.2020.07.016
26. Liu J, Li H, Luo M, Liu J, Wu L, Lin X, et al. Lymphopenia predicted illness severity and recovery in patients with COVID-19: A single-center, retrospective study. *PLoS ONE.* (2020) 15:e0241659. doi: 10.1371/journal.pone.0241659
27. Lombardi A, Trombetta E, Cattaneo A, Castelli V, Palomba E, Tirone M, et al. Early phases of COVID-19 are characterized by a reduction in lymphocyte populations and the presence of atypical monocytes. *Front Immunol.* (2020) 11:560330. doi: 10.3389/fimmu.2020.560330
28. Qin S, Jiang Y, Wei X, Liu X, Guan J, Chen Y, et al. Dynamic changes in monocytes subsets in COVID-19 patients. *Hum Immunol.* (2020) 82:170–6. doi: 10.1016/j.humimm.2020.12.010
29. Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. *Nat Rev Immunol.* (2011) 11:762–74. doi: 10.1038/nri3070
30. Rodriguez L, Pekkarinen PT, Lakshmikanth T, Tan Z, Consiglio CR, Pou C, et al. Systems-level immunomonitoring from acute to recovery phase of severe COVID-19. *Cell Reports Med.* (2020) 1:100078. doi: 10.1016/j.xcrm.2020.100078
31. De Roux A, Cavalcanti M, Marcos MA, Garcia E, Ewig S, Mensa J, et al. Impact of alcohol abuse in the etiology and severity of community-acquired pneumonia. *Chest.* (2006) 129:1219–25. doi: 10.1378/chest.129.5.1219
32. Jerrells TR, Pavlik JA, DeVasure J, Vidlak D, Costello A, Strachota JM, et al. Association of chronic alcohol consumption and increased susceptibility to and pathogenic effects of pulmonary infection with respiratory syncytial virus in mice. *Alcohol.* (2007) 41:357–69. doi: 10.1016/j.alcohol.2007.07.001
33. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol.* (2020) 146:110–8. doi: 10.1016/j.jaci.2020.04.006
34. Pasala S, Barr T, Messaoudi I. Impact of alcohol abuse on the adaptive immune system. *Alcohol Res.* (2015) 37:185–97.
35. Szabo G. Monocytes, alcohol use, and altered immunity. *Alcohol Clin Exp Res.* (1998) 22:216S–9S. doi: 10.1111/j.1530-0277.1998.tb04005.x
36. Lippai D, Bala S, Catalano D, Kodys K, Szabo G. Micro-RNA-155 deficiency prevents alcohol-induced serum endotoxin increase and small bowel inflammation in mice. *Alcohol Clin Exp Res.* (2014) 38:2217–24. doi: 10.1111/acer.12483
37. Marshall SA, McKnight KH, Blose AK, Lysle DT, Thiele TE. Modulation of binge-like ethanol consumption by IL-10 signaling in the basolateral amygdala. *J Neuroimmune Pharmacol.* (2017) 12:249–59. doi: 10.1007/s11481-016-9709-2
38. Litwinowicz K, Choroszy M, Waszczuk E. Changes in the composition of the human intestinal microbiome in alcohol use disorder: a systematic review. *Am J Drug Alcohol Abuse.* (2020) 46:4–12. doi: 10.1080/00952990.2019.1669629
39. Saltzman ET, Palacios T, Thomsen M, Vitetta L. Intestinal microbiome shifts, dysbiosis, inflammation, and non-alcoholic fatty liver disease. *Front Microbiol.* (2018) 30:61. doi: 10.3389/fmicb.2018.00061
40. Zuo T, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, et al. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. *Gastroenterology.* (2020) 159:944–55.e8. doi: 10.1053/j.gastro.2020.05.048
41. Gupta S, Parker J, Smits S, Underwood J, Dolwani S. Persistent viral shedding of SARS-CoV-2 in faeces – a rapid review. *Color Dis.* (2020) 22:611–20. doi: 10.1111/codi.15138
42. Alberca GGF, Solis-Castro RL, Solis-Castro ME, Alberca RW. Coronavirus disease−2019 and the intestinal tract: An overview. *World J Gastroenterol.* (2021) 27:1255–66. doi: 10.3748/wjg.v27.i13.1255
43. Anand S, Mande SS. Diet, microbiota and gut-lung connection. *Front Microbiol.* (2018) 19:2147. doi: 10.3389/fmicb.2018.02147
44. Gibson A, Woodside JV, Young IS, Sharpe PC, Mercer C, Patterson CC, et al. Alcohol increases homocysteine and reduces B vitamin concentration in healthy male volunteers - A randomized, crossover intervention study. *QJM.* (2008) 101:881–7. doi: 10.1093/qjmed/hcn112

45. Clugston RD, Blaner WS. The adverse effects of alcohol on vitamin A metabolism. *Nutrients*. (2012) 4:356–71. doi: 10.3390/nu4050356
46. Lieber CS. Alcohol: its metabolism and interaction with nutrients. *Annu Rev Nutr*. (2000) 20:395–430. doi: 10.1146/annurev.nutr.20.1.395
47. Penkert RR, Smith AP, Hrincius ER, McCullers JA, Vogel P, Smith AM, et al. Effect of Vitamin A deficiency in dysregulating immune responses to influenza virus and increasing mortality rates after bacterial coinfections. *J Infect Dis*. (2020). doi: 10.1093/infdis/jiaa597. [Epub ahead of print].
48. Schmidt R, Luboeinski T, Markart P, Ruppert C, Daum C, Grimminger F, et al. Alveolar antioxidant status in patients with acute respiratory distress syndrome. *Eur Respir J*. (2004) 24:994–9. doi: 10.1183/09031936.04.00120703
49. Dancer RCA, Parekh D, Lax S, D'Souza V, Zheng S, Bassford CR, et al. Vitamin D deficiency contributes directly to the acute respiratory distress syndrome (ARDS). *Thorax*. (2015) 70:617–24. doi: 10.1136/thoraxjnl-2014-206680
50. Ali N. Role of vitamin D in preventing of COVID-19 infection, progression and severity. *J Infect Public Health*. (2020) 13:1373–80. doi: 10.1016/j.jiph.2020.06.021
51. Radujkovic A, Hippchen T, Tiwari-Heckler S, Dreher S, Boxberger M, Merle U. Vitamin D deficiency and outcome of COVID-19 patients. *Nutrients*. (2020) 12:2757. doi: 10.3390/nu12092757
52. Okuno F, Arai M, Ishii H, Shigeta Y, Ebihara Y, Takagi S, et al. Mild but prolonged elevation of serum angiotensin converting enzyme (ACE) activity in alcoholics. *Alcohol*. (1986) 3:357–9. doi: 10.1016/0741-8329(86)90053-4
53. Bechara RI, Brown LAS, Eaton DC, Roman J, Guidot DM. Chronic ethanol ingestion increases expression of the angiotensin II Type 2 (AT2) receptor and enhances tumor necrosis factor-alpha- and angiotensin II-induced cytotoxicity via AT2 signaling in rat alveolar epithelial cells. *Alcohol Clin Exp Res*. (2003) 27:1006–14. doi: 10.1097/01.alc.0000071932.56932.53
54. Castelo Branco ACC, Sato MN, Alberca RW. The possible dual role of the ACE2 receptor in asthma and SARS-COV2 infection. *Front Cell Infect Microbiol*. (2020) 10:550571. doi: 10.3389/FCIMB.2020.550571
55. Jacobs M, van Eeckhoutte HP, Wijnant SRA, Janssens W, Brusselle GG, Joos GF, et al. Increased expression of ACE2, the SARS-CoV-2 entry receptor, in alveolar and bronchial epithelium of smokers and COPD subjects. *Eur Respir J*. (2020) 56:2002378. doi: 10.1183/13993003.02378-2020
56. Alberca RW, Yendo T, Aoki V, Sato MN. Asthmatic patients and COVID-19: different disease course? *Allergy*. (2020) 76:963–5. doi: 10.1111/all.14601

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Alberca, Rigato, Ramos, Teixeira, Branco, Fernandes, Pietrobon, Duarte, Aoki, Orfali and Sato. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

REVIEW
published: 18 June 2021
doi: 10.3389/fimmu.2021.649359

COVID-19 Severity in Obesity: Leptin and Inflammatory Cytokine Interplay in the Link Between High Morbidity and Mortality

Radheshyam Maurya[1]*, *Prince Sebastian*[1], *Madhulika Namdeo*[1], *Moodu Devender*[1] *and Arieh Gertler*[2]

[1] Department of Animal Biology, School of Life Sciences, University of Hyderabad, Hyderabad, India, [2] Institute of Biochemistry, Food Science and Nutrition, The Hebrew University of Jerusalem, Rehovot, Israel

OPEN ACCESS

Edited by:
Alexandros Tsoupras,
University of Limerick, Ireland

Reviewed by:
Markus Xie,
Genentech, United States
Viviana Parreño,
National Agricultural Technology Institute, Argentina
Matteo A. Russo,
San Raffaele Pisana (IRCCS), Italy
Nicolò Merendino,
University of Tuscia, Italy

***Correspondence:**
Radheshyam Maurya
rmusl@uohyd.ac.in;
rmusl@uohyd.ernet.in

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: 04 January 2021
Accepted: 31 May 2021
Published: 18 June 2021

Citation:
Maurya R, Sebastian P, Namdeo M, Devender M and Gertler A (2021) COVID-19 Severity in Obesity: Leptin and Inflammatory Cytokine Interplay in the Link Between High Morbidity and Mortality. Front. Immunol. 12:649359. doi: 10.3389/fimmu.2021.649359

Obesity is one of the foremost risk factors in coronavirus infection resulting in severe illness and mortality as the pandemic progresses. Obesity is a well-known predisposed chronic inflammatory condition. The dynamics of obesity and its impacts on immunity may change the disease severity of pneumonia, especially in acute respiratory distress syndrome, a primary cause of death from SARS-CoV-2 infection. The adipocytes of adipose tissue secret leptin in proportion to individuals' body fat mass. An increase in circulating plasma leptin is a typical characteristic of obesity and correlates with a leptin-resistant state. Leptin is considered a pleiotropic molecule regulating appetite and immunity. In immunity, leptin functions as a cytokine and coordinates the host's innate and adaptive responses by promoting the Th1 type of immune response. Leptin induced the proliferation and functions of antigen-presenting cells, monocytes, and T helper cells, subsequently influencing the pro-inflammatory cytokine secretion by these cells, such as TNF-α, IL-2, or IL-6. Leptin scarcity or resistance is linked with dysregulation of cytokine secretion leading to autoimmune disorders, inflammatory responses, and increased susceptibility towards infectious diseases. Therefore, leptin activity by leptin long-lasting super active antagonist's dysregulation in patients with obesity might contribute to high mortality rates in these patients during SARS-CoV-2 infection. This review systematically discusses the interplay mechanism between leptin and inflammatory cytokines and their contribution to the fatal outcomes in COVID-19 patients with obesity.

Keywords: COVID-19, leptin, obesity, inflammation, cytokine, mortality

INTRODUCTION

Obesity is marked as redundant fat accumulation in the body. Obesity is considered an increased circulating fatty acid that is causing low-grade chronic inflammation due to macrophages' chemoattraction and its expansion in the adipose tissue (1, 2). An individual with obesity presents with increased TNF-α cytokine, changed T-cell subset, and suppressed T-cell responses

driving to a high possibilities of communicable diseases (3). Obesity has been shown to be associated with the production of dysregulated cytokines and augment the synthesis of acute-phase reactants, such as IL-6, IL-8, TNF-α, C-reactive protein (CRP), and monocyte chemotactic protein-1 (MCP-1) in patients with obesity and various animal models of obesity (4–7). Pre-conditioned chronic inflammation directly or indirectly impacts the immune response to infection in individuals with obesity (8). Several viruses, such as influenza A virus, Adenovirus Ad 36, cytomegalovirus (CMV), and HIV utilize adipocytes as cellular reservoirs for viral shedding (9). In the 2009 influenza pandemic, individuals with obesity exhibited more severe illness from the H1N1 virus and were reported as a nonpartisan threat for severe influenza infection and appended death (10). Many other reports have been confirmed the severity of disease and death from H1N1 influenza linked in a patient with obesity (11–13). Experimental studies have confirmed that obese mice show increased susceptibility to influenza, including increased lethality with H1N1 and H3N2 strains (14–17). A precise study of MERS-CoV cases reveals that diabetes and hypertension are equally predominant in 50% of the patients. In contrast, the chances of cardiac diseases and obesity are 30% and 16%, respectively. These conditions affect the pro-inflammatory cytokine responses, resulting in impairment of the host's innate and humoral immune response among these patients (18). However, the critical mechanism for more inclusive COVID-19 severity and mortality in obesity remains unknown, despite how other viral infections can be providing some thought of COVID-19 illnesses and the severity of disease in an individual with obesity.

The connection between obesity and severity of viral infection, such as SARS and MERS, may be paramount in COVID-19 disease because of the genetic resemblance between the SARS-CoV-2 virus with SARS-CoV and MERS-CoV which is 80% and 50%, respectively (19). Patients with obesity in SARS-CoV-2 infection show severe clinical complications in the case of acute respiratory distress syndrome (ARDS), cardiac injury, thromboembolic disease, disseminated intravascular coagulation (DIC), and in severe chronic obstructive pulmonary disease (COPD) (20, 21). Acute lung injury (ALI) is another primary reason of severe illness and mortality among SARS-CoV-2–infected patients (22). Display of ARDS and ALI is cited as lung failure because of the hyper inflammatory response rather than the direct viral cytopathic injury (23, 24). Another important factor that could be contributing to the immune imbalance in COVID-19 patients with obesity is chronically higher adipokines, such as leptin. In influenza infection, leptin resistance is a major infection susceptibility factor in individuals with obesity by quashing the countenance of IFN-α, IFN-β, IFN-γ mRNAs, and memory T cells (25). It is thoroughly documented that leptin regulates the pro-inflammatory cytokines response to maintain the Th1/Th2 dichotomy. This review highlights the distinctive facts regarding the link between obesity and inflammation, specifically the interplay of leptin and inflammatory cytokine response leading severity and mortality among COVID-19 patients.

OBESITY LINKED COVID-19 PATHOGENESIS IN HUMAN

A study reported from China showed COVID-19 patients with body mass index (BMI) oF 28.0 ± 2.5 display severe signs of symptoms compared with patients with BMI of 21.2 ± 2.7. It also showed that a cohort of patients with obesity has a high frequency of getting severe symptoms and admitted to the intensive care unit (26). Two independent studies of hospitalized COVID-19 patients in New York City reported that obesity is one of the utmost shared comorbidities along with hypertension and diabetes and is independently linked to worse in-hospital outcomes and higher death (27, 28). A meta-analysis of pooled data of 22 and 35 independent studies showed that persons with obesity increase the probabilities of staying more in an intensive care unit by 74% and likely to have unfavorable fallouts with a 48% upsurge in deaths of COVID-19 patients, respectively (29). As leptin levels are vastly associated with obesity, there is the yet unanswered question of whether leptin alone is responsible for a bad prognosis. Preliminary data from various studies revealed that younger adults with obesity are more vulnerable to a higher risk for intubation or mortality in COVID-19 infection (30, 31). The morbidity and mortality in a young patient with obesity are mainly because of the visceral adiposity, leading to multiple organ failure in severe COVID-19 illness (32). A retrospective cohort study of 65 admitted patients aged 18 to 40 years in Zhongnan Hospital, Wuhan showed that obesity is a vital parameter to determine the severity of COVID-19 infection among young patients (33). The computed tomography (CT) analysis of visceral fat revealed that severity was linked to the accumulation of ectopic fat in several visceral organs, such as the liver, epicardial, and kidney (33, 34). Other studies that included a cross-sectional analysis of fat distribution showed that it is critical to consider BMI as a risk factor apart from the overall obesity in COVID-19 patients. CT-based quantification of fat showed the increment in the visceral fat area (VFA) and upper abdominal perimeter augmented the COVID-19 severity in patients (35).

The SARS-CoV-1 and SARS-CoV-2 virus entry into the adipocytes is mediated through the membrane protein angiotensin-converting enzyme 2 (ACE2) receptor (36, 37). It is vastly expressed in vascular tissues, such as the lungs and adipose tissue (38). ACE2 is expressed by a majority of cells in human at varying levels, including the intestinal enterocytes, liver, heart, and kidneys (39). The SARS-CoV-2 virus binds to the ACE2 receptors present in the host's cell membrane. Subsequently, transmembrane serine protease (TMPRSS2) cleaves the viral capsid spike (S) protein and favoring the viral particle to infiltrates into host cells (40). The ACE2 expression in adipocytes appears to be stimulated by high-fat diets (41, 42). Adipocytes of individuals with obesity express a high level of ACE2 receptor, shedding more viral load than lean individuals, which exacerbate the susceptibility and severity of patients during COVID-19 infection (43–45). Several reports suggest that drugs used to treat patients with obesity complications (antihypertensives, statins, thiazolidinediones) could induce the

overexpression of ACE2 receptor in adipocytes, leading to more viral uptake and severity of the disease (46–48) (**Figure 1**). Similarly, various extracellular vehicles (EVs) could also carry viral particles and ACE2 receptors from infected to healthy cells that could enhance COVID-19 susceptibility (43, 49). Fatty adipocytes in the visceral depots allow the SARS-CoV-2 entry through the ACE2 receptor (50), resulting in necrotic death of adipocytes in AT, which leads to a meta-inflammatory condition called fat embolism syndrome (FES). Strikingly, lung autopsy of an overweight patient with COVID-19 infection showed fat embolism macrophages infiltration in the AT (51, 52). The significant increase of inflammatory biomarkers, such as serum amyloid A, IL-6, and CRP in severe COVID-19 patients (53), are directly or indirectly linked to adipocytes with sub-clinical low-grade inflammation that further exacerbates COVID-19 severity in individuals with obesity (54, 55). Adipocytes is known to secrete leptin, which is an imperative mark of obesity and is directly proportional to body fat mass. Leptin involves distinct endocrine, paracrine, and autocrine signaling mechanism and cytokine-mediated inflammatory changes in the body (56). Therefore, leptin could play a significant role in developing severe COVID-19 infection in patients with obesity.

ADIPOSE TISSUE LINKED INFLAMMATION

Adipose tissue is typically heeded as an inert tissue and functionally considered as an energy store house. It also deeds a central role in the immune and endocrine function that regulates inflammatory responses against infection. Visceral adiposity is associated with metabolic disorder and is recognized as a high-risk factor during SARS-CoV-2 infections (34). However, the exact mechanisms lying behind it are partly explained. Adipose tissue–linked inflammation is marked by the secretion of inflammatory cytokines, such as TNF-α, IL-6, and IL-1, from adipocytes and resident macrophages in adipose tissue (2). However, a typical feature of adipose tissue is adipocyte hypertrophy due to the accumulation of extended fat droplets. Adipocytes can reach a diameter of 150 to 200 microns

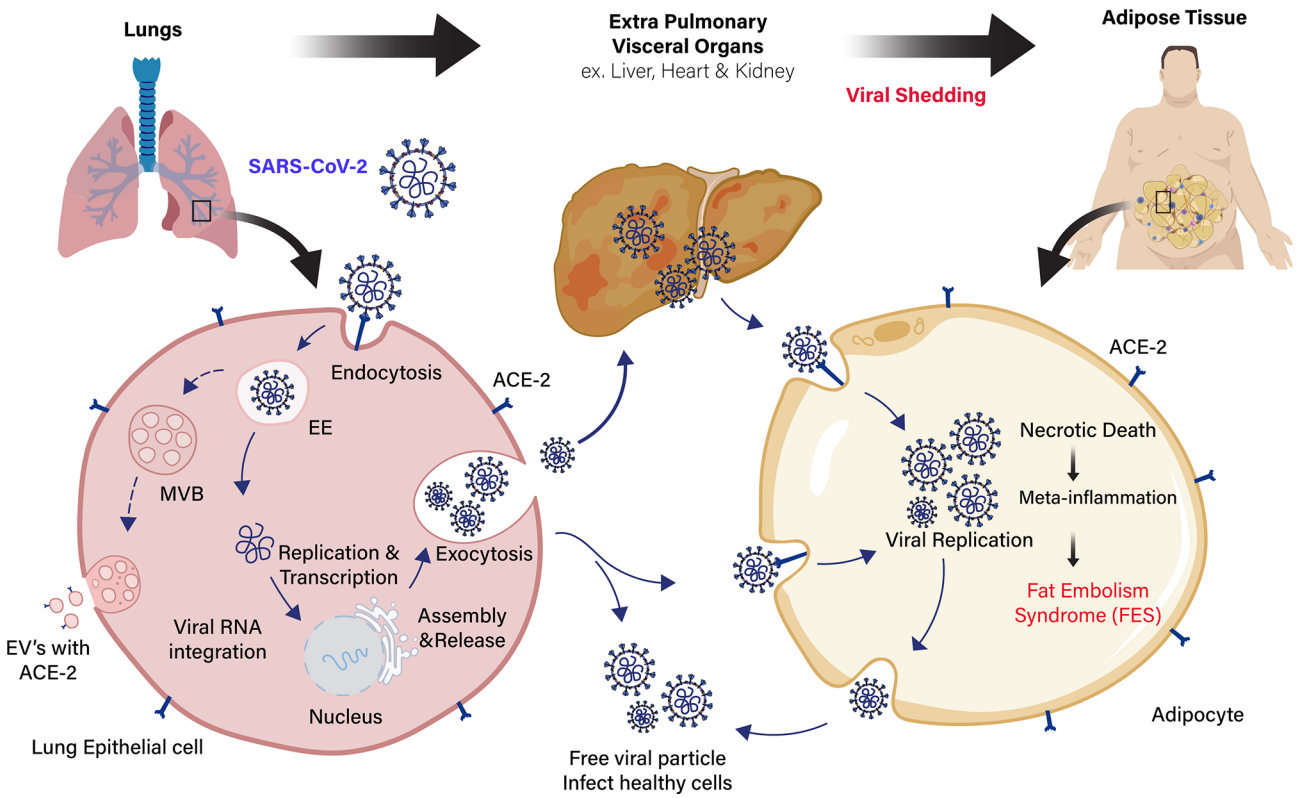


FIGURE 1 | Schematic representation of extra pulmonary viral shedding and adipose tissue (AT) inflammation in COVID-19 patients with obesity. Angiotensin-converting enzyme 2 (ACE2) receptor in the lung epithelial cells recognizes the COVID-19 spike protein and the virus with receptor taken into the cells by receptor-mediated endocytosis. Then, viral RNA integrates into the host genome for viral replication and assembly. The free viral particles inside the host cell are released through exocytosis to infect healthy cells. Extracellular vesicles (EVs) containing viral proteins, and ACE2 receptors are also carried over to cells of extra pulmonary fatty visceral organs. The fatty adipose tissue in patients with obesity is a link to extra pulmonary viral shedding through the ACE2 expressing adipocytes. The adipocytes in the fatty AT of visceral organs (liver, heart, and kidney) act as a reservoir for viral replication and assembly. Apoptotic and necrotic death of the virus inside the adipocytes leads to meta-inflammation and subsequent fat embolism syndrome (FES) in COVID-19 patients with obesity.

in size, which limits the diffusion/exchange of gas (O_2/CO_2) in the adipose tissue. This produces a progressive local hypoxia by activating the hypoxia-inducible factor 1α (HIF-1α) and NF-κβ expression (57). The HIF-1α is a molecular sensor of hypoxia, and it transactivates the leptin gene expression by HIF-1α transcription-dependent activity (58). In addition to this, hundreds of immuno-inflammatory-reparative genes are also expressed, profoundly changing the adipocyte phenotype and functionality. Leptin is a product of a hypoxia-inducible gene, and hypoxia differentiates the preadipocytes into leptin secreting endocrine cells in an mTOR-dependent manner. Preadipocyte-derived leptin activates inflammatory cytokines by a specific local autocrine/paracrine signaling (57, 59) (**Figure 2**).

Adipocytes also secrete numerous adipocytokines, such as adiponectin, resistin, visfatin, and leptin. These molecules are believed to be linked with insulin resistance, inflammatory disorders, and obesity (60–62). Adiponectin plays a vital role in the secretion of anti-inflammatory cytokines, like IL-10 and IL-1 receptor antagonist (IL-1RA), by inducing the monocytes, macrophages, and dendritic cells (DCs). Adiponectin also plays a significant role in suppressing the interferon-γ (IFN-γ) secretion in LPS-stimulated macrophages (63, 64). Human resistin incites the secretion of the proinflammatory cytokines TNF-α, IL-1, IL-6, and IL-12 by several immune cell *via* NF-κB–dependent pathway (65, 66). It also upregulates the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and CC-chemokine ligand-2(CCL-2) by human endothelial cells to release endothelin-1 (67). Serum amyloid A is another adipokines produced by adipocytes that enhances inflammatory cytokines production in individuals with obesity (68).

The primary function of leptin is to regulate appetite and energy (60). Nonetheless, leptin is also thought to be a proinflammatory cytokine, and it has a structural similarity with other inflammatory cytokines, such as IL-6, IL-12, and granulocyte colony-stimulating factor. Leptin promotes the migration of resident macrophages in the AT and stimulates their polarization toward a proinflammatory profile or a classically activated macrophage (M1) phenotype. It overturns the T helper cell phenotype by diminishing regulatory T-cells (Treg) activity and favoring Th17 polarization (69). Leptin modulates macrophage and T-cell function through the activation JAK-STAT pathway, leading to immune dysregulation and

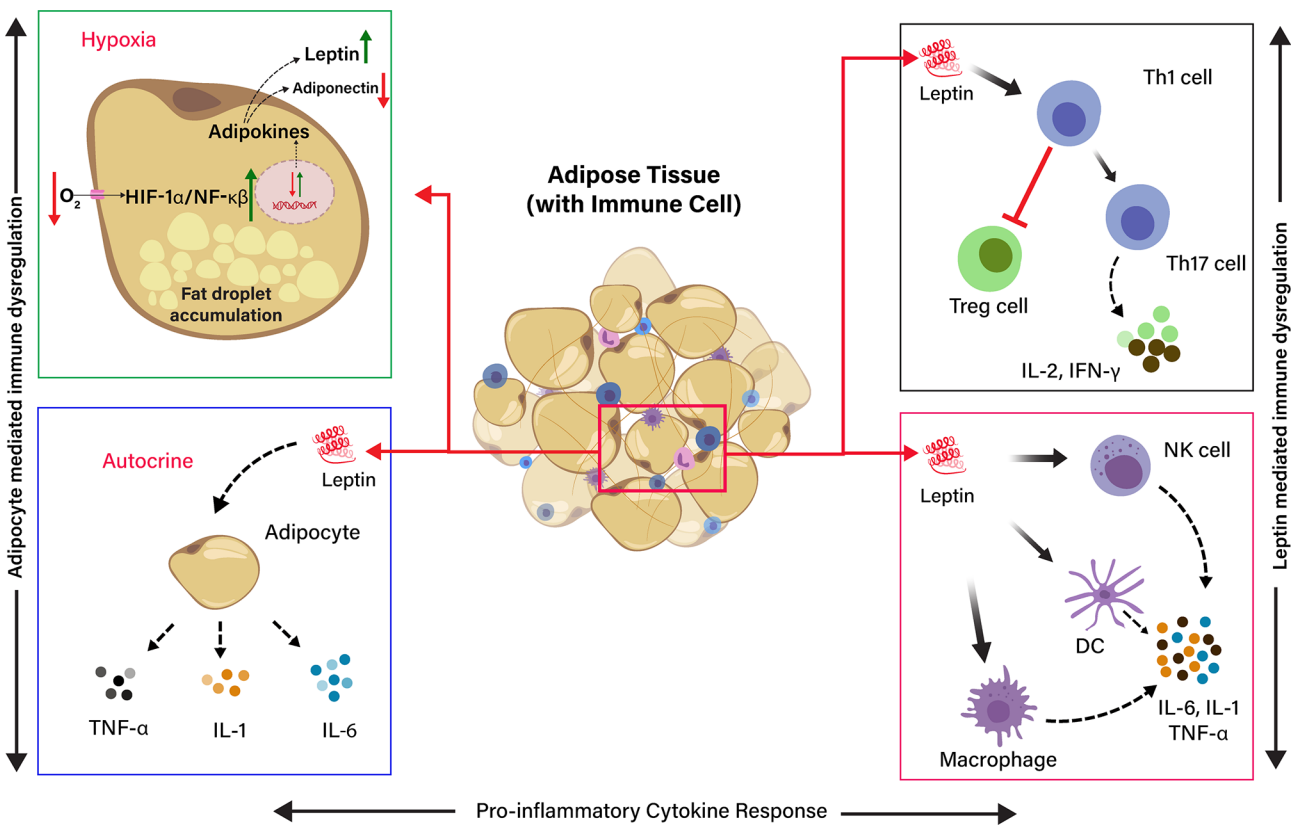


FIGURE 2 | Schematic representation of the effects of Hypoxia and Leptin on pro-inflammatory cytokine response in adipose tissue (AT). The effect of low O_2 tension on the production of adipokines in hypoxia condition. The adipocyte in the adipose tissue release leptin, which induces the release of pro-inflammatory cytokine by endocrine, paracrine, and autocrine in the AT. Leptin induces Th1 cells, and it inhibits T regulatory cells (Treg cells) and induces Th17 cells differentiation to produce IL-2 and IFN-γ. Leptin directly activates macrophages, dendritic cells (DC), and natural killer (NK) cells to produce pro-inflammatory cytokines, such as IL-6, IL-1, and TNF-α. Adipocyte-derived leptin induces the adipocytes to produce pro-inflammatory cytokines (IL-6, IL-1, and TNF-α) through autocrine signaling.

proinflammatory cytokine production (70, 71). Hyperleptinemia is another complication due to leptin resistance or leptin-impaired signaling. It interferes with the leptin receptor, contributing to the inflammatory response in obesity (72). Moreover, various other fatty tissue products have been characterized, such as TNF-α, IL-6, IL-1, CCL2, and plasminogen activator inhibitor type-1, which play a crucial role in immune dysregulation in individuals with obesity (68). Therefore, adipocyte interactions with immune cells (**Figure 2**) could be facilitating the disease severity in COVID-19 patients linked with obesity.

LEPTIN DYSREGULATION LINKED TO CYTOKINE STORM IN COVID-19 INFECTION

Leptin is a non-glycosylated 16-kDa protein encoded by the obese (ob) gene and mainly secreted by adipocytes to the circulation. Leptin acts as a pleiotropic protein, like hormone, as well as a cytokine. The circulatory leptin binds to the long form of the leptin receptor (Lep-R), class I receptor cytokine family mediates the phosphorylation and activation of STAT3 signaling pathway (71, 73). Lep-R is expressed in most of the immune cells, and leptin binds and triggers the immune response mainly through the JAK-STAT– and NF-kB–dependent pathway (69, 71, 74). Leptin functions in immune response have been confirmed in leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice (75–77). The genetically leptin-deficient or leptin-resistant animals or humans develop profound obesity because of hormonal and immune system abnormalities (77–79). Leptin secretion in individuals with obesity is chronically higher than lean subjects. The higher level of pro-inflammatory leptin and lower anti-inflammatory adiponectin ratio could be contributing to the obesity-associated complication due to biased immune response (80). Leptin can tempt the stimulation and proliferation of various immune cells, including circulating monocytes, natural killer cells (NK) cells, and T helper cells by increasing the release of inflammatory cytokines, such as TNF–α, IL–1, and IL–6 (81–83). Leptin has structural homology with the long-chain helical family's cytokines, such as IL-6, IL-11, IL-12 cytokine, and oncostatin M (84). Leptin has a shared signal-transducing peptide portion for the IL-6-related cytokines, such as IL-6, IL-11, leukocyte inhibitory factor (LIF), and granulocyte colony-stimulating factor (GCSF) (69, 85). Along with these, certain inflammatory and infectious stimuli are also enhancing the leptin expression, such as TNF-α, IL-1, IL-6, and lipopolysaccharide (LPS) (86). Therefore, the crucial question is whether or not the cytokine storm in COVID-19 patients with obesity is linked to leptin dysregulation.

Adipocytes engage in a cross-talk with various immune cells and could be directly involved in their activation and proliferation, resulting in the production of inflammatory cytokines (87, 88). Adipocytes add nearly one third of the IL-6 concentration to the circulation, along with macrophages that are recruited into the AT by leptin (89). IL-6 is the foremost cytokine in acute phase inflammation, an innate immune response elicited by infection. IL-6 also plays an indispensable role in improving acquired immunity against pathogens, including cytokine and chemokine expressions, stimulation of antibody production from B cells, macrophage regulation, and dendritic cell differentiation (90, 91). The macrophage deposition and its chronic activation cause the abnormal secretion of IL-6, TNF-α during SARS-CoV-2 infection in a patient with obesity (51, 92). An altered pro-inflammatory profile and hyperleptinemia lead to thrombotic risk in these patients that are linked to coagulation of blood, resulting in visceral organ failures (90, 93). In addition, leptin modulates the gene expression in cardiomyocytes *via* hypoxia-mediated HIF-1α factors, which is the leading cause of heart ischemia (94). In a recent cross-sectional study in 31 COVID-19–positive patients, interestingly, the serum leptin levels of ventilated patients were significantly higher compared with the control groups (95). Adipocyte dysfunction linked to leptin could be contributing to the expansion of ARDS in COVID-19 patients. Therefore, a strong possibility of neutralizing the circulatory leptin could control the hyper cytokine responses that are linked to obesity in COVID-19 patients.

INTERPLAY BETWEEN LEPTIN AND IL-6 IN COVID-19 INFECTION

Severe COVID-19 patients show an expressively higher serum IL-6 than those with mild infection (96). High level of IL-6 is also another key signature of severe SARS-CoV, MERS-CoV, and pandemic H1N1 influenza A viral infections (97–99) In individuals with obesity, adipocytes secrete pleiotropic leptin and pleiotropic adipocyte-specific IL-6 cytokine (91, 100). IL-6–mediated inflammation in obesity is source-specific, and the IL-6 secreted by adipocytes or infection of non-resident adipocyte cells, including adipose tissue macrophage (ATM) and T cells in a non-canonical mechanism (101). Visceral adipose tissue (VAT) produces higher inflammatory cytokines (TNF-α, IL-6, and IL-1β) in comparison to subcutaneous adipose tissue (102), suggesting that cytokine storm in COVID-19 infection could be independent of obesity. IL-6 is released from adipocytes, either binding to a soluble or membrane-bound receptor, and forms an IL-6 ligand/receptor complex. IL-6 is also associated with a homodimer of glycoprotein 130 for downstream signaling in adipocytes (103, 104). Leptin and IL-6 act in agreement with partly sharing a common downstream target mediated by phosphorylation of STAT3 in ventromedial hypothalamic leptin signaling (105, 106). Furthermore, the structural and functional similarity of leptin and IL-6 receptor-mediated signaling *in-vitro* showed that both are activating the JAK-STAT pathway (82, 107). Leptin induces IL-6 expression through the binding to the leptin receptor (LEPR) and activates JAK2/STAT-3–dependent phosphorylation of IRS-1, PI3K, Akt signaling pathway (72). The binding of p65 and p50 transcription factor to the NF-κB element enhances the recruitment of p300 to the IL-6 promoter, resulting in up-regulation of IL-6 expression (91, 104). Conversely, IL-6

upregulates the expression of TNF-α and IL-1 through JAK/STAT-dependent phosphorylation of PKA signaling pathway (108). The dose-dependent effects of TNF-α and IL-1 cytokines on leptin gene overexpression revealed that their anorectic effects might be arbitrated in bit by expression of the leptin gene in the adipocyte (82, 109). Leptin also inhibits the apoptosis through the activation of PI3K-dependent Bcl-2 and Bcl-xL signaling pathways (110). The high leptin concentration in serum is correlated with incremental death in patients with ARDS caused by an infectious agent (111). It has also been stated that leptin induces an inflammatory phenotype in murine alveolar macrophages, hinting that a higher concentration of leptin in obesity might be potentiating the incidence of pulmonary inflammation in COVID-19 infection (112). The dysregulation of leptin in COVID-19 patients could be linked to IL-6 cytokine signaling because it shares the IL-6 receptors. The mechanism of leptin and IL-6 signaling pathways are summarized in **Figure 3**.

Another interesting study showed that leptin and IL-6 induce anorexia by acting on the lateral parabrachial nucleus (lPBN) in the brain, suggesting that targeting leptin might be controlling the IL-6 signaling in individuals with obesity (113). The elevated level of IL-6 in serum due to increased production of adipokines leads to induce low-grade chronic inflammation (114). Although high IL-6 in COVID-19 infection has a prominent role in inflammation linked mortality, recent studies showed that colchicine treatment decreased the concentrations of multiple inflammatory molecules, including IL-6, CRP, and resistin in patient with obesity (115). Tocilizumab is used to treat cytokine release syndrome (CRS) in patients with cancer treated with chimeric antigen receptor-modified (CAR) T cells. It inhibits the expression of IL-6 through the blocking of the IL-6, receptor resulting in inhibitions of proinflammatory cytokine, such as TNF-α, IL-1, and IFNγ (116, 117). In an early pandemic, it was reported that IL-6 plays a significant role in the cytokine storm in

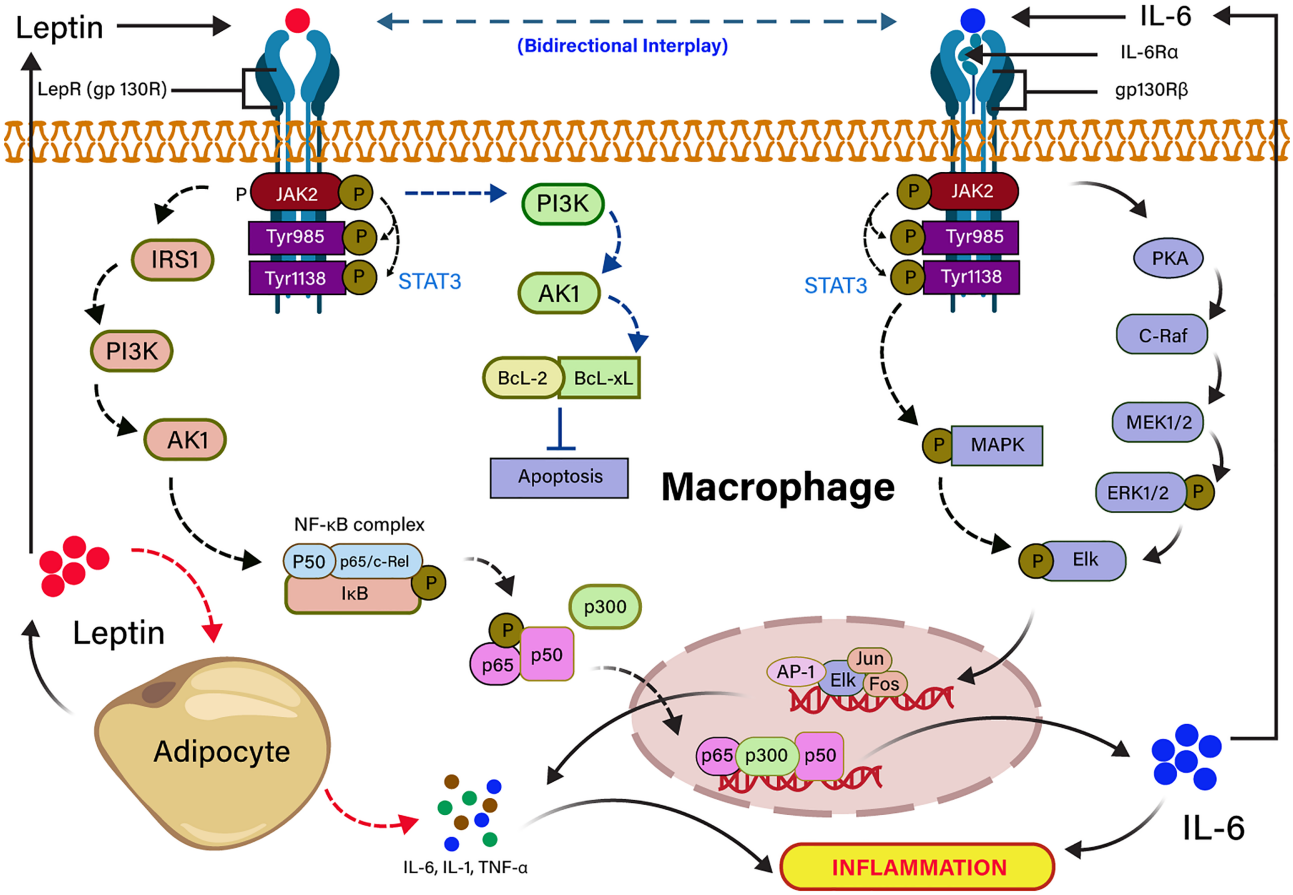


FIGURE 3 | Signal transduction cascades of leptin and IL-6 interplay in inflammation. Leptin binding to the long-form leptin receptor (LRb or gp130R) results in the activation of the Janus kinase 2 (JAK2) signaling pathway by autophosphorylation, which subsequently phosphorylates tyrosine 985 and 1138 residues of the intracellular receptor tail. The phosphorylated Tyr 1138 activates the transcription factor STAT3. The activated JAK/STAT3 activates IRS1 and PI3K downstream signaling pathway, which recruits NF-kB complex (P50, P65/c-Rel, IkB), resulting in the transcription of IL-6 expressing genes in the nucleus. JAK/STAT3 directly activates PI3k inhibits apoptosis by the activation of anti-apoptotic factors (BCL-2, BCL-XL). Pleiotropic leptin and IL-6 follow similar signaling through gp130R and gp130Rβ, respectively. Leptin-induced IL-6 binding to the gp130Rβ results in the activation of JAK/STAT followed by the activation of PKA- and MAPK-mediated Elk phosphorylation. The phosphorylated Elk induces the transcription factors for TNF-α and IL-1. IL-6–mediated TNF-α and IL-1 induce the production of leptin from adipocytes.

COVID-19 patients, opening new hopes to treat the cytokine storm by blocking the IL-6 cytokine receptor. However, later on, multicenter studies reported that there is no significant improvement after blocking the IL-6 receptor in these patients (118). Subsequently, IL-6 is not the only cytokine, several other cytokines, such as TNF-α, IL-1, and IL-8, have also been elevated in CRS and are equally important in acute inflammatory reactions that contribute to tissue injury. TNF-α and IL-1 blockade might be another probable therapeutic approach to restrained organ damage in patients with SARS-CoV-2 infection (119, 120). In vitro studies showed that adiponectin also inhibits IL-6 expression by murine pulmonary endothelial cells and reduces lung inflammation in mice during ARDS induced by bacterial liposaccharides (121). However, data if adipokines can regulate pulmonary inflammation in COVID-19 infection are lacking so far. Despite the fact that blocking the specific cytokines could be a strategy to treat COVID-19 patients, there is a possibility it may lead to severe outcomes. Therefore, the bidirectional interplay of cytoadipokines might play a role in non-specific immune response, such as cytokine storm in obesity-related mortality in severe COVID-19 patients.

PROSPECTIVE OF LEPTIN TREATMENT IN COVID-19 INFECTION

Because obesity is strongly correlated with poor prognosis in COVID-19 infection and because hyperleptinemia is characteristic of obesity, we suggest that a possibility lowering leptin activity may improve such bad prognosis. This can be achieved by blocking leptin action using leptin receptor antagonists (L39A/D40A/F41A mutants of human/mouse/rat/ovine leptins) (122) developed by our groups. The antagonistic activity of those mutants was up to 60-fold elevated by additional mutation (D23L), resulting in a respective super active D23L/L39A/D40A/F41A mutants (123). In view of the potential pharmaceutical uses of recombinant leptin mutants acting as antagonists, the basic question of how the biopotency of recombinant proteins can be elevated *in vivo* needs to be investigated. The capability of a hormone or hormone antagonists to elicit a biological effect *in vivo* depends not only on the affinity for its receptor but also on its clearance from the circulation. The measured half-life in circulation of leptin is or leptin antagonist only ~ 60 min (123, 124). As kidney-mediated clearance is mostly reliant on molecular mass and proteins greater than 70 to 80 kDa are cleared at a remarkably slower rate, increasing protein size will prolong its *in vivo* half-life. This can be attained by increasing the size of the hormone without affecting its activity, *via* attachment of polyethylene glycol (PEG) molecule. Therefore, to extend the biological activity of those mutants in animals by prolonging their *in vivo* half-life in circulation, they were mono-pegylated at their amino-terminus by 20-kDa PEG (123, 124) and termed PEG-SMLA (mouse) or PEG-SHLA (human) or PEG-SRLA (rat). Although the molecular weight of those analogs is ~ 36 kDa, due to the very large hydrodynamic volume of PEG, they behave like 220-kDa proteins, and their half-life in mice circulation was extended to ~ 14 to 15 h. The *in vivo* effect of those mono-pegylated analogs was well-documented in Prof. Gertler's and in other laboratories (125–129). By applying different amounts of leptin antagonists, it is possible to control the leptin activity in serum.

CONCLUSION

The interplays between leptin and inflammatory cytokine response that regulate the immune-metabolic feats of leptin on immune cells are required to explore the insight of their relation. The past two decades of studies about leptin reveals that adipose tissues play a crucial role in regulating metabolism and immune cell function. Adipose tissue inflammation is marked by infiltration of immune cells, such as lymphocytes, macrophages, dendritic cells, and neutrophils into it. Leptin expands the monocyte, macrophage, and T-cell proliferation by enhancing inflammatory cytokines secretion. Leptin also plays a major role in the anorexia and cachexia condition of inflammatory diseases caused by pathogens, collagen vascular disease, and even cancer. However, chronic inflammation, either from autoimmune or infectious diseases, or impaired leptin response to CNS (hypothalamus)/leptin resistance, induce resistance to the weight control, which is a leading cause of obesity and anorexia. The absence of leptin triggers the immune defects that eventually translate into exaggerated death due to infections. Therefore, high circulating leptin might involve dysregulation of proinflammatory cytokine in obesity, which is the leading cause of high morbidity and mortality in patients with SARS-CoV-2 infection. Individuals with obesity suggest that metabolic consequences of obesity compromise host antiviral defenses, leading to critical outcomes from infection. This could be one of the reasons why the virus has taken its toll to damaged individuals with obesity more aggressively. Therefore, more studies in the mechanism of leptin are warranted to investigate the immune response in COVID-19 patients with obesity.

AUTHOR CONTRIBUTIONS

RM and AG conceived and designed the study. PS, MN, and MD have done literature collection and wrote the initial version of the draft. RM and PS have prepared the figures and finalized the manuscript. All authors contributed to the article and approved the submitted version. RM is the guarantor and takes all responsibility for the integrity and accuracy of the data.

ACKNOWLEDGMENTS

We sincerely thank Dr. David Sacks, LPD/NIAID, NIH, Bethesda, for his valuable comments and suggestions during the manuscript preparation. We acknowledge the ICMR for providing ICMR-DHR International Fellowship, DST-SERB, DST-FIST, DST-Purse, and University of Hyderabad for the subscription of scientific journal and books to compile the review.

REFERENCES

1. Ervin RB. Prevalence of Metabolic Syndrome Among Adults 20 Years of Age and Over, by Sex, Age, Race and Ethnicity, and Body Mass Index: United States, 2003-2006. *Natl Health Stat Rep* (2009) (13):2003–6.
2. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity Is Associated With Macrophage Accumulation in Adipose Tissue. *J Clin Invest* (2003) 112(12):1796–808. doi: 10.1172/JCI200319246
3. Schaible UE, Kaufmann SHE. Malnutrition and Infection: Complex Mechanisms and Global Impacts. *PLoS Med* (2007) 4(5):0806–12. doi: 10.1371/journal.pmed.0040115
4. Sudhakar M, Silambanan S, Chandran AS, Prabhakaran AA, Ramakrishnan R. C-Reactive Protein (CRP) and Leptin Receptor in Obesity: Binding of Monomeric CRP to Leptin Receptor. *Front Immunol* (2018) 9(MAY):1–13. doi: 10.3389/fimmu.2018.01167
5. Hotamisligil GS. Inflammation, Metaflammation and Immunometabolic Disorders. *Nat Publ Gr* (2017) 542(7640):177–85. doi: 10.1038/nature21363
6. Ellulu MS, Patimah I, Khaza H, Rahmat A, Abed Y, Sci AM. Obesity and Inflammation : The Linking Mechanism and the Complications. *Arch Med Sci* (2016) 13(4):851–63. doi: 10.5114/aoms.2016.58928
7. Greenberg AS, Obin MS. Obesity and the Role of Adipose Tissue in Inflammation and Metabolism. *Am J Clin Nutr* (2006) 83(2):461–5. doi: 10.1093/ajcn/83.2.461S
8. Ramos-Nino ME. The Role of Chronic Inflammation in Obesity-Associated Cancers. *ISRN Oncol* (2013) 2013:697521. doi: 10.1155/2013/697521
9. Bourgeois C, Gorwood J, Barrail-Tran A, Lagathu C, Capeau J, Desjardins D, et al. Specific Biological Features of Adipose Tissue, and Their Impact on HIV Persistence. *Front Microbiol* (2019) 10:2837. doi: 10.3389/fmicb.2019.02837
10. Louie JK, Acosta M, Samuel MC, Schechter R, Vugia DJ, Harriman K. Major Article A Novel Risk Factor for a Novel Virus : Obesity and 2009 Pandemic Influenza A (H1N1). *Clin Infect Dis* (2011) 52(3):301–12. doi: 10.1093/cid/ciq152
11. Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al. Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans. *N Engl J Med* (2009) 360(25):2605–15. doi: 10.1056/NEJMoa0903810
12. Fuhrman C, Bonmarin I, Bitar D, Cardoso T, Duport N, Herida M, et al. Adult Intensive-Care Patients With 2009 Pandemic Influenza A(H1N1) Infection. *Epidemiol Infect* (2011) 139(8):1202–9. doi: 10.1017/S0950268810002414
13. Vaillant L, La Ruche G, Tarantola A, Barboza P. Epidemiology of Fatal Cases Associated With Pandemic H1N1 Influenza 2009. *Euro Surveill* (2009) 14 (33):19309. doi: 10.2807/ese.14.33.19309-en
14. Smith AG, Sheridan PA, Harp JB, Beck MA. Diet-Induced Obese Mice Have Increased Mortality and Altered Immune Responses When Infected With Influenza Virus. *J Nutr* (2007) 137(5):1236–43. doi: 10.1093/jn/137.5.1236
15. Radigan KA, Morales-Nebreda L, Soberanes S, Nicholson T, Nigdelioglu R, Cho T, et al. Impaired Clearance of Influenza a Virus in Obese, Leptin Receptor Deficient Mice Is Independent of Leptin Signaling in the Lung Epithelium and Macrophages. *PLoS One* (2014) 9(9):1–8. doi: 10.1371/journal.pone.0108138
16. Easterbrook JD, Dunfee RL, Schwartzman LM, Jagger BW, Sandouk A, Kash JC, et al. Obese Mice Have Increased Morbidity and Mortality Compared to Non-Obese Mice During Infection With the 2009 Pandemic H1N1 Influenza Virus. *Influenza Other Respir Viruses* (2011) 5(6):418–25. doi: 10.1111/j.1750-2659.2011.00254.x
17. O'Brien KB, Vogel P, Duan S, Govorkova EA, Webby RJ, McCullers JA, et al. Impaired Wound Healing Predisposes Obese Mice to Severe Influenza Virus Infection. *J Infect Dis* (2012) 205(2):252–61. doi: 10.1093/infdis/jir729
18. Badawi A, Ryoo SG. Prevalence of Comorbidities in the Middle East Respiratory Syndrome Coronavirus (MERS-CoV): A Systematic Review and Meta-Analysis. *Int J Infect Dis* (2016) Aug49:129–33. doi: 10.1016/j.ijid.2016.06.015
19. Muniyappa R, Gubbi S. COVID-19 Pandemic, Coronaviruses, and Diabetes Mellitus. *Am J Physiol Endocrinol Metab* (2020) 318(5):E736–41. doi: 10.1152/ajpendo.00124.2020
20. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* (2020) 71(15):762–8. doi: 10.1093/cid/ciaa248
21. Ye Q, Wang B, Mao J. The Pathogenesis and Treatment of the 'Cytokine Storm'' in COVID-19. *J Infect* (2020) 80(6):607–13. doi: 10.1016/j.jinf.2020.03.037
22. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical Predictors of Mortality Due to COVID-19 Based on an Analysis of Data of 150 Patients From Wuhan, China. *Intensive Care Med* (2020) 46(5):846–8. doi: 10.1007/s00134-020-05991-x
23. Fanelli V, Ranieri VM. Mechanisms and Clinical Consequences of Acute Lung Injury. *Ann Am Thorac Soc* (2015) 12:S3–8. doi: 10.1513/AnnalsATS.201407-340MG
24. Bhattacharyya R, Iyer P, Phua GC, Lee JH. The Interplay Between Coagulation and Inflammation Pathways in COVID-19-Associated Respiratory Failure : A Narrative Review. *Pulm Ther* (2020) 6(2):215–31. doi: 10.1007/s41030-020-00126-5
25. Hur SJ, Kim DH, Chun SC, Lee SK. Effect of Adenovirus and Influenza Virus Infection on Obesity. *Life Sci* (2013) 93(16):531–5. doi: 10.1016/j.lfs.2013.08.016
26. Cai Q, Chen F, Wang T, Luo F, Liu X, Wu Q, et al. Obesity and COVID-19 Severity in a Designated Hospital in Shenzhen, China. *Diabetes Care* (2020) 43(7):1392–8. doi: 10.2337/dc20-0576
27. Palaiodimos L, Kokkinidis DG, Li W, Karamanis D, Ognibene J, Arora S, et al. Severe Obesity Is Associated With Higher in-Hospital Mortality in a Cohort of Patients With COVID-19 in the Bronx, New York. *Metabolism* (2020) 108:154262. doi: 10.1016/j.metabol.2020.154262
28. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA - J Am Med Assoc* (2020) 323(20):2052–9. doi: 10.1001/jama.2020.6775
29. Popkin BM, Du S, Green WD, Beck MA, Algaith T, Herbst CH, et al. Individuals With Obesity and COVID-19: A Global Perspective on the Epidemiology and Biological Relationships. *Obes Rev* (2020) 21(11):1–17. doi: 10.1111/obr.13128
30. Anderson MR, Geleris J, Anderson DR, Zucker J, Nobel YR, Freedberg D, et al. Body Mass Index and Risk for Intubation or Death in SARS-CoV-2 Infection. *Ann Intern Med* (2020) 6:782–90. doi: 10.7326/M20-3214
31. Lighter J, Phillips M, Hochman S, Sterling S, Johnson D, Francois F, et al. Obesity in Patients Younger Than 60 Years Is a Risk Factor for COVID-19 Hospital Admission. *Clin Infect Dis* (2020) 71(15):896–7. doi: 10.1093/cid/ciaa415
32. Nogueira-de-Almeida CA, Del Ciampo LA, Ferraz IS, Del Ciampo IRL, Contini AA, Ued F da V. COVID-19 and Obesity in Childhood and Adolescence: A Clinical Review. *J Pediatr (Rio J)* (2020) 96(5):546–58. doi: 10.1016/j.jped.2020.07.001
33. Deng M, Qi Y, Deng L, Wang H, Xu Y, Li Z, et al. Obesity as a Potential Predictor of Disease Severity in Young COVID-19 Patients: A Retrospective Study. *Obesity* (2020) 8(10):1815–25. doi: 10.1002/oby.22943
34. Iacobellis G, Malavazos AE, Ferreira T. COVID-19 Rise in Younger Adults With Obesity: Visceral Adiposity Can Predict the Risk. *Obesity* (2020) 28 (10):1795. doi: 10.1002/oby.22951
35. Petersen A, Bressem K, Albrecht J, Thieß H-M, Vahldiek J, Hamm B, et al. The Role of Visceral Adiposity in the Severity of COVID-19: Highlights From a Unicenter Cross-Sectional Pilot Study in Germany. *Metabolism* (2020) 110:154317. doi: 10.1016/j.metabol.2020.154317
36. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A Crucial Role of Angiotensin Converting Enzyme 2 (ACE2) in SARS Coronavirus-Induced Lung Injury. *Nat Med* (2005) 11(8):875–9. doi: 10.1038/nm1267
37. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* (2020) 181(2):271–80.e8. doi: 10.1016/j.cell.2020.02.052
38. Kruglikov IL, Scherer PE. The Role of Adipocytes and Adipocyte-Like Cells in the Severity of COVID-19 Infections. *Obesity* (2020) 28(7):1187–90. doi: 10.1002/oby.22856
39. Li MY, Li L, Zhang Y, Wang XS. Expression of the SARS-CoV-2 Cell Receptor Gene ACE2 in a Wide Variety of Human Tissues. *Infect Dis Poverty* (2020) 9(1):1–7. doi: 10.1186/s40249-020-00662-x

40. O'Rourke RW, Lumeng CN. Pathways to Severe COVID-19 for People With Obesity. *Obesity (Silver Spring)* (2020) 29(4):645–53. doi: 10.1002/oby.23099
41. Méry G, Epaulard O, Borel A, Toussaint B, Lucas R. COVID-19 : Underlying Adipokine Storm and Angiotensin 1-7 Umbrella. *Front Immunol* (2020) 11:1–10. doi: 10.3389/fimmu.2020.01714
42. Gupte M, Boustany-Kari CM, Bharadwaj K, Police S, Thatcher S, Gong MC, et al. ACE2 Is Expressed in Mouse Adipocytes and Regulated by a High-Fat Diet. *Am J Physiol Regul Integr Comp Physiol* (2008) 295(3):781–8. doi: 10.1152/ajpregu.00183.2008
43. Kass DA, Duggal P, Cingolani O. Obesity Could Shift Severe COVID-19 Disease to Younger Ages. *Lancet* (2020) 395(10236):1544–5. doi: 10.1016/S0140-6736(20)31024-2
44. Ryan PM, Caplice NM. Is Adipose Tissue a Reservoir for Viral Spread, Immune Activation, and Cytokine Amplification in Coronavirus Disease 2019? *Obesity* (2020) 28(7):1–4. doi: 10.1002/oby.22843
45. Luzi L, Radaelli MG. Influenza and Obesity: Its Odd Relationship and the Lessons for COVID-19 Pandemic. *Acta Diabetol* (2020) 57(6):759–64. doi: 10.1007/s00592-020-01522-8
46. Romaní-Pérez M, Outeiriño-Iglesias V, Moya CM, Santisteban P, González-Matías LC, Vigo E, et al. Activation of the GLP-1 Receptor by Liraglutide Increases ACE2 Expression, Reversing Right Ventricle Hypertrophy, and Improving the Production of SP-A and SP-B in the Lungs of Type 1 Diabetes Rats. *Endocrinology* (2015) 156(10):3559–69. doi: 10.1210/en.2014-1685
47. Tikoo K, Patel G, Kumar S, Karpe PA, Sanghavi M, Malek V, et al. Tissue Specific Up Regulation of ACE2 in Rabbit Model of Atherosclerosis by Atorvastatin: Role of Epigenetic Histone Modifications. *Biochem Pharmacol* (2015) 93(3):343–51. doi: 10.1016/j.bcp.2014.11.013
48. Zhang W, Xu Y-Z, Liu B, Wu R, Yang Y-Y, Xiao X-Q, et al. Pioglitazone Upregulates Angiotensin Converting Enzyme 2 Expression in Insulin-Sensitive Tissues in Rats With High-Fat Diet-Induced Nonalcoholic Steatohepatitis. *SciWorld J* (2014) 2014:603409. doi: 10.1155/2014/603409
49. Wang J, Chen S, Bihl J. Exosome-Mediated Transfer of ACE2 (Angiotensin-Converting Enzyme 2) From Endothelial Progenitor Cells Promotes Survival and Function of Endothelial Cell. *Oxid Med Cell Longev* (2020) 2020:4213541. doi: 10.1155/2020/4213541
50. Al-Benna S. Association of High Level Gene Expression of ACE2 in Adipose Tissue With Mortality of COVID-19 Infection in Obese Patients. *Obes Med* (2020) 19:100283. doi: 10.1016/j.obmed.2020.100283
51. Cinti S, Graciotti L, Giordano A. COVID-19 and Fat Embolism : A Hypothesis to Explain the Severe Clinical Outcome in People With Obesity. *Int J Obes* (2020) 44(8):1800–2. doi: 10.1038/s41366-020-0624-5
52. Meng Y, Yu W, Liu Z, Zhang M, Chen Y, Li S, et al. The Inflammation Patterns of Different Inflammatory Cells in Histological Structures of Hyperplasic Prostatic Tissues. *Transl Androl Urol* (2020) 9(4):1639–49. doi: 10.21037/tau-20-448
53. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The Role of Biomarkers in Diagnosis of COVID-19 - a Systematic Review. *Life Sci* (2020) 254:117788. doi: 10.1016/j.lfs.2020.117788
54. Docea AO, Tsatsakis A, Albulescu D, Cristea O, Zlatian O, Vinceti M, et al. A New Threat From an Old Enemy: Re–Emergence of Coronavirus (Review). *Int J Mol Med* (2020) 45(6):1631–43. doi: 10.3892/ijmm.2020.4555
55. Petrakis D, Margină D, Tsarouhas K, Kouretas D, Spandidos DA, Tsatsakis A. Obesity – A Risk Factor for Increased COVID – 19 Prevalence, Severity and Lethality (Review). *Mol Med Rep* (2020) 22(1):9–19. doi: 10.3892/mmr.2020.11127
56. Mangge H, Almer G, Truschnig-Wilders M, Schmidt A, Gasser R, Fuchs D. Inflammation, Adiponectin, Obesity and Cardiovascular Risk. *Curr Med Chem* (2010) 17(36):4511–20. doi: 10.2174/092986710794183006
57. Trayhurn P. Hypoxia and Adipose Tissue Function and Dysfunction in Obesity. *Physiol Rev* (2013) 93(1):1–21. doi: 10.1152/physrev.00017.2012
58. Grosfeld A, André J, De Mouzon SH, Berra E, Pouysségur J, Guerre-Millo M. Hypoxia-Inducible Factor 1 Transactivates the Human Leptin Gene Promoter. *J Biol Chem* (2002) 277(45):42953–7. doi: 10.1074/jbc.M206775200
59. Palhinha L, Liechocki S, Hottz ED, Pereira JA da S, de Almeida CJ, Moraes-Vieira PMM, et al. Leptin Induces Proadipogenic and Proinflammatory Signaling in Adipocytes. *Front Endocrinol (Lausanne)* (2019) 10:841. doi: 10.3389/fendo.2019.00841
60. La Cava A, Matarese G. The Weight of Leptin in Immunity. *Nat Rev Immunol* (2004) 4(5):371–9. doi: 10.1038/nri1350
61. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, et al. CCR2 Modulates Inflammatory and Metabolic Effects of High-Fat Feeding Find the Latest Version: CCR2 Modulates Inflammatory and Metabolic Effects of High-Fat Feeding. *J Clin Invest* (2006) 1(116):115–24. doi: 10.1172/JCI24335
62. Kusminski CM, McTernan PG, Schraw T, Kos K, O'Hare JP, Ahima R, et al. Adiponectin Complexes in Human Cerebrospinal Fluid: Distinct Complex Distribution From Serum. *Diabetologia* (2007) 50(3):634–42. doi: 10.1007/s00125-006-0577-9
63. Wolf AM, Wolf D, Rumpold H, Moschen AR, Kaser A, Obrist P, et al. Overexpression of Indoleamine 2,3-Dioxygenase in Human Inflammatory Bowel Disease. *Clin Immunol* (2004) 113(1):47–55. doi: 10.1016/j.clim.2004.05.004
64. Kershaw EE, Flier JS. Adipose Tissue as an Endocrine Organ. *J Clin Endocrinol Metab* (2004) 89(6):2548–56. doi: 10.1210/jc.2004-0395
65. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, An Adipokine With Potent Proinflammatory Properties. *J Immunol* (2005) 174(9):5789–95. doi: 10.4049/jimmunol.174.9.5789
66. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. Human Resistin Stimulates the Pro-Inflammatory Cytokines TNF-α and IL-12 in Macrophages by NF-κB-Dependent Pathway. *Biochem Biophys Res Commun* (2005) 334(4):1092–101. doi: 10.1016/j.bbrc.2005.06.202
67. Verma S, Buchanan MR, Anderson TJ. Endothelial Function Testing as a Biomarker of Vascular Disease. *Circulation* (2003) 108(17):2054–9. doi: 10.1161/01.CIR.0000089191.72957.ED
68. Tilg H, Moschen AR. Adipocytokines : Mediators Linking Adipose Tissue, Inflammation and Immunity. *Nat Rev Immunol* (2006) 6(September):772–83. doi: 10.1038/nri1937
69. Abella V, Scotece M, Conde J, Pino J, Gonzalez-Gay MA, Gómez-Reino JJ, et al. Leptin in the Interplay of Inflammation, Metabolism and Immune System Disorders. *Nat Rev Rheumatol* (2017) 13(2):100–9. doi: 10.1038/nrrheum.2016.209
70. Rebello CJ, Kirwan JP, Greenway FL. Obesity, the Most Common Comorbidity in SARS-CoV-2: Is Leptin the Link? *Int J Obes* (2020) 44(9):1810–7. doi: 10.1038/s41366-020-0640-5
71. Maurya R, Bhattacharya P, Dey R, Nakhasi HL. Leptin Functions in Infectious Diseases. *Front Immunol* (2018) 9:1–15. doi: 10.3389/fimmu.2018.02741
72. Pérez-Pérez A, Vilariño-García T, Fernández-Riejos P, Martín-González J, Segura-Egea JJ, Sánchez-Margalet V. Role of Leptin as a Link Between Metabolism and the Immune System. *Cytokine Growth Factor Rev* (2017) 35(2016):71–84. doi: 10.1016/j.cytogfr.2017.03.001
73. Li M-D. Leptin and Beyond: An Odyssey to the Central Control of Body Weight. *Yale J Biol Med* (2011) 84(1):1–7.
74. Procaccini C, Jirillo E, Matarese G. Molecular Aspects of Medicine Leptin as an Immunomodulator. *Mol Aspects Med* (2012) 33(1):35–45. doi: 10.1016/j.mam.2011.10.012
75. Ikejima S, Sasaki S, Sashinami H, Mori F, Ogawa Y, Nakamura T, et al. Impairment of Host Resistance to Listeria Monocytogenes Infection in Liver of Db/Db and Ob/Ob Mice. *Diabetes* (2005) 54(1):182–9. doi: 10.2337/diabetes.54.1.182
76. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin Modulates the T-Cell Immune Response and Reverses Starvation-Induced Immunosuppression. *Nature* (1998) 394(6696):897–901. doi: 10.1038/29795
77. Busso N, So A, Chobaz-Péclat V, Morard C, Martinez-Soria E, Talabot-Ayer D, et al. Leptin Signaling Deficiency Impairs Humoral and Cellular Immune Responses and Attenuates Experimental Arthritis. *J Immunol* (2002) 168(2):875–82. doi: 10.4049/jimmunol.168.2.875
78. Matarese G, Moschos S, Mantzoros CS. Leptin in Immunology. *J Immunol* (2005) 174(6):3137–42. doi: 10.4049/jimmunol.174.6.3137
79. Faggioni R, Fantuzzi G, Gabay C, Moser A, Dinarello CA, Feingold KR, et al. Leptin Deficiency Enhances Sensitivity to Endotoxin-Induced Lethality. *Am J Physiol Regul Integr Comp Physiol* (1999) 276(1 45-1):136–42. doi: 10.1152/ajpregu.1999.276.1.R136

80. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in Inflammation and Metabolic Disease. *Nat Rev Immunol* (2011) 11(2):85–97. doi: 10.1038/nri2921
81. Yong S, Hyun J, Won S, Kim M, Kim S, Kim M, et al. Biochemical and Biophysical Research Communications Preferential Effects of Leptin on CD4 T Cells in Central and Peripheral Immune System Are Critically Linked to the Expression of Leptin Receptor. *Biochem Biophys Res Commun* (2010) 394(3):562–8. doi: 10.1016/j.bbrc.2010.03.019
82. Paz-Filho G, Mastronardi C, Franco CB, Wang KB, Wong M-L, Licinio J. Leptin: Molecular Mechanisms, Systemic Pro-Inflammatory Effects, and Clinical Implications. *Arq Bras Endocrinol Metabol* (2012) 56(9):597–607. doi: 10.1590/S0004-27302012000900001
83. Acedo SC, Gambero S, Cunha FGP, Lorand-Metze I, Gambero A. Participation of Leptin in the Determination of the Macrophage Phenotype: An Additional Role in Adipocyte and Macrophage Crosstalk. *In Vitro Cell Dev Biol Anim* (2013) 49(6):473–8. doi: 10.1007/s11626-013-9629-x
84. Faggioni R, Feingold KR, Grunfeld C. Leptin Regulation of the Immune Response and the Immunodeficiency of Malnutrition 1. *FASEB J* (2001) 15 (14):2565–71. doi: 10.1096/fj.01-0431rev
85. Baumann H, Morella KK, White DW, Dembski M, Bailon PS, Kim H, et al. The Full-Length Leptin Receptor has Signaling Capabilities of Interleukin 6-Type Cytokine Receptors. *Proc Natl Acad Sci USA* (1996) 93(16):8374–8. doi: 10.1073/pnas.93.16.8374
86. Maury E, Brichard SM. Adipokine Dysregulation, Adipose Tissue Inflammation and Metabolic Syndrome. *Mol Cell Endocrinol* (2010) 314:1–16. doi: 10.1016/j.mce.2009.07.031
87. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose Expression of Tumor Necrosis Factor-Alpha: Direct Role in Obesity-Linked Insulin Resistance. *Science* (1993) 259(5091):87–91. doi: 10.1126/science.7678183
88. Huh JY, Park YJ, Ham M, Kim JB. Crosstalk Between Adipocytes and Immune Cells in Adipose Tissue Inflammation and Metabolic Dysregulation in Obesity. *Mol Cells* (2014) 37(5):365–71. doi: 10.14348/molcells.2014.0074
89. Sonnenberg GE, Krakower GR, Kissebah AH. Perspective A Novel Pathway to the Manifestations of Metabolic Syndrome. *Obes Res* (2004) 12(2):180–6. doi: 10.1038/oby.2004.24
90. Mülberg J, Schooltink H, Stoyan T, Günther M, Graeve L, Buse G, et al. The Soluble Interleukin-6 Receptor Is Generated by Shedding. *Eur J Immunol* (1993) 23(2):473–80. doi: 10.1002/eji.1830230226
91. Tang C-H, Lu D-Y, Yang R-S, Tsai H-Y, Kao M-C, Fu W-M, et al. Leptin-Induced IL-6 Production Is Mediated by Leptin Receptor, Insulin Receptor Substrate-1, Phosphatidylinositol 3-Kinase, Akt, NF-κB, and P300 Pathway in Microglia. *J Immunol* (2007) 179(2):1292–302. doi: 10.4049/jimmunol.179.2.1292
92. Tan Y, Lim SG, Hong W. Microreview Regulation of Cell Death During Infection by the Severe Acute Respiratory Syndrome Coronavirus and Other Coronaviruses. *Cell Microbiol* (2007) 9:2552–61. doi: 10.1111/j.1462-5822.2007.01034.x
93. Konstantinides S, Schäfer K, Koschnick S, Loskutoff DJ. Leptin-Dependent Platelet Aggregation and Arterial Thrombosis Suggests a Mechanism for Atherothrombotic Disease in Obesity. *Curr Pharm Des* (2001) 24(9):1012–8. doi: 10.1172/JCI13143
94. Abd Alkhaleq H, Kornowski R, Waldman M, Levy E, Zemel R, Nudelman V, et al. Leptin Modulates Gene Expression in the Heart and Cardiomyocytes Towards Mitigating Ischemia-Induced Damage. *Exp Cell Res* (2020) 397 (2):112373. doi: 10.1016/j.yexcr.2020.112373
95. van der Voort PHJ, Moser J, Zandstra DF, Muller Kobold AC, Knoester M, Calkhoven CF, et al. Leptin Levels in SARS-CoV-2 Infection Related Respiratory Failure: A Cross-Sectional Study and a Pathophysiological Framework on the Role of Fat Tissue. *Heliyon* (2020) 6(8):e04696. doi: 10.1016/j.heliyon.2020.e04696
96. Chiappetta S. COVID-19 and the Role of Chronic Inflammation in Patients With Obesity. *Int J Obes* (2020) 6(8):e04696. doi: 10.1038/s41366-020-0597-4
97. Paquette SG, Banner D, Zhao Z, Fang Y, Huang SSH, León AJ, et al. Interleukin-6 Is a Potential Biomarker for Severe Pandemic H1N1 Influenza a Infection. *PLoS One* (2012) 7(6). doi: 10.1371/journal.pone.0038214
98. Fehr AR, Channappanavar R, Perlman S. Middle East Respiratory Syndrome: Emergence of a Pathogenic Human Coronavirus. *Annu Rev Med* (2017) 68:387–99. doi: 10.1146/annurev-med-051215-031152
99. Channappanavar R, Perlman S. Pathogenic Human Coronavirus Infections: Causes and Consequences of Cytokine Storm and Immunopathology. *Semin Immunopathol* (2017) 39(5):529–39. doi: 10.1007/s00281-017-0629-x
100. Fuster JJ, Walsh K. The Good, the Bad, and the Ugly of Interleukin-6 Signaling. *EMBO J* (2014) 33(13):1425–7. doi: 10.15252/embj.201488856
101. Han MS, White A, Perry RJ, Camporez JP, Hidalgo J, Shulman GI, et al. Regulation of Adipose Tissue Inflammation by Interleukin 6. *Proc Natl Acad Sci USA* (2020) 117(6):2751–60. doi: 10.1073/pnas.1920004117
102. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the Release of Adipokines by Adipose Tissue, Adipose Tissue Matrix, and Adipocytes From Visceral and Subcutaneous Abdominal Adipose Tissues of Obese Humans. *Endocrinology* (2004) 145(5):2273–82. doi: 10.1210/en.2003-1336
103. Wueest S, Konrad D. The Role of Adipocyte-Specific IL-6-Type Cytokine Signaling in FFA and Leptin Release. *Adipocyte* (2018) 7(3):226–8. doi: 10.1080/21623945.2018.1493901
104. Febbraio MA. Gp130 Receptor Ligands as Potential Therapeutic Targets for Obesity. *J Clin Invest* (2007) 117(4):841–9. doi: 10.1172/JCI30453
105. Mishra D, Richard JE, Maric I, Porteiro B, Häring M, Kooijman S, et al. Parabrachial Interleukin-6 Reduces Body Weight and Food Intake and Increases Thermogenesis to Regulate Energy Metabolism. *Cell Rep* (2019) 26(11):3011–26.e5. doi: 10.1016/j.celrep.2019.02.044
106. Dunn-Meynell AA, Le Foll C, Johnson MD, Lutz TA, Hayes MR, Levin BE. Endogenous VMH Amylin Signaling Is Required for Full Leptin Signaling and Protection From Diet-Induced Obesity. *Am J Physiol Regul Integr Comp Physiol* (2016) 310(4):R355–65. doi: 10.1152/ajpregu.00462.2015
107. Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC. Defective STAT Signaling by the Leptin Receptor in Diabetic Mice. *Proc Natl Acad Sci USA* (1996) 93(13):6231–5. doi: 10.1073/pnas.93.13.6231
108. Zhao T, Hou M, Xia M, Wang Q, Zhu H, Xiao Y, et al. Globular Adiponectin Decreases Leptin-Induced Tumor Necrosis Factor-α Expression by Murine Macrophages: Involvement of cAMP-PKA and MAPK Pathways. *Cell Immunol* (2005) 238(1):19–30. doi: 10.1016/j.cellimm.2005.12.002
109. Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet DJ, et al. Multiple Cytokines and Acute Inflammation Raise Mouse Leptin Levels: Potential Role in Inflammatory Anorexia. *J Exp Med* (1997) 185(1):171–5. doi: 10.1084/jem.185.1.171
110. Guo X, Roberts MR, Becker SM, Podd B, Zhang Y, Chua SC, et al. Leptin Signaling in Intestinal Epithelium Mediates Resistance to Enteric Infection by Entamoeba Histolytica. *Mucosal Immunol* (2011) 4(3):294–303. doi: 10.1038/mi.2010.76
111. Ubags NDJ, Stapleton RD, Vernooy JHJ, Burg E, Bement J, Hayes CM, et al. Hyperleptinemia Is Associated With Impaired Pulmonary Host Defense. *JCI Insight* (2016) 1(8):294–303. doi: 10.1172/jci.insight.82101
112. Mancuso P, Canetti C, Gottschalk A, Tithof PK, Peters-Golden M. Leptin Augments Alveolar Macrophage Leukotriene Synthesis by Increasing Phospholipase Activity and Enhancing Group IVC iPLA2 (cPLA2gamma) Protein Expression. *Am J Physiol Lung Cell Mol Physiol* (2004) 287(3):L497–502. doi: 10.1152/ajplung.00010.2004
113. Larsen L, Le Foll C, Dunn-Meynell AA, Levin BE. IL-6 Ameliorates Defective Leptin Sensitivity in DIO Ventromedial Hypothalamic Nucleus Neurons. *Am J Physiol Regul Integr Comp Physiol* (2016) 311(4):R764–70. doi: 10.1152/ajpregu.00258.2016
114. Roytblat L, Rachinsky M, Fisher A, Greemberg L, Shapira Y, Douvdevani A, et al. Raised Interleukin-6 Levels in Obese Patients. *Obes Res* (2000) 8 (9):673–5. doi: 10.1038/oby.2000.86
115. Demidowich AP, Levine JA, Apps R, Cheung FK, Chen J, Fantoni G, et al. Colchicine ' s Effects on Metabolic and Inflammatory Molecules in Adults With Obesity and Metabolic Syndrome : Results From a Pilot Randomized Controlled Trial. *Obes Res* (2020) 8(9):673–5. doi: 10.1038/s41366-020-0598-3
116. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and Toxicity Management of 19-28z CAR T Cell Therapy in B Cell Acute Lymphoblastic Leukemia. *Sci Transl Med* (2014) 6(224):224ra25. doi: 10.1126/scitranslmed.3008226
117. Moore JB, June CH. Cytokine Release Syndrome in Severe COVID-19. *Science* (2020) 368(6490):473–4. doi: 10.1126/science.abb8925
118. Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al. Effective Treatment of Severe COVID-19 Patients With Tocilizumab. *Science* (2020) 117(20):10970–5. doi: 10.1073/pnas.2005615117

119. Del Valle DM, Kim-Schulze S, Huang H-H, Beckmann ND, Nirenberg S, Wang B, et al. An Inflammatory Cytokine Signature Predicts COVID-19 Severity and Survival. *Nat Med* (2020) 26(10):1636–43. doi: 10.1038/s41591-020-1051-9
120. Dinarello CA, Simon A, van der Meer JWM. Treating Inflammation by Blocking Interleukin-1 in a Broad Spectrum of Diseases. *Nat Rev Drug Discov* (2012) 11(8):633–52. doi: 10.1038/nrd3800
121. Konter JM, Parker JL, Baez E, Li SZ, Ranscht B, Denzel M, et al. Adiponectin Attenuates Lipopolysaccharide-Induced Acute Lung Injury Through Suppression of Endothelial Cell Activation. *J Immunol* (2012) 188(2):854–63. doi: 10.4049/jimmunol.1100426
122. Niv-Spector L, Gonen-Berger D, Gourdou I, Biener E, Gussakovsky EE, Benomar Y, et al. Identification of the Hydrophobia Strand in the A-B Loop of Leptin as Major Binding Site III: Implications for Large-Scale Preparation of Potent Recombinant Human and Ovine Leptin Antagonists. *Biochem J* (2005) 391(2):221–30. doi: 10.1042/BJ20050457
123. Shpilman M, Niv-Spector L, Katz M, Varol C, Solomon G, Ayalon-Soffer M, et al. Development and Characterization of High Affinity Leptins and Leptin Antagonists. *J Biol Chem* (2011) 286(6):4429–42. doi: 10.1074/jbc.M110.196402
124. Elinav E, Niv-Spector L, Katz M, Price TO, Ali M, Yacobovitz M, et al. Pegylated Leptin Antagonist Is a Potent Orexigenic Agent: Preparation and Mechanism of Activity. *Endocrinology* (2009) 150(7):3083–91. doi: 10.1210/en.2008-1706
125. Ottaway N, Mahbod P, Rivero B, Norman LA, Gertler A, D'Alessio DA, et al. Diet-Induced Obese Mice Retain Endogenous Leptin Action. *Cell Metab* (2015) 21(6):877–82. doi: 10.1016/j.cmet.2015.04.015
126. Banks WA, Gertler A, Solomon G, Niv-Spector L, Shpilman M, Yi X, et al. Principles of Strategic Drug Delivery to the Brain (SDDB): Development of Anorectic and Orexigenic Analogs of Leptin. *Physiol Behav* (2011) 105 (1):145–9. doi: 10.1016/j.physbeh.2011.05.024
127. Gertler A, Solomon G. Leptin-Activity Blockers: Development and Potential Use in Experimental Biology and Medicine. *Can J Physiol Pharmacol* (2013) 91(11):873–82. doi: 10.1139/cjpp-2013-0012
128. Solomon G, Atkins A, Shahar R, Gertler A, Monsonego-Ornan E. Effect of Peripherally Administered Leptin Antagonist on Whole Body Metabolism and Bone Microarchitecture and Biomechanical Properties in the Mouse. *Am J Physiol Endocrinol Metab* (2014) 306(1):E14–27. doi: 10.1152/ajpendo.00155.2013
129. Mak RH, Cheung WW, Solomon G, Gertler A. Preparation of Potent Leptin Receptor Antagonists and Their Therapeutic Use in Mouse Models of Uremic Cachexia and Kidney Fibrosis. *Curr Pharm Des* (2018) 24 (9):1012–8. doi: 10.2174/1381612824666180125094921

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Maurya, Sebastian, Namdeo, Devender and Gertler. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 18 June 2021
doi: 10.3389/fnut.2021.683261

Effects of the Mediterranean Lifestyle During the COVID-19 Lockdown in Spain: Preliminary Study

Ana Zaragoza-Martí[1], Miriam Sánchez-SanSegundo[2], Rosario Ferrer-Cascales[2], Eva Maria Gabaldón-Bravo[1], Ana Laguna-Pérez[1] and Lorena Rumbo-Rodríguez[1]*

[1] Nursing Department, Health Psychology and Human Behavior Research Group, Faculty of Health Sciences, University of Alicante, Institute for Health and Biomedical Research (ISABIAL), Alicante, Spain, [2] Department of Health Psychology, Health Psychology and Human Behavior Research Group, Faculty of Health Sciences, University of Alicante, Alicante, Spain

We aim to assess the beneficial effects of the Mediterranean style-diet before and after the period of confinement due to COVID-19 in a sample of 51 older patients who were part of a clinical trial of the Instituto de Investigación sanitaria y Biomédica de Alicante (ISABIAL, CEIM). Participants were randomly assigned to two conditions: experimental vs. a control group. A pre-test survey assessment was conducted before confinement, while a post-test survey was conducted after the confinement period. Adherence to Mediterranean Diet and nutritional status were evaluated through self-reported questionnaires. Individuals who initiated the Mediterranean Diet intervention program before confinement increased 3.5% their level of adherence to the Mediterranean Diet and maintained their nutritional status after the confinement. In the case of BMI, there no were statistically significant differences between groups before and after confinement. These results suggest that adherence to the Mediterranean Diet may play an important role in the establishment of appropriate dietary guidelines in confinement situations such as the COVID-19.

Keywords: COVID-19, mediterranean diet, confinement, nutritional status, older

OPEN ACCESS

Edited by:
Ioannis Zabetakis, University of Limerick, Ireland

Reviewed by:
Jose Paulo Andrade, Universidade Do Porto, Portugal
Leila Lujan-Barroso, Bellvitge Biomedical Reserach Institute -IDIBELL, Spain

****Correspondence:***
Miriam Sánchez-SanSegundo
miriam.sanchez@ua.es

Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: *20 March 2021*
Accepted: *26 May 2021*
Published: *18 June 2021*

Citation:
Zaragoza-Martí A, Sánchez-SanSegundo M, Ferrer-Cascales R, Gabaldón-Bravo EM, Laguna-Pérez A and Rumbo-Rodríguez L (2021) Effects of the Mediterranean Lifestyle During the COVID-19 Lockdown in Spain: Preliminary Study. Front. Nutr. 8:683261. doi: 10.3389/fnut.2021.683261

INTRODUCTION

The Coronavirus disease (SARS-COV2), initially originated in the Chinese city of Wuhan in late 2019, has become a global health alarm due to its high infection potential and rapid spread. This situation has led governments of most nations around the world, including Spain, to take exceptional measures, such as the state of alarm, confining the entire population to their homes to control the progress of the contagionS (1).

The limitation of the social and personal life of the population has had a significant impact on people's health, especially in terms of habits of physical activity, psychological health levels, and eating patterns. Different studies have shown that the lockdown produced by the SARS-COV2 disease has triggered levels of anxiety, depression, perceived stress, and fear in the population, and has led to greater alterations in food and nutritional status in general (2, 3). These lifestyle changes caused by the lockdown can increase the risk of developing pathologies traditionally associated with populational sedentary lifestyles such as obesity, type II diabetes, cardiovascular disease, or chronic degenerative diseases (4). These diseases are particularly prevalent in the group of older people, who are more vulnerable, due to physiological changes caused by age in different systems such as basal metabolism, digestive system, cardiovascular system, nervous system, and motor system (5, 6).

Hence, the importance of maintaining an active lifestyle and healthy eating habits has been highlighted, especially in situations of confinement (7). In this sense, one of the best dietary patterns with the greatest health benefits is the Mediterranean Diet (MD) model. The evidence accumulated to date suggests that the MD plays a protective role in the prevention of numerous pathologies. The MD is characterized by being rich in fruits and vegetables, legumes, whole grains, nuts, and olive oil; because it is moderate in dairy, fish and lean meats and because it includes occasional consumption of red meats and derivatives and processed foods. Besides, the MD pattern is also characterized by having a social nature, that is, eating at the table with family, sharing experiences, respecting local gastronomy and seasonal products, as well as promoting an active lifestyle (8).

The benefits of the MD have been highlighted in numerous individual studies and meta-analyses showing that it reduces mortality rates by 8%, the risk of developing cardiovascular disease by 10%, and other diseases by 4% (9). The MD has also been shown to have a protective effect against the development of some cancers and the delay in the onset of neurodegenerative diseases associated with aging (10). The MD has been considered to be the most beneficial diet due to anti-inflammatory and antioxidative properties, particularly during aging. For example, the effect of the Mediterranean Diet on Metabolic Health has been found in a recent meta-analysis of controlled trials of 36,983 participants, which showed the beneficial changes in 18 of 28 metabolic syndrome components (11). Also, a recent meta-analysis of cross-section studies comparing 13,733 participants from 5 countries has shown a positive association between adherence to the MD and longer telomere length. It has been suggested that telomere shortening is the primary cause of aging. Thus, MD may play an important role in the preservation of telomere length and longevity (12).

Thus, following a healthy diet pattern such as the Mediterranean diet, is important, given that the levels of gene expression of cytokinins can be modified according to the foods that are ingested and they may be able to modulate the inflammatory processes, oxidative stress, and telomere length related which have been associated with aging and multiples diseases including COVID-19. There is evidence showing that the key to the Mediterranean diet in modulating the inflammatory response is due to the MD is characterized by low cholesterol levels, high levels of antioxidants from fruits and vegetables and high content of monounsaturated fatty acids and polyunsaturated precedents from olive oil, nuts, and oily fish (13).

From this perspective of promoting Mediterranean health and lifestyle, the objective of this work was to determine the degree of adherence to the MD and the changes in nutritional status before and after the lockdown in a sample of older citizens residing in the Spanish Mediterranean. To date, the PREDIMED multicenter, randomized prevention trial is the most important study conducted in Spain that demonstrates the benefits of the MD diet on health and in particular in cardiovascular disease (14). A previous study conducted in Spain has demonstrated that the COVID-19 confinement in Spain has led to the adoption of better healthy dietary habits, including a higher adherence to the MedDiet (15). However, a more recent work has also found that the consumption of unhealthy food has also increased during the pandemic situation caused by the COVID-19 infection in Spain (4).

Given the previous positive findings of the MD we hypothesize that individuals who initiated an intervention program based on the benefits of the MD before the lockdown will show adequate adherence to the MD and maintenance of their nutritional status. To confirm this hypothesis, we evaluated a sample of 51 patients who were part of the clinical trial of the Alicante Institute for Health and Biomedical Research (ISABIAL, CEIM).

MATERIALS AND METHODS

Sample and Procedure

The sample included 51 older people from the Mediterranean city of Alicante (Spain). Participants ranged in age from 60 to 74 years ($M = 65$, $SD = 4$, 67). Inclusion criteria were: (1) being over 60 years of age and having attended the scheduled consultation of the health care professional of the health center, (2) having a Body Mass Index (BMI) >25 Kg/m^2. Exclusion criteria were: patients with a score of 3 or more errors (if they had studies) in the Pfeiffer's Short Portable Mental Status Questionnaire which evaluates the cognitive deterioration by means of 10 questions (i.e., who is the current president?) (16) and four or more errors (no studies), having reported reading and writing difficulties, having followed dietary-nutritional treatment supervised by a nutritionist over the past year, and being considered too fragile according to medical or nursing criteria. Participants were randomly assigned to two conditions: experimental vs. control wait-list group. Ten participants were excluded from the initial sample. Five participants (2.5%) were excluded due to having followed a dietary treatment over the past year, 2 (1%) participants scored above three errors in the Pleiffer's questionnaire and 3 (1.5%) declined participation. The remaining 51 participants were assigned to the experimental group ($n = 25$; 96% females; 4% males) or the control group ($n = 26$; 84.6% females; 15.4% males). During the follow-up (Time 2 and Time 3), two participants from the experimental group dropped out of the intervention program and were classified as non-completers, mostly because they refused to be part of the follow-up data collection.

The study was approved by the Ethics Committee of the Instituto de Investigación Sanitaria y Biomédica de Alicante [ISABIAL (Health and Biomedical Research Institute of Alicante)] (CEIm: PI2019/057). After being informed about the study, their voluntary participation, and that they could withdraw from the study with no consequences, participants were requested to participate. Consents were obtained from the entire sample. Several additional attempts were made by the project staff to engage participants who did not return a signed form.

Experimental Design

Participants were recruited from the Department of Health Alicante—General Hospital (Spain). Demographic data, including age, sex, nationality, employment status, financial

income, weight, size, BMI, and hip-waist perimeter were gathered from the participants. All participants completed a pre-/post-test battery of questionnaires which included adherence levels to the MD, anthropometric data, lifestyle, and physical activity. For the experimental group, the pre-test battery was followed by the implementation of the intervention program over the subsequent 6 months, between September 2019 and June 2020 and 3 months of follow-up. The program consisted of nine 1-h individual sessions and six 2-h group sessions distributed across 6 months. A cut-off $\geq$ 7 sessions of the total number of sessions was used to classify participants as completers (representing at least 80% attendance of the program).

Intervention Program

A multi-component intervention was performed for 6 months and 3 of follow-up. Participants in the experimental group received personalized training for weight management, food education, psychological support, and self-care recommendations. During the individual sessions, each participant received a menu adapted to their needs based on the MD. Besides, three group sessions were conducted. During the group sessions, general training in MD, nutritional labeling, and healthy recipes were provided Psychological support sessions throughout the intervention included issues related to self-control, anxiety management, emotional feeding, achievements, and difficulties encountered during treatment, review of weekly action plans, relaxation techniques, consolidation of the new image, cognitive distortions, change management, coping with uncertainty, and motivation to change. These sessions were conducted by highly qualified psychology professionals. For physical activity, at least 150 min per week was recommended, following the recommendations of the World Health Organization (WHO). In these sessions, in addition to the practice of physical exercise, training was included in sedentarism (epidemiology and consequences), health benefits of physical exercise, types of physical exercise, and community benefits and resources for the practice of physical exercise. The intervention program was conducted from September 2019 to March 2020. The follow-up was conducted from March to June 2020 through several methods: telephone, whatsApp^{TM}, mail and a short electronic questionnaire which was shared *via* Google survey platform. Also, all participants who received the intervention were invited to participate several online courses about healthy diet during confinement within the Aula de la Salud of the University of Alicante (see https://mayoresalud.ua.es/formacion/#alimentacion).

Measures

Eating Habits

To determine the degree of adherence to the MD, we employed a specific short questionnaire of 14 items validated for the Spanish population and used by the Mediterranean Diet Prevention group (PREDIMED) (17). To obtain the score, each item with a positive connotation for the MD is assigned a value of +1 and, if the item has a negative connotation, it receives a value of −1. From the sum of the values obtained in the 14 items, the degree of adherence is determined, establishing two levels, such that if the total score is ≥9, the score reflects a good level of adherence and if the total sum is <9, the score shows low adherence. To determine participants' eating habits, a dietary interview was prepared, following the guidelines of the Spanish Society of Dietitians-Nutritionists.

Sociodemographic and Clinical Variables

An *ad hoc* questionnaire was used to collect sociodemographic, clinical, and lifestyle data. The sociodemographic data contemplated in our study were age, sex, marital status, years of schooling, and place of residence. The clinical variables studied were systolic and diastolic blood pressure, weight, and height. Finally, the lifestyle variables studied were actual alcohol consumption (never or yes on a regular basis) tobacco use (never or yes on a regular basis), and physical activity (hours/weeks).

Anthropometric Variables

Standardized methods were used to measure anthropometric data. Bodyweight was measured using a vertical mechanical scale with SECA700® sliding weights, with an accuracy of 100 g. Height was measured with an accuracy of 0.2 cm, using the vertical meter. With the data of weight in kg and height in cm, the BMI was calculated (BMI = weight/height^2; that is, Kg/m^2). BMI was interpreted using the WHO classification (BMI < 18.8 = low weight; BMI between 18.5 and 24.99 = normal weight; BMI between 25 and 29.9 = overweight; and BMI > 30 = obesity). Body perimeters were measured in triplicate (later obtaining the mean) with an extendable tape measure. The waist perimeter measurement was performed below the rib cage and above the navel (the narrowest waist circumference). The hip perimeter was taken horizontally at the maximum extension area of the buttocks (largest posterior protrusion). With the result of the two measurements, the waist-hip index (ICC = waist/hip) was calculated. To assess the presence of cardiovascular risk, participants were classified according to the result of the waist-hip index. In the case of women, cardiovascular risk was determined when the index was 0.85 or more and, for men, when it was 0.94 or more.

Statistical Analysis

Descriptive statistics were used to define the proportion of responses for each question and the total distribution of the questionnaire. The normality of the data was confirmed using the Shapiro-Wilks *W*-test. Values were reported as means and percentage. To assess the intervention effect before and after the confinement period, two independent Generalized Estimating Equations (GEE) for the dichotomous variables "adherence to MD" and "obesity" were applied using the binomial link function with an exchangeable correlation structure and adjusted by age. These analyses were performed using the geepack R program v.4.0.2. Furthermore, the percentage change between scores from T1 to T2 was calculated following this formula: [(T2-T1/T1)]*100. These analyses were performed using IBM SPSS, Statistics for Windows, Version 24.0, considering $p < 0.05$ as significant.

TABLE 1 | Sociodemographic characteristics of the participants.

	Control group $N = 26$	Experimental group $N = 25$	Total	*P*
Age	66.69 ± 5.14	63.44 ± 3.40	65.1 ± 4.67	0.01
Sex				0.61
Female	24 (92.3)	22 (88.0)	46 (90.2)	
Male	2 (7.7)	13 (12.0)	15 (9.8)	
Marital status				0.54
Married	17 (65.4)	19 (76.0)	36 (70.6)	
Others	9 (34.6)	6 (24.0)	15 (29.4)	
Nationality				
Spanish	26 (100.0)	25 (100.0)	51 (100.0)	
Occupational situation				0.12
Full-Party time/employee	5 (19.2)	11 (44.0)	16 (31.3)	
Retired	16 (61.6)	9 (36.0)	25 (49.0)	
Other	5 (2, 18)	5 (20.0)	10 (19.7)	
Coexistence				
At home alone	6 (23.1)	3 (12.0)	9 (17.6)	0.58
At home with my partner	19 (73.1)	21 (84.0)	40 (78.4)	
Others	1 (3.8)	1 (4.0)	2 (3.9)	
Educational level				0.19
No studies	1 (3.8)	1 (4.0)	2 (3.9)	
Primary studies	11 (42.3)	4 (16.0)	15 (29.4)	
Middle studies	10 (38.5)	12 (48.0)	22 (43.1)	
Higher education	4 (15.4)	8 (32,0)	12 (23.5)	
Income				0.25
<1000€	13 (50.0)	8 (32.0)	21 (42.0)	
>1000€	13 (50.0)	17 (68.0)	30 (58.0)	
Tobacco use				0.66
Yes	2 (7.7)	3 (12.0)	5 (9, 8)	
No tobacco use	24 (92.3)	22 (88.0)	46 (90,2)	
Alcohol consumption				0.78
Yes	12 (46.2)	13 (52.0)	25 (49.0)	
Doesn't drink alcohol	14 (53.8)	12 (48.0)	26 (51.0)	

TABLE 2 | GEE models for high adherence to MD and for Obesity.

		OR	IC 95%	*P*-value
High adherence to MD				
GROUP	Control	1		
	Experimental	9.83	(3.61–26.78)	<0.001
Age	(years)	1.01	(0.92–1.12)	0.779
Obesity				
GROUP	Control	1		
	Experimental	0.90	(0.38–2.14)	0.812
Age	(years)	0.96	(0.87–1.05)	0.371

TABLE 3 | Mean of percentage change by groups in the score of Adherence to the MD and nutritional status before and after the lockdown.

	Mean (SD) before lockdown	Mean (SD) after lockdown	Percentage change
Adherence to MD (PREDIMED total score)			
Control group	7.88 ± 1.75	7.88 ± 2.75	0.00%
Experimental group	9.00 ± 1.68	9.32 ± 1.68	3.50%
BMI			
Control group	31.98 ± 3.88	32.05 ± 3.95	0.21%
Experimental group	31.05 ± 4.38	31.08 ± 4.44	0.09%

MD, Mediterranean diet; BMI, body mass index.

RESULTS

All participants in the study had Spanish nationality and lived in the province of Alicante. The average age of the control group was 66.60 years vs. 63.44 years of the experimental group. Regarding marital status, there were no single participants, and more than 65% of the sample, both in the experimental group and the control group, was married. In the case of employment, 40% of the participants in the experimental group were active full-time compared to 7.7% of the control group. Regarding coexistence, more than 70% of the participants lived with their partner, and none of the participants lived in residences or in the home of other relatives. All the other sociodemographic and lifestyle variables are shown in **Table 1**.

As displayed in **Table 2**, there were a significant effect of intervention in the experimental group after adjusted for age (OR 9.8, 95% CI 3.61-26.78), $p < 0.001$. However, there were no significant effect of the intervention regarding the BMI, again after adjusted for age (OR 0.90, 95% CI 0.38-2.14), $p < 0.812$.

Table 3 shows adherence to MD and nutritional status before and after the lockdown, taking into account the distribution into the experimental group or the control group. Regarding adherence to MD, in the control group, no variation was observed before and after the lockdown, but in the case of the experimental group, there was a significant improvement of 3.5% of percentage of change to adherence to MD. Concerning nutritional status, the experimental there were few variations after the lockdown with no significant effect before and after lockdown.

Table 4 shows the dietary behavior of participants during the lockdown. We observed that more than 60% of the control group participants ingested more food during the lockdown compared to 48% of the experimental group. Concerning the way of cooking, we found that the experimental group changed the way they cooked to a healthy cooking style in 84% of cases. Finally, concerning snacking throughout the day, we observed that more than 50% of the participants in both groups performed some type of snacking, in 56% of the cases due to anxiety in the experimental group vs. 34.6% in the control group.

DISCUSSION

The COVID-19 lockdown has significantly affected the population's dietary patterns and physical activity, with most studies showing that it is strongly associated with increased weight gain and BMI. These negative effects have been associated with high levels of isolation, fear, anxiety, and depression

TABLE 4 | Eating behavior during the lockdown in the control group and the experimental group.

	Control group		Experimental group	
	Yes *n* (%)	No *n* (%)	Yes *n* (%)	No *n* (%)
Have you eaten more food than normal during the lockdown?	16 (61.5)	10 (38.5)	12 (48.0)	13 (52.0)
If yes, was it healthy food?	15 (57.7)	11 (42.3)	16 (64.0)	9 (36.0)
Have you changed the way you cook during the lockdown?	5 (19.2)	21 (80.8)	8 (32.0)	17(68.0)
If so, has it been healthy?	22 (84.6)	4 (15.4)	21 (84.0)	4 (16.0)
Have you snacked throughout the day during the lockdown?	14 (53.8)	12 (46.2)	15 (60.0)	10 (40.0)
If yes, was it due to anxiety?	9 (34.6)	17 (65.4)	14 (56.0)	11 (44.0)

generated by the current emergency crisis. However, while lockdown is a necessary measure to protect public health, it is also necessary to provide global interventions to mitigate the negative lifestyle patterns that are commonly manifested during confinement periods, particularly among older people, who are more vulnerable to adverse outcomes (18).

In the current study, we examined the changes in nutritional status in a sample of older residents of the Spanish Mediterranean area before and after confinement due to COVID-19. Also, we examined how the adherence to the Mediterranean diet may play a protective role in nutritional status during the lockdown. As we hypothesized, individuals who began an intervention program based on the Mediterranean diet before confinement could maintain their nutritional status and increase their level of adherence to the MD after confinement. Similar to our findings, the beneficial effects of the MD has been recently found in a Spanish study examining dietary changes of Spanish adult population during the COVID. Authors found that the adherence to the MD increased by 0.8 points during the three first weeks of confinement period as compared with previous habits (15).

Most studies have reported that diets that reduce calorie intake for weight loss are frequently unbalanced, given that these diets are mainly aimed at the reduction of intake of carbohydrates and an increase of fats and proteins (19). In contrast, the MD can help in weight control and generate a balanced nutritional status, as it has been reported as one of the best models of healthy eating due to its contribution to good health (20). Mediterranean diet, which has been considered as rich in nutrients with antioxidant and anti-inflammatory effect may have health benefits for older people, including the prevention of neurodegenerative disease and the modification of the immune and inflammatory response (21). Mediterranean diet has been associated with beneficial effects on weight circumference, blood lipids, and blood pressure (13). It also plays an important role in bone mineralization, which is particularly significant in lockdown situations due to the reduction of physical activity and mobility. Unfortunately, nutritional status as a preventive measure is not well-considered in the current emergency crisis (22, 23).

We have reported how educational interventions based on the beneficial effects of the Mediterranean diet can create an environment in which people can evaluate the positive outcomes of following the recommended lifestyle patterns of (24) as well as receiving guidance to maintain healthy choices under adverse situations such as the COVID-19. This set of circumstances is particularly significant in older people, given that body composition tends to change with aging, and the lockdown can lead to a loss of muscle mass and functional mobility due to lack of physical activity and weight gain. Additional studies are needed to support the current findings in Spain and worldwide, given the current emergency crisis due to COVID-19.

There are several limitations in the current study that suggest areas for future research. Firstly, we used a small sample size conducted in a single city in Spain, and therefore, researchers and interventionists must use caution when generalizing the findings to other areas in Spain or Europe. Second, measures were estimated based on self-reported data. Thus, some patients may underestimate their responses to the questionnaire. Also, many of the variables assessed in this study were derived from single-item measures, a common technique in epidemiological research. Therefore, future studies could be addressed using validated multi-item measures of the constructs and including possible determinants of the MD and nutritional status. Finally, future research could evaluate the effectiveness and feasibility of culturally tailored interventions for older Spanish citizens and families, which target some of the variables associated with nutritional status found in this study. Despite these limitations, this study provides solid evidence of the effectiveness of the MD on the maintenance of weight and nutritional status of the older population. Future research should seek to replicate the study in Spain and also explore the impact of the MD in clinical and mental health outcomes.

CONCLUSION

In conclusion, adherence to the Mediterranean-style diet can play an important role in the establishment of appropriate dietary guidelines in lockdown situations. To achieve nutritional and health improvements global interventions are needed to mitigate the negative lifestyle patterns that are commonly shown during confinement periods, particularly among older people, who are more vulnerable to adverse outcomes. Research of the beneficial effects of MD has shown that the MD can contribute to the prevention of physical and neurodegenerative diseases and also to the modification of the immune and inflammatory response. Educational strategies of recommended

intake should be specific, measurable, achievable, realistic, and timely (SMART), based on the effects of the MD on health, and they should be accompanied by physical activity to create an environment in which people can evaluate the positive outcomes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL) (CEIm: PI2019/057). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AZ-M and MS-S: conceptualization, writing—original draft preparation, and writing—review and editing. AZ-M: methodology, software, formal analysis, resources, data curation, project administration, and funding acquisition. AZ-M, MS-S, and LR-R: validation. AZ-M, LR-R, RF-C, AL-P, and EG-B: research, visualization, and supervision. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was funded by the Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL) (CEIm: PI2019/057).

ACKNOWLEDGMENTS

We thank the Alicante-General Hospital Department of Health.

REFERENCES

1. Bao Y, Sun Y, Meng S, Shi J, Liu L. 2019-Ncov epidemic: address mental health care to empower society. *Lancet.* (2020) 22:e37-8. doi: 10.1016/S0140-6736(20)30309-3
2. Abbas AM, Mohsen Kamel M. Dietary habits in adults during quarantine in the context of COVID-19 pandemic. *Obes Med.* (2020) 19:100254. doi: 10.1016/j.obmed.2020.100254
3. Pellegrini M, Ponzo V, Rosato R, Scumaci E, Goitre I, Benso A, et al. Changes in weight and nutritional habits in adults with obesity during the "lockdown" period caused by the COVID-19 virus emergency. *Nutrients.* (2020) 12:2016. doi: 10.3390/nu12072016
4. Sánchez-Sánchez E, Ramírez-Vargas G, Avellaneda-López Y, Orellana-Pecino JI, García-Marín E, Díaz-Jimenez J. Eating habits and physical activity of the Spanish population during the COVID-19 pandemic period. *Nutrients.* (2020) 12:E2826. doi: 10.3390/nu12092826
5. Zaragoza-Martí A, Ferrer-Cascales R, Hurtado-Sánchez JA, Laguna-Pérez A, Cabañero-Martínez MJ. Relationship between adherence to the Mediterranean diet and health-related quality of life and life satisfaction among older adults. *J Nutr Health Aging.* (2018) 22:89-96. doi: 10.1007/s12603-017-0923-2
6. Ruiz-Roso MB, Knott-Torcal C, Matilla-Escalante DC, Garcimartín A, Sampedro-Nuñez MA, Dávalos A, et al. COVID-19 lockdown and changes of the dietary pattern and physical activity habits in a cohort of patients with Type 2 diabetes mellitus. *Nutrients.* (2020) 12:2327. doi: 10.3390/nu12082327
7. Martinez-Ferran M, Guía-Galipienso F, Sanchis-Gomar F, Pareja-Galeano H. Metabolic impacts of confinement during the COVID-19 pandemic due to modified diet and physical activity habits. *Nutrients.* (2020) 12:1549. doi: 10.3390/nu12061549
8. Zaragoza-Martí A, Cabañero-Martínez MJ, Hurtado-Sánchez JA, Laguna-Pérez A, Ferrer-Cascales R. Evaluation of Mediterranean diet adherence scores: a systematic review. *BMJ Open.* (2018) 1–8. doi: 10.1136/bmjopen-2017-019033
9. Sofi F, Macchi C, Abbate R, Gensini GF, Casini A. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. *Public Health Nutr.* (2014) 17:2769-82. doi: 10.1017/S1368980013003169
10. Sofi F, Abbate R, Franco Gensini G, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *J Clin Nutr.* (2010) 92:1189-96. doi: 10.3945/ajcn.2010.29673
11. Papadaki A, Nolen-Doerr E, Mantzoros CS. The effect of the Mediterranean diet on metabolic health: a systematic review and meta-analysis of controlled trials in adults. *Nutrients.* (2020) 12:3342. doi: 10.3390/nu12113342
12. Canudas S, Becerra-Tomás N, Hernández-Alonso P, Serena Galié, Leung C, Crous-Bou M, et al. Mediterranean diet and telomere length: a systematic review and meta-analysis. *Adv Nutr.* (2020) 11:1544-54. doi: 10.1093/advances/nmaa079
13. Maiorino MA, Bellastella G, Longo M, Caruso P, Esposito K. Mediterranean diet and COVID-19: hypothesizing potential benefits in people with diabetes. *Front Endocrinol.* (2020) 16:574315. doi: 10.3389/fendo.2020.574315
14. Martínez-González MA, Salas-Salvadó J, Estruch R, Corella D, Fitó M, et al. Benefits of the Mediterranean diet: insights from the PREDIMED study. *Prog Cardiovasc Dis.* (2015) 58:50-60. doi: 10.1016/j.pcad.2015.04.003
15. Rodríguez-Pérez C, Molina-Montes E, Verardo V, Artacho R, García-Villanova B, Guerra-Hernández EJ, et al. Changes in dietary behaviours during the COVID-19 outbreak confinement in the Spanish COVIDiet study. *Nutrients.* (2020) 12:1730. doi: 10.3390/nu12061730
16. Martínez de la Iglesia J, Dueñas Herrero R, Onís Vilches MC, Aguado Taberné C, Albert Colomer C, Luque Luque R. Cross-cultural adaptation and validation of Pfeiffer's test (Short Portable Mental Status Questionnaire [SPMSQ]) to screen cognitive impairment in general population aged 65 or older. *Med Clin.* (2001) 117:129–34.
17. Martínez-González MA, Fernández-Jarne E, Serrano-Martínez M, Gomez-García E. Development of a short dietary intake questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. *Eur J Clin Nutr.* (2004) 58:1550–2. doi: 10.1038/sj.ejcn.1602004
18. Achraf Ammar A, Brach M, Trabelsi K, Chtourou H, Boukhris O, Masmoudi L, et al. Effects of COVID-19 home confinement on eating behaviour and physical activity: results of the ECLB-COVID19 international online survey. *Nutrients.* (2020) 12:1583. doi: 10.3390/nu12061583
19. Ortega RM. Importan**ce** of functional foods in the Mediterranean diet. *Public Health Nutr.* (2006) 9:1136-40. doi: 10.1017/S1368980007668530
20. Serra-Majem L, Roman B, Estruch R. Scientific evidence of interventions using the Mediterranean diet: a systematic review. *Nutr Rev.* (2006) 64(suppl_1): S27-47. doi: 10.1111/j.1753-4887.2006.tb00232.x
21. Stark AH, Madar Z. Olive oil as a functional food: epidemiology and nutritional approaches. *Nutr Rev.* (2002) 60:170–6. doi: 10.1301/002966402320243250

22. Azzolino D, Saporiti E, Proietti M, Cesari M. Nutritional considerations in frail older patients with COVID-19. *J Nutr Health Aging*. (2020) 24:696-8. doi: 10.1007/s12603-020-1400-x
23. Scarmozzino F, Visioli F. Covid-19 and the subsequent lockdown modified dietary habits of almost half the population in an Italian sample. *Foods*. (2020) 9:675. doi: 10.3390/foods 9050675
24. Mazloomy-Mahmoodabad SS, Navabi ZS, Ahmadi A, Askarishahi M. The effect of educational intervention on weight loss in adolescents with overweight and obesity: application of the theory of planned behavior. *ARYA Atheroscler*. (2017) 13:176-83.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Zaragoza-Martí, Sánchez-SanSegundo, Ferrer-Cascales, Gabaldón-Bravo, Laguna-Pérez and Rumbo-Rodríguez. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Immunology

ORIGINAL RESEARCH
published: 24 June 2021
doi: 10.3389/fimmu.2021.648250

A Computational Approach Identified Andrographolide as a Potential Drug for Suppressing COVID-19-Induced Cytokine Storm

Mohd Rehan[1,2*†], *Firoz Ahmed*[3,4*†], *Saad M. Howladar*[5], *Mohammed Y. Refai*[3,4], *Hanadi M. Baeissa*[3], *Torki A. Zughaibi*[1,2], *Khalid Mohammed Kedwa*[6] *and Mohammad Sarwar Jamal*[7]

[1] King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia, [2] Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia, [3] Department of Biochemistry, College of Science, University of Jeddah, Jeddah, Saudi Arabia, [4] University of Jeddah Center for Research and Product Development, University of Jeddah, Jeddah, Saudi Arabia, [5] Department of Biology, College of Science, University of Jeddah, Jeddah, Saudi Arabia, [6] Hematology Department, Regional Lab Makkah, Haddah, Saudi Arabia, [7] Integrative Biosciences Center, Wayne State University, Detroit, MI, United States

Background: The newly identified betacoronavirus SARS-CoV-2 is the causative pathogen of the coronavirus disease of 2019 (COVID-19) that killed more than 3.5 million people till now. The cytokine storm induced in severe COVID-19 patients causes hyper-inflammation, is the primary reason for respiratory and multi-organ failure and fatality. This work uses a rational computational strategy to identify the existing drug molecules to target host pathways to reduce the cytokine storm.

Results: We used a "*host response signature network*" consist of 36 genes induced by SARS-CoV-2 infection and associated with cytokine storm. In order to attenuate the cytokine storm, potential drug molecules were searched against *"host response signature network"*. Our study identified that drug molecule andrographolide, naturally present in a medicinal plant *Andrographis paniculata*, has the potential to bind with crucial proteins to block the TNF-induced NFkB1 signaling pathway responsible for cytokine storm in COVID-19 patients. The molecular docking method showed the binding of andrographolide with TNF and covalent binding with NFkB1 proteins of the TNF signaling pathway.

Conclusion: We used a rational computational approach to repurpose existing drugs targeting host immunomodulating pathways. Our study suggests that andrographolide could bind with TNF and NFkB1 proteins, block TNF-induced cytokine storm in COVID-19 patients, and warrant further experimental validation.

Keywords: SARS-CoV-2, COVID-19, cytokine storm, systems bioinformatics, drug-repurposing, andrographolide, TNF signaling pathway, molecular docking

OPEN ACCESS

Edited by:
Ioannis Zabetakis, University of Limerick, Ireland

Reviewed by:
Shima Shahjouei, Geisinger Health System, United States
Hifzur Rahman Ansari, King Abdullah International Medical Research Center (KAIMRC), Saudi Arabia
Naoshad Muhammad, Washington University in St. Louis, United States

***Correspondence:**
Mohd Rehan
mrehan786@gmail.com
Firoz Ahmed
fahmed1@uj.edu.sa

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Immunology

Received: *31 December 2020*
Accepted: *31 May 2021*
Published: *24 June 2021*

Citation:
Rehan M, Ahmed F, Howladar SM, Refai MY, Baeissa HM, Zughaibi TA, Kedwa KM and Jamal MS (2021) A Computational Approach Identified Andrographolide as a Potential Drug for Suppressing COVID-19-Induced Cytokine Storm. Front. Immunol. 12:648250. doi: 10.3389/fimmu.2021.648250

INTRODUCTION

The ongoing pandemic by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected more than 168 million people worldwide, causing more than 3.5 million deaths as of 26 May 2021 (https://www.worldometers.info/coronavirus/). SARS-CoV-2 infects the lower respiratory tract resulting in a severe respiratory disease called coronavirus disease of 2019 (COVID-19) (1). The majority of COVID-19 patients have mild symptoms, including cough, fever, body pain, which recover in a few days. However, a significant number of patients developed severe cases with difficulty in breath, respiratory and lung failure, organ damage, and even death (2–4). Accumulating studies found that an excessively high level of pro-inflammatory cytokines released, called cytokine storm, in response to SARS-CoV-2 infection triggers acute respiratory distress syndrome (ARDS) and multi-organ failure in COVID-19 patients (2, 4). The most prominent cytokines elevated in the blood plasma of severe COVID-19 patients include TNF, IL-6, IL-8, IL-10, and IL-2 (5, 6).

SARS-CoV-2 is classified as betacoronavirus closely related to two previously identified human pathogenic SARS-CoV and MERS-CoV, which caused the epidemics in 2002 and 2012, respectively (7). The SARS-CoV-2 infects the host cell with its spike protein that binds to the ACE2 receptor on the host cell surface and facilitates viral entry. SARS-CoV-2 has a plus-strand RNA genome of about 29,900 nucleotides that work as mRNA to encode viral proteins using host machinery. The genome also contains several *cis*-acting RNA elements that could be involved in viral infection and replications (8). Studies indicated that SARS-CoV-2 has evolutionary closely related to bat-derived SARS-CoV (8, 9).

Till now, no safe and effective therapy has been approved to cure COVID-19. However, life support treatment is recommended for the management and treatment of COVID-19 patients based on the symptoms and severity of the cases.

Based upon multiple clinical trial data on mild-to-severe COVID-19 patients, the US Food and Drug Administration (FDA) has approved only a single antiviral drug Veklury (remdesivir), for the treatment of COVID-19 on 22 October 2020. Furthermore, the FDA authorized eight drugs for emergency use to treat severe COVID-19 patients, and numerous other therapeutics are currently being under clinical trials (https://www.fda.gov/media/136832/download). The FDA is working with different organizations to facilitate the development of safe and effective drug molecules in combating COVID-19.

The elevated level of cytokines is the primary cause of severity in COVID-19 patients, and hence, attenuating the level of these cytokines would be a better approach to manage and treat the COVID-19 patients. Therefore, it is urgently required to identify the drug molecules for targeting the critical pathways in the human that could minimize cytokine storm in COVID-19. However, developing a new medicine is a scientifically challenging process that requires a very long time and massive money with the risk of failure. With the high rate of SARS-CoV-2 infection and the rise of new variant strains, drug repurposing became an extremely important process to identify the potential candidate drugs for treating the severe symptoms of COVID-19. Drug repurposing refers to an approach to identifying new therapeutic uses for approved or investigational drugs other than the scope of the original medical indication (10, 11). Since the repurpose drugs already passed the early-stage trial, including safety assessment, efficacy, toxicity, pharmacokinetic, pharmacodynamic, and preclinical testing, this approach has several advantages: drugs are sufficiently safe, low risk of failure, less investment, and shorten the timeline, could instantly start the tested trials in patients (11). Several drugs were repurposed against numerous diseases (12, 13). Furthermore, various computational methods, including molecular docking and dynamic simulations, have increasingly being used for structural insights into the action mechanism of existing drugs and for novel drug designing (14–18).

This study used a rational computational approach to repurpose drug molecules to minimize the cytokine storm associated with COVID-19 patients. Our previous study identified the SARS-CoV-2-mediated activation of the "*host response signature network*" responsible for cytokine storm (5). In this work, we identified andrographolide to target the "*host response signature network*" through a virtual drug screening method. Finally, we employed a structure-based molecular docking method to demonstrate that the andrographolide could bind with TNF and NFkB1, potentially blocking the TNF-mediated NFkB1 pathway underpinning cytokine storm in severe patients with COVID-19.

MATERIALS AND METHODS

Host Regulatory Network and Biological Pathway Analysis

This study used the "*host response signature network*" identified in the human cells infected with SARS-CoV-2 in our previous study (5). Briefly, transcriptome data of SARS-CoV-2 infected normal human bronchial epithelial (NHBE) cells and controlled NHBE cells were used to identify the differentially expressed host genes (DEHGs) (19). A network was created by integrating DEHGs with human protein-protein interaction data. Furthermore, analysis of the network revealed an important sub-network of highly inter-connected 31 proteins (IL6, TNF, CXCL8, CXCL3, CXCL5, IRF9, SAA1, OAS3, CSF2, IFI6, OAS2, CSF3, IRF7, ICAM1, CXCL2, MX1, OAS1, MMP9, IL1A, IL1B, C3, TLR2, IFI27, CXCL6, CXCL1, CCL20, XAF1, IFI44L, MX2, BST2, IFITM1) predominantly involved in cytokine storm and under the regulation of transcription factors STAT1, STAT2, STAT3, POU2F2, and NFkB1. The sub-network of 31 proteins and their five master regulators are called as "*host response signature network*". In order to understand the biological pathways associated with the "*host response signature network*", KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis was performed using DAVID 6.8 (https://david.ncifcrf.gov/home.jsp).

Potential Drugs Against "Host Response Signature Network"

The "*host response signature network*" was analyzed to find potential drug molecules using the "Gene Association" module of

Network Data Exchange (NDEx version 2.4.5) (https://ndexbio.org/) (20, 21). NDEx is an open-source platform for biological network analysis and knowledge discovery. The output of "Gene Association" analysis gives networks of query gene/protein and its direct or indirect interaction with chemical and drug molecules. All output networks of drug-target interaction were merged to make a non-redundant network and visualized with Cytoscape software version 3.7. Subsequently, we analyzed the potential drug molecules targeting the "*host response signature network*". For further study, we selected a drug molecule, andrographolide, naturally present in a medicinal plant used to treat common cold and inflammation (22).

Data Retrieval

The three-dimensional coordinates of andrographolide were retrieved from the PubChem database with PubChem CID, 5318517. The crystal structure of human NFkB1, p50 subunit, with bound native DNA was selected and retrieved from the PDB database with PDB Id, 2V2T. The crystal structure of human TNF as a dimer with bound native inhibitor was selected and retrieved from the PDB database with PDB Id, 5MU8.

Predicting the Drug-Likeness and Pharmacokinetic Properties

The drug-likeness and pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity, were analyzed using the online tool "pkCSM-pharmacokinetics" (http://biosig.unimelb.edu.au/pkcsm/). This machine learning-based method uses the various graph-based signature of chemical compounds having sets of distance patterns between atoms (23).

Covalent Docking of Andrographolide to NFkB1

It was reported that andrographolide is a covalent inhibitor of NFkB1 and makes a covalent bond with Cys-62 of NFkB1 (24, 25). Autodock Vina (26) was used for covalent docking of andrographolide to NFkB1. To perform covalent docking, the reported atom of the andrographolide was linked to the sulfide atom of Cys-62 through a covalent bond (25). The adduct Cys-andrographolide was made flexible for rotation through various bonds using Autodock Tools (27). Then the docking of a very small molecule (e.g., water molecule) was performed, leading to the relaxed conformation of the flexible adduct Cys-andrographolide. Finally, this relaxed conformation of the adduct Cys-andrographolide was reported as the final covalent docking of andrographolide. Autodock Tools was used to prepare the protein, the ligand, and the grid box required for docking by Autodock Vina.

Molecular Docking and Protein-Ligand Complex Analysis

For normal molecular docking, we used Dock version 6.5 (28). For TNF, the homodimer (Chains A and B) was used for molecular docking, and the native inhibitor bound within the binding site formed by both monomers (Chains A and B) was used as a probe for the TNF homodimer binding site. The protein and ligand preparation required for molecular docking and visualization at different stages of docking was performed using Chimera version 1.6.2 (29). The illustrations for binding poses were generated using Pymol version 2.4.0 (Schrödinger, LLC) and the protein-ligand interaction plots were prepared using Ligplot+ version 2.1 (30). The binding energy and dissociation constant scores were predicted using XScore version 1.2.11 (31).

RESULTS AND DISCUSSION

The integration and analysis of high-throughput biological data are extensively used to identify altered regulatory networks and the potential target molecules of complex diseases (5, 32). Furthermore, virtual drug screening and molecular docking were used to find therapeutic drug molecules against target molecules to treat the disease (17, 18). Therapeutic molecules against SARS-CoV-2 infection could be developed using two major approaches: (i) design the therapeutic molecules against the crucial targets of the virus (33–36); (ii) identify the disease-related gene regulatory network and then design the therapeutic molecules against it. Our previous work identified the "*host response signature network*" associated with the influx of cytokine storm through the TNF-induced NFkB1 signaling pathway (5). Our study also suggested vitamin D's role in reducing the cytokine storm and viruses (5). In this work, we employed the later drug development strategy in which the "*host response signature network*" induced in the SARS-CoV-2 infection was used as targets to repurpose drug molecules to reduce the cytokine storm responsible for severe COVID-19.

Biological Pathways of "Host Response Signature Network"

The "*host response signature network*" was analyzed with the KEGG pathway, which identified several significantly associated biological pathways. We found that TNF signaling pathway is significantly associated with IL6, CSF2, CCL20, IL1B, CXCL1, CXCL3, TNF, CXCL2, MMP9, NFkB1, and ICAM1 of the "*host response signature network*" and responsible for the induction of cytokine storm (**Figure 1**). The complete list of all KEGG pathways is provided in the **Supplementary Table S1**.

Potential Drug Molecules Targeting "Host Response Signature Network"

Analysis of potential drug molecules for "*host response signature network*" with the "Gene Association" module of online tool NDEx returns a list of drug-target interactions networks (**Table 1**). The list of drug-target interactions from different networks was merged to create a non-redundant drug-target network (**Figure 2**). The detailed information of drug molecules is provided in the **Supplementary Table S2**. The data was further investigated to find a potential drug for better management of the cytokine storm in severe COVID-19 patients. We have selected andrographolide for further study based upon the following criteria from the list of repurposed drugs.

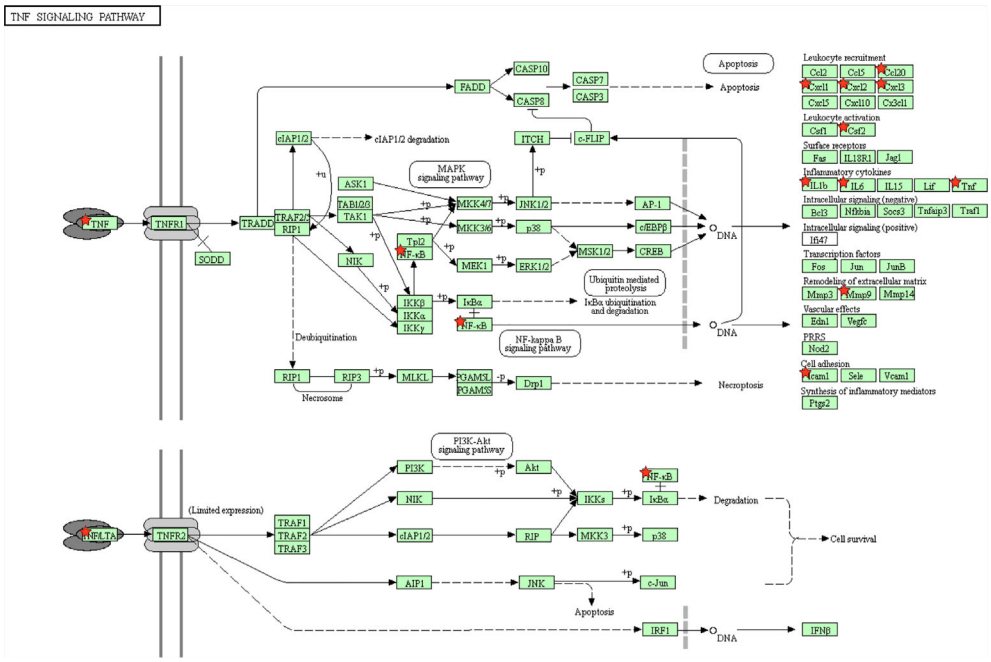


FIGURE 1 | The KEGG pathway enrichment analysis showed that the *"host response signature network"* is associated with the TNF signaling pathway. The proteins IL6, CSF2, CCL20, IL1B, CXCL1, CXCL3, TNF, CXCL2, MMP9, NFKB1, and ICAM1 of *the "host response signature network"* are represented as red stars.

TABLE 1 | Drug-target networks were retrieved using the "Gene Association" module of NDEx v2.4.5.

Network Name	Query result	
	Network Properties	**Number of overlapping gene**
DrugBank - Combined Network **Parent:** Nodes:11994; Egde:27799	Nodes:106; Egde:119	14 genes
DisGeNET - Gene-Disease Associations (Score >=0.5) **Parent:** Nodes:8631; Egde:8273	Nodes:75; Egde:79	14 genes
DrugBank - Target drugs **Parent:** Nodes:11407; Egde:19650	Nodes:104; Egde:118	13 genes
BioGRID: Protein-Chemical Interactions (H. sapiens) **Parent:** Nodes:6776; Egde:10854	Nodes: 52; Egde:45	12 genes
BindingDB - High Affinity Compounds vs. human targets (Commercially available) **Parent:** Nodes:1374; Egde:3371	Nodes: 7; Egde:6	2 genes
DrugBank - Enzyme drugs **Parent:** Nodes:1984; Egde:4905	Nodes: 2; Egde:1	1 gene

The "host response signature network" was used as a query to find the potential drug molecules.

(a) The drug must target/suppress the highest number of proteins of the "*host response signature network*"; (b) targeted proteins associate with the same biological pathway; (c) targeted proteins play a crucial role in the cytokine storm; and (d) a medicinal plant could be a source of the drug.

We found that andrographolide can target four different proteins IL1B, NFkB1, TNF, and IL6 (**Table S2A**) of the TNF signaling pathway **(Figure 1)**, which might result in blockage of the cytokine storm in COVID-19. Andrographolide is a labdane diterpenoid isolated from *Andrographis paniculata*, also known as the "king of bitters", a medicinal plant that mainly grows in Asian countries **(Figure 3)**. *Andrographis paniculata* traditionally used in Unani, Ayurvedic, and Chinese herbal medicines with a broad range of therapeutic applications, including treating a common cold, upper respiratory tract infections, and inflammation (22).

Drug-Likeness and Pharmacokinetic Properties

As shown in **Table 2**, the andrographolide was having a low molecular weight of 350.46 (< 500) and the lipophilicity (LogP) value of 1.96 (<5), H-bond donors 3 (<5) and acceptors 5 (<10), and three rotatable bonds (<10). The values for all five conditions (Lipinski's Rule of Five) for the andrographolide were well within the desired range of a drug molecule.

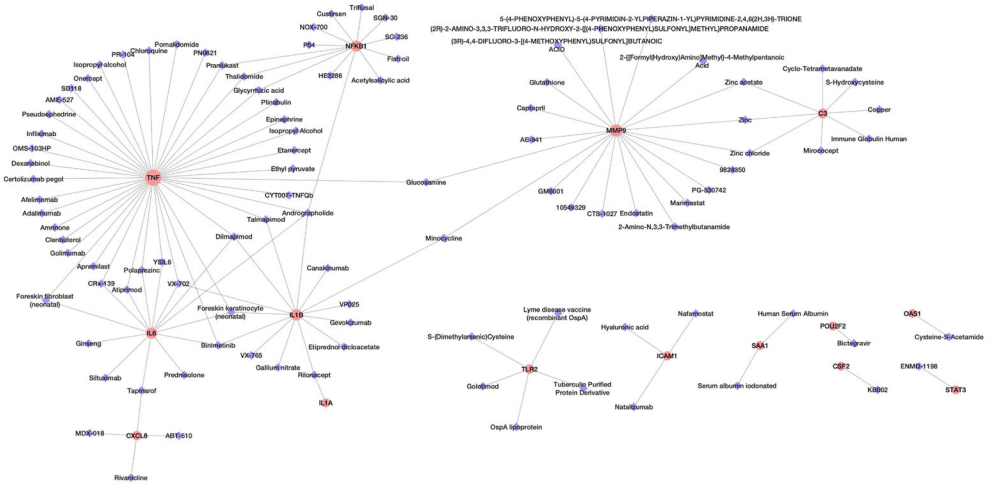


FIGURE 2 | The network of drug molecules targeting the *"host response signature network"*.

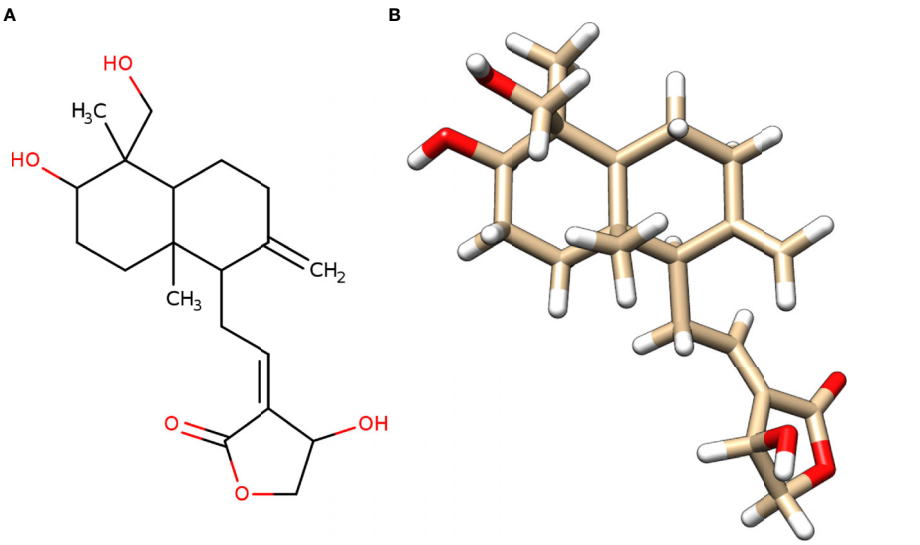


FIGURE 3 | Molecular structure of andrographolide. **(A)** Two-dimensional sketch of andrographolide with O-atoms and -OH groups in red color. **(B)** Three-dimensional diagram of andrographolide with O-atoms in red and H-atoms in white colors.

TABLE 2 | Drug-likeness (Lipinski rule of five) for andrographolide.

Lipinski rule of five		
Property	**Desired value**	**Andrographolide**
Mol Wt.	<500	350.46
H-Bond Donors	<5	3
H-Bond Acceptors	<10	5
Rotatable Bonds	<10	3
Lipophilicity (LogP)	<5	1.96

While most of the pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity, were acceptable for andrographolide (**Table 3**). Therefore, this suggests that andrographolide is a potentially safe drug candidate for use in humans.

Covalent Docking Analysis of Andrographolide With NFkB1

The PDB structures of IL1B and IL6 are available as a complex with their receptors (for example, PDB Ids: 3O4O and 1P9M, respectively) and with the antibody (for example, PDB Ids: 4G6J and 4CNI, respectively). The information for the binding site of ILIB and IL6 for a chemical compound was not obvious. So, we

TABLE 3 | Pharmacokinetic properties (ADMET) prediction for andrographolide.

Pharmacokinetic properties (ADMET)				
Property	**Model Name**	**Desired value**	**Unit**	**Andrographolide**
ABSORPTION	Water solubility		log mol/L	-3.494
	Caco2 permeability	>0.90	log Papp in 10-6 cm/s	1.07
	Intestinal absorption (human)	>>30	% Absorbed	95.357
	Skin Permeability	>-2.5	log Kp	-3.794
	P-glycoprotein substrate	No	Yes/No	No
	P-glycoprotein I inhibitor		Yes/No	No
	P-glycoprotein II inhibitor		Yes/No	No
DISTRIBUTION	VDss (human)	0.71<VDss<2.81	log L/kg	-0.286
	Fraction unbound (human)		Fu	0.281
	BBB permeability	<0.3	log BB	-0.598
	CNS permeability	>-2	log PS	-2.691
METABOLISM	CYP2D6 substrate	No	Yes/No	No
	CYP3A4 substrate	No	Yes/No	Yes
	CYP1A2 inhibitor		Yes/No	No
	CYP2C19 inhibitor		Yes/No	No
	CYP2C9 inhibitor		Yes/No	No
	CYP2D6 inhibitor		Yes/No	No
	CYP3A4 inhibitor		Yes/No	No
EXCRETION	Total Clearance		log ml/min/kg	1.183
	Renal OCT2 substrate	No	Yes/No	No
TOXICITY	AMES toxicity	No	Yes/No	No
	Max. tolerated dose (human)	<0.477	log mg/kg/day	0.128
	hERG I inhibitor	No	Yes/No	No
	hERG II inhibitor	No	Yes/No	No
	Oral Rat Acute Toxicity (LD50)		mol/kg	2.162
	Oral Rat Chronic Toxicity (LOAEL)		log mg/kg_bw/day	1
	Hepatotoxicity	No	Yes/No	No
	Skin Sensitization	No	Yes/No	No
	T. pyriformis toxicity	<-0.5	log ug/L	0.491
	Minnow toxicity	>-0.3	log mM	1.37

ADMET, absorption, distribution, metabolism, excretion, and toxicity; BBB, blood-brain barrier; CNS, central nervous system; CYP, cytochrome P; hERG, human ether-a-go-go-related gene; LD50, lethal dose 50%; LOAEL, lowest observed adverse effect level; OCT2, organic cation transporter 2; VDss, steady-state volume of distribution.

proceeded with the docking of andrographolide to NFkB1 and TNF only.

The experimental laboratories showed that andrographolide reacted with the sulfhydryl group (-SH) of Cys-62 of NFkB1 and formed a covalent bond (24, 25). One of the studies mentioned above further proceed to a normal docking of andrographolide to NFkB1, and they reported Cys-62 and Arg-57 as the interacting residues forming hydrogen bonds (25). We tried normal docking considering Cys-62 as a clue for the binding site and failed to see a binding site cavity close to Cys-62 residue where this compound may fit. Therefore, we proceeded with the covalent docking of andrographolide to NFkB1. The docking results showed that the andrographolide formed a covalent bond and a hydrogen bond (2.86 Å) with the sulfide atom of Cys-62 (**Figure 4**). The Cys-62 was also involved in 13 non-bonding contacts with andrographolide (**Table 4**). Another interacting residue Glu-63 was involved in another 13 non-bonding contacts. Thus, the andrographolide formed an adduct with Cys-62 and became part of the protein. In addition, it formed a hydrogen bond and 26 non-bonding interactions with the protein (**Figure 4** and **Table 4**). Thus, our study suggests andrographolide could block the DNA binding site, interfering with the binding of DNA, and thus the transcription factor NFkB1 would not be able to carry out its function. This is in agreement with previous findings, which revealed that andrographolide binds with NFkB1, which blocks the binding of NFkB1 to DNA and prevents transcriptional activity (24, 25).

Molecular Docking Analysis of Andrographolide With TNF Homodimer

The andrographolide docked well within the binding site formed by two monomeric units of TNF (**Figure 5A**) and stabilized by 20 non-bonded contacts and one hydrogen bond through nine interacting residues (**Figure 5B** and **Table 5**). The absolute values of binding scores, including dock score (-32.67), binding energy (-7.52 Kcal/mol), and dissociation constant measure pK_d (5.51), were also reasonably high required for the stable protein-ligand complex. The nine interacting residues include four residues from chain A (Tyr-59, Tyr-119, Leu-120, and Gly-121) and five residues from chain B (Leu-94, Tyr-119, Leu-120, Gly-121, and Gly-122). Interestingly, the three identical residues Tyr-119, Leu-120, and Gly-121 from both the monomers, played a role in binding. Of nine interacting residues, Gly-121(B) formed hydrogen bonding interaction with the andrographolide with bond length 3.06 Å and also formed eight (maximum) non-bonded contacts. Therefore, the Gly-121(B) is proposed as the key residue playing a role in binding. While comparing the binding of andrographolide to that of the native inhibitor JNJ525 (**Figures 5B, C**), it was found that the

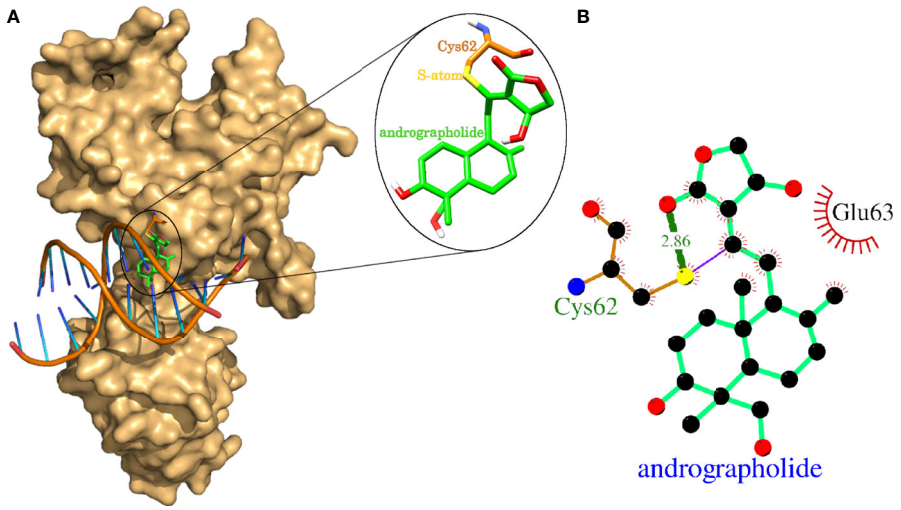


FIGURE 4 | Covalent docking of andrographolide to human NFkB1. **(A)** NFkB1 is shown in surface representation colored light orange with bound native double-helical DNA and docked andrographolide. The docked andrographolide forming Cys-andrographolide adduct was shown as a close-up in the inset. The andrographolide, part of the adduct, was shown in sticks representation having carbon backbone in green color with heteroatoms oxygen, nitrogen, sulfur, and hydrogen atoms in red, blue, yellow, and white colors respectively. The Cys-62 part of the adduct forming the covalent bond through sulfur atom was also shown in sticks representation with the backbone in orange color. **(B)** Protein-ligand interaction plot of andrographolide with NFkB1. The andrographolide and hydrogen bonding residues are shown in ball and sticks representation with balls representing atoms and sticks representing the bond between two atoms. The color of balls distinguishes among atom types as C-atom in black, O-atoms in red, N-atoms in blue, and S-atom in yellow colors. The non-bonded interactions labeled with interacting residue are shown as red arcs with bristles, covalent bonds as purple line, and hydrogen bonds as thick green lines labeled with bond length in Å.

TABLE 4 | The NFkB1 residues interacting with andrographolide.

Interacting residue	Covalent bond	Hydrogen bond	Non-bonded contacts
Cys-62	1	1	13
Glu-63	–	–	13

The interacting residues are listed with a number of interactions (covalent bond, hydrogen bonds and non-bonded contacts).

andrographolide was also binding to the same site and sharing common six interacting residues Tyr-59 (A), Tyr-119 (A), Gly-121 (A), Tyr-119 (B), Gly-121 (B), and Gly-122 (B). Thus, the andrographolide was binding in the site where the TNF inhibitor is binding and inhibiting TNF activity.

Previous studies showed that andrographolide has a potent inhibitor of wide verities of viruses, including influenza virus (37, 38), hepatitis C virus (39), herpes simplex virus type 1 (40), Chikungunya virus (41). It also has the potential to inhibit the proliferation of lung carcinoma (42) and nasopharyngeal carcinoma (43), and tumor metastasis (44). Another report demonstrated that mice treated with andrographolide reduce the inflammation and fibrosis of damaged liver (45). It also prevents liver neutrophil infiltration and reduced TNF and COX-2 signaling (45). A recent study with the molecular docking method suggested andrographolide as a potential inhibitor of SARS-CoV-2 main protease M^{pro} (47). Another computational-based study found that andrographolide and its derivative, 14-deoxy-11,12-didehydroandrographolide, have a high binding affinity with three target proteins of SARS-CoV-2, *i.e.*, main protease, papain-like protease (PL^{pro}), and spike protein (48). In addition, the same study used a DIGEP-Pred tool and predicted that both compounds induce the level of several proteins involved in regulating immune systems, including the NFkB signaling pathway (48).

The NFkB1 is a family of transcription factors, which regulate the expression of various genes of pro-inflammatory cytokines, including IL6 and IL8 (CXCL8), mainly responsible for cytokine storm in COVID-19 (**Figure 1**) (5, 49). NFkB1 inhibitory protein (IkB) binds with NFkB1 dimer in an unstimulated cell, which prevents movement of NFkB1 from the cytoplasm to the nucleus. In response to SARS-CoV-2 infection, the TNF signaling pathway is activated, which causes degradation of IkB, resulting in the release and translocation of NFkB1 to the nucleus for transcription of various cytokine genes (5, 49). Our study found that andrographolide bound well with TNF and NFkB1 and provided structural insights into their binding. This binding of andrographolide blocks the TNF signaling pathways at these two critical steps, thus prevents the expression of various cytokine genes responsible for the influx of cytokine in COVID-19.

In addition, our study identified Dilmapimod as potential compounds to reduce the cytokine storm through targeting/suppressing IL1B, TNF, and IL6 (**Table S2A**). Activation of the p38 MAPK signaling pathway is associated with lung inflammation in asthma and chronic obstructive pulmonary disease (COPD) (50). The p38 MAPK regulated NFkB dependent transcription of multiple inflammatory cytokines (51). Clinical studies showed that Dilmapimod, a novel inhibitor of p38 MAPK, decreases the TNF and IL1B level in the blood of COPD patients (52, 53). Another study also suggested the potential use of Dilmapimod for COVID-19 management (54). Furthermore, our analysis identified that Prednisolone, a corticosteroid, has the potential to suppress the level

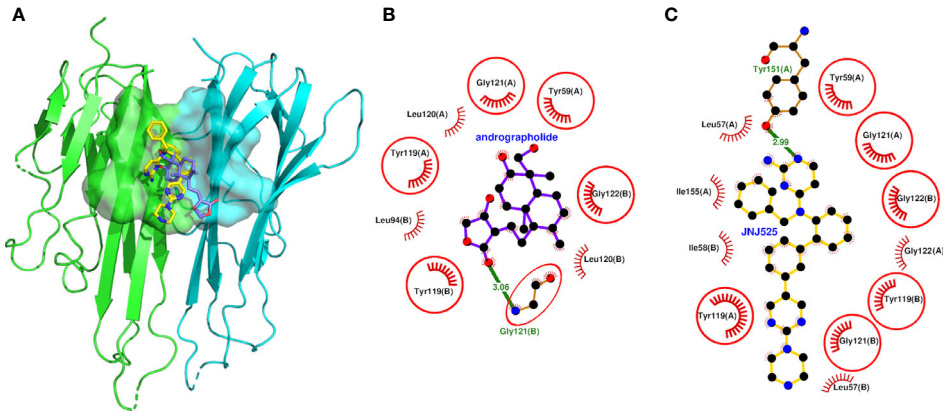


FIGURE 5 | Molecular docking of andrographolide with human TNF homodimer. **(A)** The pose of andrographolide within the binding site of TNF homodimer. The NFkB1 is shown in cartoon representation colored green (Chain A) and cyan (Chain B) with the binding site in surface representation. The andrographolide and the native inhibitor are shown as sticks colored blue and yellow, respectively, with red oxygen and blue nitrogen atoms. **(B, C)** Protein-ligand interaction plots of andrographolide and the native inhibitor JNJ525 with TNF homodimer. The andrographolide and hydrogen bonding residue are shown in ball and sticks representation, with balls representing atoms and sticks representing the bond between two atoms. The color of balls distinguishes among atom types as C-atom in black, O-atoms in red, and N-atoms in blue colors. The non-bonded interactions labeled with interacting residue are shown as red arcs with bristles, while the hydrogen bond is shown as a green line labeled with bond length in Å. The residues common among interacting residues of both the native inhibitor and the andrographolide are encircled.

TABLE 5 | The residues of TNF homodimer interacting with andrographolide.

Interacting residue	No. of hydrogen bonds	No. of non-bonded contacts
Tyr-59(A)		1
Tyr-119(A)		3
Leu-120(A)		1
Gly-121(A)		1
Leu-94(B)		1
Tyr-119(B)		1
Leu-120(B)		2
Gly-121(B)	1	8
Gly-122(B)		2

The interacting residues of both chains (A and B) are listed with a number of interactions (hydrogen bonds and non-bonded contacts).

of IL6 (**Table S2A**); thus, supported previous work indicating that Prednisolone could be useful in treating severe COVID-19 (55).

Previous studies showed the anti-inflammatory and anticancer effects of Dehydroxymethylepoxyquinomicin (DHMEQ), a new inhibitor of NFkB (56, 57). Another study found that DHMEQ reduces allergic airway inflammation and cytokines in a mice model of asthma (58). An investigation conducted in Jurkat T-lymphoblastic leukemia cells found that DHMEQ prevents the TNF-α-induced nuclear translocation of NFkB (46). These findings also raised the potential use of DHMEQ in inhibiting cytokine storms and thus need to be studied in more detail in treating COVID-19.

Our study used a rational computational approach to identify the potential drug molecule for COVID-19. Though it is associated with the following limitations: (i) we used the "*host response signature network*" identified in the NHBE cells infected with SARS-CoV-2 (5). The work included a limited sample size of transcriptomic data with three infected and three controlled groups (19). (ii) This study lacks *in vitro* drug screening data on human lung cells infected with SARS-CoV-2. Therefore, it is difficult to draw the effect of andrographolide on the "*host response signature network*" in SRAS-CoV-2 infected cells. (iii) Furthermore, our study lacks the experimental evaluation of the dose-dependent effect of the drug and its associated risk.

CONCLUSION

The SARS-CoV-2 infection resulted in the COVID-19 pandemic killed millions of people around the world. Therefore, it is urgently needed to develop safe and effective drug molecules in a limited time to combat COVID-19. The influx of cytokines in COVID-19 patients is the prominent reason for organ damage and death. Thus, our study used the information of altered regulatory network inducing cytokine storm in COVID-19 and then repurposed andrographolide as a potential drug molecule to reduce the cytokine storm. Molecular docking analysis showed that andrographolide could inhibit NFkB1 and TNF, and thus block the pathways responsible for cytokine storm. This naturally occurring compound possesses drug-like properties and could be a promising drug for further biochemical and cell-based experimental validation for combating severe COVID-19.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

FA conceived the idea, generated the data, analyzed and interpreted the results, wrote and revised the manuscript, and designed and guided the project. MR improved the idea,

generated the data, analyzed and interpreted the results, wrote, and revised the manuscript. SH and MYR verified the generated data and wrote and revised the manuscript. HB and TZ verified the computational methods, wrote and revised the manuscript. KK and MJ wrote and revised the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to acknowledge the technical support provided by the King Fahd Medical Research Center, King Abdulaziz University; Department of Biochemistry, Department of Biology, University of Jeddah; and University of Jeddah Center for Research and Product Development (UJCRPD), University of Jeddah.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.648250/full#supplementary-material

REFERENCES

1. Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, Xu T, et al. Identification of a Novel Coronavirus Causing Severe Pneumonia in Human: A Descriptive Study. *Med J (Engl)* (2020) 133:1015–24. doi: 10.1097/CM9.0000000000000722
2. Moore BJB, June CH. Cytokine Release Syndrome in Severe COVID-19. *Science* (2020) 368:eabb8925. doi: 10.1126/science.abb8925
3. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical Course and Outcomes of Critically Ill Patients With SARS-Cov-2 Pneumonia in Wuhan, China: A Single-Centered, Retrospective, Observational Study. *Lancet Respir Med* (2020) 8:475–81. doi: 10.1016/S2213-2600(20)30079-5
4. Wang T, Du Z, Zhu F, Cao Z, An Y, Gao Y, et al. Comorbidities and Multi-Organ Injuries in the Treatment of COVID-19. *Lancet* (2020) 395(10228):e52. doi: 10.1016/S0140-6736(20)30558-4
5. Ahmed F. A Network-Based Analysis Reveals the Mechanism Underlying Vitamin D in Suppressing Cytokine Storm and Virus in SARS-Cov-2 Infection. *Front Immunol* (2020) 11(3084):590459. doi: 10.3389/fimmu.2020.590459
6. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine Release Syndrome in Severe COVID-19: Interleukin-6 Receptor Antagonist Tocilizumab May Be the Key to Reduce Mortality. *Int J Antimicrob Agents* (2020) 55(5):105954. doi: 10.1016/j.ijantimicag.2020.105954
7. Choudhry H, Bakhrebah MA, Abdulaal WH, Zamzami MA, Baothman OA, Hassan MA, et al. Middle East Respiratory Syndrome: Pathogenesis and Therapeutic Developments. *Future Virol* (2019) 14(4):237–46. doi: 10.2217/fvl-2018-0201
8. Ahmed F, Sharma M, Al-Ghamdi AA, Al-Yami SM, Al-Salami AM, Refai MY, et al. A Comprehensive Analysis of Cis-Acting RNA Elements in the SARS-Cov-2 Genome by a Bioinformatics Approach. *Front Genet* (2020) 11 (1385):1385. doi: 10.3389/fgene.2020.572702
9. Tang X, Wu C, Li X, Song Y, Yao X, Wu X, et al. On the Origin and Continuing Evolution of SARS-Cov-2. *Natl Sci Rev* (2020) 7(6):1012–23. doi: 10.1093/nsr/nwaa036
10. Ashburn TT, Thor KB. Drug Repositioning: Identifying and Developing New Uses for Existing Drugs. *Nat Rev Drug Discovery* (2004) 3(8):673–83. doi: 10.1038/nrd1468
11. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, et al. Drug Repurposing: Progress, Challenges and Recommendations. *Nat Rev Drug Discov* (2019) 18(1):41–58. doi: 10.1038/nrd.2018.168
12. Kale VP, Habib H, Chitren R, Patel M, Pramanik KC, Jonnalagadda SC, et al. Old Drugs, New Uses: Drug Repurposing in Hematological Malignancies. *Semin Cancer Biol* (2020) 68:242–8. doi: 10.1016/j.semcancer.2020.03.005
13. Kumar R, Harilal S, Gupta SV, Jose J, Thomas Parambi DG, Uddin MS, et al. Exploring the New Horizons of Drug Repurposing: A Vital Tool for Turning Hard Work Into Smart Work. *Eur J Med Chem* (2019) 182:111602. doi: 10.1016/j.ejmech.2019.111602
14. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular Docking and Structure-Based Drug Design Strategies. *Molecules* (2015) 20(7):13384–421. doi: 10.3390/molecules200713384
15. Rehan M, Mahmoud MM, Tabrez S, Hassan HMA, Ashraf GM. Exploring Flavonoids for Potential Inhibitors of a Cancer Signaling Protein PI3Kγ Kinase Using Computational Methods. *Anticancer Res* (2020) 40(8):4547–56. doi: 10.21873/anticanres.14460
16. Rehan M. Anticancer Compound XL765 as PI3K/mTOR Dual Inhibitor: A Structural Insight Into the Inhibitory Mechanism Using Computational Approaches. *PloS One* (2019) 14(6):e0219180. doi: 10.1371/journal.pone.0219180
17. Rehan M, Mostafa M. Virtual Screening of 1,4-Naphthoquinone Derivatives for Inhibition of a Key Cancer Signaling Protein, AKT1 Kinase. *Anticancer Res* (2019) 39(7):3823–33. doi: 10.21873/anticanres.13532
18. Rehan M. A Structural Insight Into the Inhibitory Mechanism of an Orally Active PI3K/Mtor Dual Inhibitor, PKI-179 Using Computational Approaches. *J Mol Graph Model* (2015) 62:226–34. doi: 10.1016/j.jmgm.2015.10.005
19. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced Host Response to SARS-Cov-2 Drives Development of COVID-19. *Cell* (2020) 181(5):1036–45.e9. doi: 10.1016/j.cell.2020.04.026
20. Pratt D, Chen J, Welker D, Rivas R, Pillich R, Rynkov V, et al. Ndex, the Network Data Exchange. *Cell Syst* (2015) 1(4):302–5. doi: 10.1016/j.cels.2015.10.001
21. Pratt D, Chen J, Pillich R, Rynkov V, Gary A, Demchak B, et al. Ndex 2.0: A Clearinghouse for Research on Cancer Pathways. *Cancer Res* (2017) 77(21): e58–61. doi: 10.1158/0008-5472.CAN-17-0606
22. Akbar S. Andrographis Paniculata: A Review of Pharmacological Activities and Clinical Effects. *Altern Med Rev* (2011) 16(1):66–77.
23. Pires DE, Blundell TL, Ascher DB. Pkcsm: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J Med Chem* (2015) 58(9):4066–72. doi: 10.1021/acs.jmedchem.5b00104
24. Xia YF, Ye BQ, Li YD, Wang JG, He XJ, Lin X, et al. Andrographolide Attenuates Inflammation by Inhibition of NF-Kappa B Activation Through Covalent Modification of Reduced Cysteine 62 of P50. *J Immunol* (2004) 173 (6):4207–17. doi: 10.4049/jimmunol.173.6.4207
25. Wang J, Tan XF, Nguyen VS, Yang P, Zhou J, Gao M, et al. A Quantitative Chemical Proteomics Approach to Profile the Specific Cellular Targets of Andrographolide, a Promising Anticancer Agent That Suppresses Tumor Metastasis. *Mol Cell Proteomics* (2014) 13(3):876–86. doi: 10.1074/mcp.M113.029793
26. Trott O, Olson AJ. Autodock Vina: Improving the Speed and Accuracy of Docking With a New Scoring Function, Efficient Optimization, and Multithreading. *J Comput Chem* (2010) 31(2):455–61. doi: 10.1002/jcc.21334
27. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. Autodock4 and Autodocktools4: Automated Docking With Selective Receptor Flexibility. *J Comput Chem* (2009) 30(16):2785–91. doi: 10.1002/jcc.21256
28. Ewing TJ, Makino S, Skillman AG, Kuntz ID. DOCK 4.0: Search Strategies for Automated Molecular Docking of Flexible Molecule Databases. *J Comput Aided Mol Des* (2001) 15(5):411–28. doi: 10.1023/a:1011115820450
29. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera–A Visualization System for Exploratory Research and Analysis. *J Comput Chem* (2004) 25(13):1605–12. doi: 10.1002/jcc.20084
30. Laskowski RA, Swindells MB. Ligplot+: Multiple Ligand-Protein Interaction Diagrams for Drug Discovery. *J Chem Inf Model* (2011) 51(10):2778–86. doi: 10.1021/ci200227u
31. Wang R, Lai L, Wang S. Further Development and Validation of Empirical Scoring Functions for Structure-Based Binding Affinity Prediction. *J Comput Aided Mol Des* (2002) 16(1):11–26. doi: 10.1023/a:1016357811882
32. Ahmed F. Integrated Network Analysis Reveals FOXM1 and MYBL2 as Key Regulators of Cell Proliferation in Non-Small Cell Lung Cancer. *Front Oncol* (2019) 9:1011. doi: 10.3389/fonc.2019.01011

33. Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. Analysis of Therapeutic Targets for SARS-Cov-2 and Discovery of Potential Drugs by Computational Methods. *Acta Pharm Sin B* (2020) 10(5):766–88. doi: 10.1016/j.apsb.2020.02.008
34. Gupta AK, Khan MS, Choudhury S, Mukhopadhyay A, Sakshi, Rastogi A, et al. Coronavr: A Computational Resource and Analysis of Epitopes and Therapeutics for Severe Acute Respiratory Syndrome Coronavirus-2. *Front Microbiol* (2020) 11:1858. doi: 10.3389/fmicb.2020.01858
35. Ahmed F, Raghava GP. Designing of Highly Effective Complementary and Mismatch SiRNAs for Silencing a Gene. *PloS One* (2011) 6(8):e23443. doi: 10.1371/journal.pone.0023443
36. Tyagi A, Ahmed F, Thakur N, Sharma A, Raghava GP, Kumar M. Hivsirdb: A Database of HIV Inhibiting SiRNAs. *PloS One* (2011) 6(10):e25917. doi: 10.1371/journal.pone.0025917
37. Chen JX, Xue HJ, Ye WC, Fang BH, Liu YH, Yuan SH, et al. Activity of Andrographolide and Its Derivatives Against Influenza Virus *In Vivo* and *In Vitro*. *Biol Pharm Bull* (2009) 32(8):1385–91. doi: 10.1248/bpb.32.1385
38. Yu B, Dai CQ, Jiang ZY, Li EQ, Chen C, Wu XL, et al. Andrographolide as an Anti-H1N1 Drug and the Mechanism Related to Retinoic Acid-Inducible Gene-I-Like Receptors Signaling Pathway. *Chin J Integr Med* (2014) 20 (7):540–5. doi: 10.1007/s11655-014-1860-0
39. Lee JC, Tseng CK, Young KC, Sun HY, Wang SW, Chen WC, et al. Andrographolide Exerts Anti-Hepatitis C Virus Activity by Up-Regulating Haeme Oxygenase-1 *via* the P38 MAPK/Nrf2 Pathway in Human Hepatoma Cells. *Br J Pharmacol* (2014) 171(1):237–52. doi: 10.1111/bph.12440
40. Seubsasana S, Pientong C, Ekalaksananan T, Thongchai S, Aromdee C. A Potential Andrographolide Analogue Against the Replication of Herpes Simplex Virus Type 1 in Vero Cells. *Med Chem* (2011) 7(3):237–44. doi: 10.2174/157340611795564268
41. Wintachai P, Kaur P, Lee RC, Ramphan S, Kuadkitkan A, Wikan N, et al. Activity of Andrographolide Against Chikungunya Virus Infection. *Sci Rep* (2015) 5:14179. doi: 10.1038/srep14179
42. Luo X, Luo W, Lin C, Zhang L, Li Y. Andrographolide Inhibits Proliferation of Human Lung Cancer Cells and the Related Mechanisms. *Int J Clin Exp Med* (2014) 7(11):4220–5.
43. Peng T, Hu M, Wu TT, Zhang C, Chen Z, Huang S, et al. Andrographolide Suppresses Proliferation of Nasopharyngeal Carcinoma Cells *via* Attenuating NF-κB Pathway. *BioMed Res Int 2015* (2015) p:735056. doi: 10.1155/2015/735056
44. Yu CC, Chen CA, Fu SL, Lin HY, Lee MS, Chiou WY, et al. Andrographolide Enhances the Anti-Metastatic Effect of Radiation in RAS-Transformed Cells *via* Suppression of ERK-Mediated MMP-2 Activity. *PloS One* (2018) 13(10): e0205666. doi: 10.1371/journal.pone.0205666
45. Lee TY, Chang HH, Wen CK, Huang TH, Chang YS. Modulation of Thioacetamide-Induced Hepatic Inflammations, Angiogenesis and Fibrosis by Andrographolide in Mice. *J Ethnopharmacol* (2014) 158 Pt A:423–30. doi: 10.1016/j.jep.2014.10.056
46. Ariga A, Namekawa J, Matsumoto N, Inoue J, Umezawa K. Inhibition of Tumor Necrosis Factor-Alpha -Induced Nuclear Translocation and Activation of NF-Kappa B by Dehydroxymethylepoxyquinomicin. *J Biol Chem* (2002) 277(27):24625–30. doi: 10.1074/jbc.M112063200
47. Enmozhi SK, Raja K, Sebastine I, Joseph J. Andrographolide as a Potential Inhibitor of SARS-CoV-2 Main Protease: An *In Silico* Approach. *J Biol Chem* (2021) 39(9):3092–8. doi: 10.1080/07391102.2020.1760136
48. Khanal P, Dey YN, Patil R, Chikhale R, Wanjari MM, Gurav SS, et al. Combination of System Biology to Probe the Anti-Viral Activity of Andrographolide and Its Derivative Against COVID-19. *RSC Adv* (2021) 11 (9):5065–79. doi: 10.1039/D0RA10529E
49. Liu T, Zhang L, Joo D, Sun SC. Nf-κb Signaling in Inflammation. *Signal Transduct Target Ther* (2017) 2:e17023. doi: 10.1038/sigtrans.2017.23
50. Pelaia C, Vatrella A, Gallelli L, Lombardo N, Sciacqua A, Savino R, et al. Role of P38 Mitogen-Activated Protein Kinase in Asthma and COPD: Pathogenic Aspects and Potential Targeted Therapies. *Drug Des Devel Ther* (2021) 15:1275–84. doi: 10.2147/DDDT.S300988
51. Carter AB, Knudtson KL, Monick MM, Hunninghake GW. The P38 Mitogen-Activated Protein Kinase Is Required for NF-Kappab-Dependent Gene Expression. The Role of TATA-Binding Protein (TBP). *J Biol Chem* (1999) 274(43):30858–63. doi: 10.1074/jbc.274.43.30858
52. Singh D, Smyth L, Borrill Z, Sweeney L, Tal-Singer R. A Randomized, Placebo-Controlled Study of the Effects of the P38 MAPK Inhibitor SB-681323 on Blood Biomarkers of Inflammation in COPD Patients. *J Clin Pharmacol* (2010) 50(1):94–100. doi: 10.1177/0091270009347873
53. Betts JC, Mayer RJ, Tal-Singer R, Warnock L, Clayton C, Bates S, et al. Gene Expression Changes Caused by the P38 MAPK Inhibitor Dilmapimod in COPD Patients: Analysis of Blood and Sputum Samples From a Randomized, Placebo-Controlled Clinical Trial. *Pharmacol Res Perspect* (2015) 3(1):e00094. doi: 10.1002/prp2.94
54. Patra T, Ray R. IL-6 Induction and Signaling: Horizons of COVID-19-Related Pathogenesis. *DNA Cell Biol* (2021) 40(5):639–42. doi: 10.1089/dna.2021.0152
55. Gopalaswamy R, Subbian S. Corticosteroids for COVID-19 Therapy: Potential Implications on Tuberculosis. *Int J Mol Sci* (2021) 22(7):3773. doi: 10.3390/ijms22073773
56. Nishioka C, Ikezoe T, Jing Y, Umezawa K, Yokoyama A. DHMEQ, a Novel Nuclear Factor-Kappab Inhibitor, Induces Selective Depletion of Alloreactive or Phytohaemagglutinin-Stimulated Peripheral Blood Mononuclear Cells, Decreases Production of T Helper Type 1 Cytokines, and Blocks Maturation of Dendritic Cells. *Immunology* (2008) 124(2):198–205. doi: 10.1111/j.1365-2567.2007.02755.x
57. Lin Y, Ukaji T, Koide N, Umezawa K. Inhibition of Late and Early Phases of Cancer Metastasis by the NF-κb Inhibitor DHMEQ Derived From Microbial Bioactive Metabolite Epoxyquinomicin: A Review. *Int J Mol Sci* (2018) 19 (3):729. doi: 10.3390/ijms19030729
58. Shimizu K, Konno S, Ozaki M, Umezawa K, Yamashita K, Todo S, et al. Dehydroxymethylepoxyquinomicin (DHMEQ), A Novel NF-Kappab Inhibitor, Inhibits Allergic Inflammation and Airway Remodelling in Murine Models of Asthma. *Clin Exp Allergy* (2012) 42(8):1273–81. doi: 10.1111/j.1365-2222.2012.04007.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Rehan, Ahmed, Howladar, Refai, Baeissa, Zughaibi, Kedwa and Jamal. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

REVIEW
published: 12 July 2021
doi: 10.3389/fnut.2021.629440

Dairy-Derived and Egg White Proteins in Enhancing Immune System Against COVID-19

Gaber El-Saber Batiha[1†], Mohammed Alqarni[2], Dina A. B. Awad[3†], Abdelazeem M. Algammal[4], Richard Nyamota[5], Mir I. I. Wahed[6], Muhammad Ajmal Shah[7], Mohammad N. Amin[8,9], Babatunde O. Adetuyi[10], Helal F. Hetta[11], Natália Cruz-Martins[12,13,14*], Niranjan Koirala[15,16], Arabinda Ghosh[17], Javier Echeverría[18], Jorge Pamplona Pagnossa[19] and Jean-Marc Sabatier[20]*

[1] Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt, [2] Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Taif, Saudi Arabia, [3] Department of Food Hygiene, Faculty of Veterinary Medicine, Benha University, Benha, Egypt, [4] Department of Bacteriology, Immunology, and Mycology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt, [5] Department of Biochemistry and Molecular Biology, Faculty of Science, Egerton University, Njoro, Kenya, [6] Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh, [7] Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad, Pakistan, [8] Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh, [9] Pratyasha Health Biomedical Research Center, Dhaka, Bangladesh, [10] Department of Natural Sciences, Faculty of Pure and Applied Sciences, Precious Cornerstone University, Ibadan, Nigeria, [11] Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Asyut, Egypt, [12] Faculty of Medicine, University of Porto, Porto, Portugal, [13] Institute for Research and Innovation in Health (i3S), University of Porto, Porto, Portugal, [14] Laboratory of Neuropsychophysiology, Faculty of Psychology and Education Sciences, University of Porto, Porto, Portugal, [15] Department of Natural Products Research, Dr. Koirala Research Institute for Biotechnology and Biodiversity, Kathmandu, Nepal, [16] Laboratory of Biotechnology, Faculty of Science and Technology, University of Macau, Taipa, Macau, [17] Microbiology Division, Department of Botany, Gauhati University, Guwahati, India, [18] Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile, [19] Biological Sciences Department, Federal University of Lavras (UFLA), Lavras, Brazil, [20] Université Aix-Marseille, Institut de Neuro-Physiopathologie (INP), UMR 7051, Faculté de Pharmacie, Marseille, France

OPEN ACCESS

Edited by:
Ioannis Zabetakis, University of Limerick, Ireland

Reviewed by:
Junpeng Wang, Henan University, China
Mengqiang Wang, Ocean University of China, China

***Correspondence:**
Gaber El-Saber Batiha
gaberbatiha@gmail.com
Natália Cruz-Martins
ncmartins@med.up.pt

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutritional Immunology, a section of the journal Frontiers in Nutrition

Received: *14 November 2020*
Accepted: *09 June 2021*
Published: *12 July 2021*

Citation:
Batiha GE-S, Alqarni M, Awad DAB, Algammal AM, Nyamota R, Wahed MII, Shah MA, Amin MN, Adetuyi BO, Hetta HF, Cruz-Martins N, Koirala N, Ghosh A, Echeverría J, Pagnossa JP and Sabatier J-M (2021) Dairy-Derived and Egg White Proteins in Enhancing Immune System Against COVID-19. Front. Nutr. 8:629440. doi: 10.3389/fnut.2021.629440

Coronavirus disease (COVID-19) is a global health challenge, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) triggers a plethora of respiratory disturbances and even multiple organs failure that can be fatal. Nutritional intervention is one of the key components toward to a proper management of COVID-19 patients, especially in those requiring medication, and should thus be considered the first-line treatment. Immuno-modulation and -stimulation are currently being explored in COVID-19 management and are gaining interest by food and pharmaceutical industries. Various dietary combinations, bioactive components, nutrients and fortified foods have been reported to modulate inflammation during disease progression. Dietary combinations of dairy-derived products and eggs are gaining an increasing attention given the huge immunomodulatory and anti-inflammatory properties attributed to some of their chemical constituents. Eggs are complex dietary components containing many essential nutrients and bioactive compounds as well as a high-quality proteins. Similarly, yogurts can replenish beneficial bacteria and contains macronutrients capable of stimulating immunity by enhancing cell immunity, reducing oxidative stress, neutralizing inflammation and regulating the intestinal barriers and gut microbiome. Thus, this review highlights the impact of nutritional intervention on COVID-19 management, focusing on the immunomodulatory and inflammatory effects of immune-enhancing nutrients.

Keywords: SARS-CoV-2, coronavirus disease 2019, immunomodulation, nutritional therapy, nutrients

INTRODUCTION

Coronavirus disease (COVID-19) triggered by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) led to multiple respiratory disturbances, multiple organs failure and even death, ultimately representing a global health challenge. The virus, first reported in December 2019, in the Wuhan city - China (1), has spread worldwide and has been classified by the World Health Organization (WHO) as a global pandemic (2, 3). Clinical presentations by most COVID-19 patients requiring admission include severe inflammation, respiratory failure and reduced appetite (4).

Nutritional intervention, also regarded as the first-line treatment, is among the core components involved in comprehensive management of COVID-19 patients under medication. Available clinical evidence reveals that although people of all ages are susceptible to infection, a poor prognosis and higher mortality rate has been reported in malnourished elderly individuals, immunocompromised patients and even in those with chronic diseases (5). In this sense, not only does good nutrition enhance body immunity against diseases, including COVID-19, but also shortens the recovery period (6). The elderly are especially susceptible to infection, owing to their body's declining physiological and immunological efficiency. This increases the likelihood of infections, being severe and refractory, thereby presenting an immunity challenge to the elderly who happens to contract an infectious pathogen. This elucidates the importance of systematic nutritional derangements in COVID-19 patients since immunity is weakened by inadequate nutrition (7, 8). Currently, immune modulation and stimulation is being increasingly explored for COVID-19 management and has gained interest by both food and pharmaceutical industries. In fact, the immune response is crucial for maintaining healthy human physiology by detecting and eliminating pathogens, aging, or cancer cells (9). Briefly, immunomodulation refers to the immune system ability to regulate fatal illnesses, such as acquired immunodeficiency syndrome caused by human immunodeficiency virus (HIV) (10, 11). Currently used therapeutic immunomodulators include penicillamine, cyclosporine A, cyclophosphamide, pidotimod, levamisole, thiocarbamate, imiquimod, prostaglandin, tilorone, and niridazole (12–16). However, these drugs are usually associated with undesired side effects. In addition, since no effective drug is currently available for COVID-19 treatment or adequate early phase vaccine supply for the entire populace, nutritional enhancement of immune system is the best immediate intervention for preventing SARS-CoV-2 infection. In this sense, this review presents different food sources and nutritional supplements that enhance immune function, such as egg derived proteins, milk and fermented dairy products containing different probiotics (**Figure 1**), that have been shown to play a key role in preventing COVID-19 and in the management of hospitalized patients with mild clinical presentations.

IMMUNE SYSTEM ACTIVITY: A SYNOPSIS

The primary host's defense from pathogens and harmful toxins emanates from the immune system (17). Two categories of the immune system include the adaptive and innate subsystems. The innate immune subsystem also referred to as native/natural immunity is non-specific and forms the layer of protection, usually through mechanical barriers of entry, such as the skin and mucosal tissues (17). Other forms of native immunity involve the non-specific inflammatory components, such as interferons, defensins, and cytokines, and also incorporate the bone marrow components, such as the basophils, monocytes, dendritic cells, macrophages, eosinophils, and neutrophils. As critical first-line of defense, neutrophils and macrophages are also of pivotal important in phagocytosis, the mechanisms through which macrophages recognize and neutralize pathogens and defective/cancer cells (18). Macrophages act also as immune regulators by producing different cytokines (i.e., interleukins, interferon gamma, tumor necrosis factors-α) (19). On the other side, an immunomodulator can be stratified as immunosuppressant, immunostimulant, or immunoadjuvant. In this perspective, immunotherapy can be defined as the host's immunity modulation with the desired outcome of disease treatment or management (20).

In contrast to innate immunity, the specific response by the immune system is referred to as adaptive/acquired/specific. This kind of immune response is usually the second-line of defense and involves B and T lymphocytes (20). Adaptive immunity can either be cell-mediated or humoral immune response, where humoral immunity is mediated by B-lymphocytes through antibodies production, that are specifically directed toward the pathogen, whereas cell-mediated immune responses are initiated by T lymphocytes complexing with infected cells or pathogens, which leads to lysis of the "unwanted" cells and cytokines release, whose primary role is immune regulation. T lymphocytes/cells fall into three subsets, termed as helper (TH), cytotoxic, and suppressor or regulatory T cells. The expression of cluster of differentiation (CD) 8+ cell surface receptors characterizes the cytotoxic lymphocytes. The CD8 surface receptors play a role in recognition of endogenous antigens presented by the major histocompatibility complex class I (MHC-I) from virus-infected cells that have been lysed (**Figure 2**). In contrast, helper T cells, express CD4+surface receptors that recognize exogenous antigens presented by MHC-II. The reaction mechanism of TH cells entails T and B lymphocytes activation by secreting cytokines that also activate other immune cells (21, 22), whereas the suppressor T cells regulate the immune response by smothering autoimmunity, thereby maintain the self-tolerance.

EGG AS AN IMMUNE-ENHANCING FOOD

It has been reported that various dietary combinations, bioactive components, nutrients, and fortified foods modulate inflammation during disease progression (23–25). Eggs represent a complex and controversial dietary component (26) that

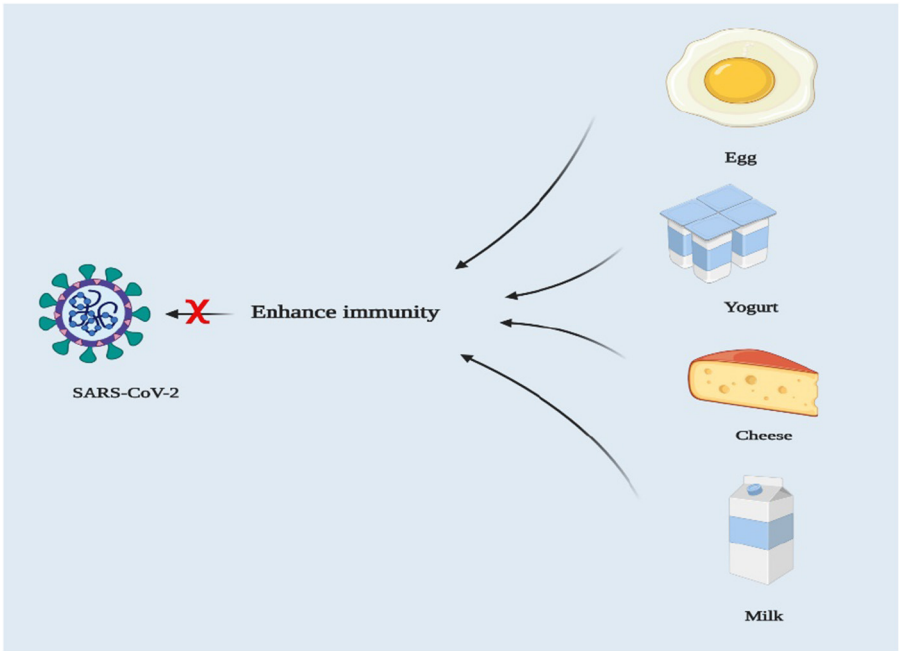


FIGURE 1 | Food sources enhancing the immune response.

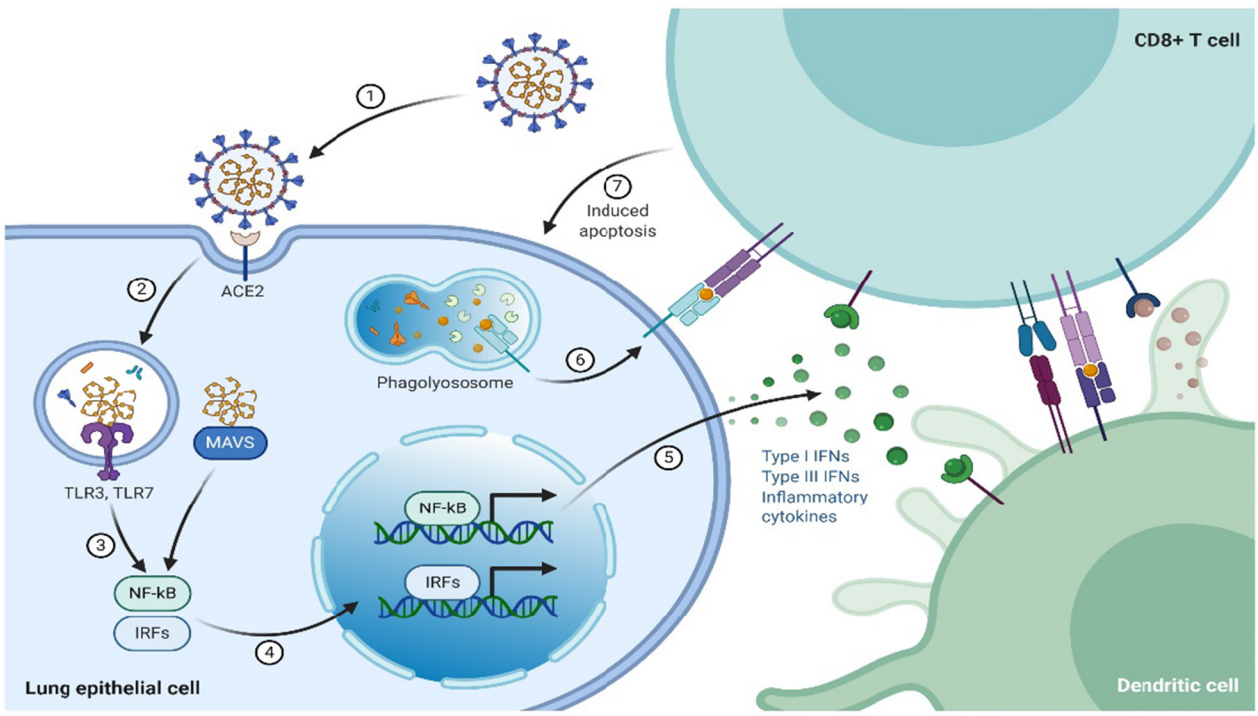


FIGURE 2 | The immune system response to SARS-CoV-2.

contains numerous essential nutrients and bioactive compounds, besides being high-quality protein source leading to divergent opinions in dietary recommendation across populations (27, 28).

Egg Structure

Structurally, eggs are composed of albumin (63%), eggshell (9.5%), and yolk (27.5%) (29). Biochemically, they comprise of 75% water, 12% proteins, 12% lipids, various minerals and carbohydrates (30, 31). Though proteins are distributed across the different egg parts, they are mainly contained in the yolk and egg white, while small proportions occur in the eggshell and shell membrane (32, 33). Lipids exclusively occur in the egg yolk mainly as lipoproteins (30, 32), while the bulk of minerals is found in the eggshell. As minor egg components, carbohydrates are found throughout the egg either as free carbohydrates or glycoconjugates (32). The bulk component of egg is the albumen or egg white that constitutes 60% of cumulative egg weight, whereas protein and water add up to other major components (31, 32). The major egg white's proteins are ovalbumin, ovotransferrin, and ovomucoid. Other proteins include ovomacroglobulin (ovostatin), cystatin, lysozyme, avidin, ovoinhibitor andovomucin that gives the albumen its characteristic viscosity (32).

Dietary Benefits of Eggs

Eggs are rich in complete proteins that promote muscle protein synthesis and maintenance of skeletal mass (34, 35). On average, one large egg provides ∼6.3 g protein that is rich in essential amino acids (36). Eggs are affordable nutritious dietary components with significant health benefits (37). These nutrients include vitamins, essential proteins, minerals, fats, and various bioactive compounds. Eggs contain high nutrients to energy density ratio per egg, while also providing numerous essential nutrients (38). For instance, a typical boiled egg weighing ∼50 g can provide as much as 78 kcal energy, 0.56 g carbohydrate, 6.29 g protein, and 5.3 g total fat. The total fats from an egg contains up to 1.6, 0.7, 2.0, and 186 mg of saturated, polyunsaturated, monounsaturated fats, and cholesterol, respectively. The micronutrients from eggs include iron, calcium, phosphorus, zinc, potassium magnesium, sodium, and most vitamins except vitamin C. These vitamins include riboflavin, niacin, thiamin, folate, and vitamins A, B6, B12, D, E, and K. Besides providing high quality proteins comparable to breast milk, eggs are also a source of antioxidants. Notable antioxidants from eggs include phosvitin rich in phosphoserines, ovotransferrin that chelates $Fe^{3+}$and ovalbumin that improves the polysaccharide's antioxidant activity through covalent binding (39). These antioxidants act by chelating metal ions and scavenging free radicals thereby inhibit the lipids oxidation. Therefore, eggs are potential sources of natural antioxidants that can be used in both cosmetic and food industries. The eggs' antioxidant activity can ameliorate many degenerative conditions, such as cardiovascular diseases (CVD) in humans (40). However, eggs are not only rich in bioactive components and vital nutrients but also in complete proteins and nutritional cholesterol (28, 41). This controversy has resulted in divergent opinions in dietary recommendation of across populations. Nonetheless, owing to their nutritive quality, eggs, and their derivatives are known to regulate inflammation and modulate immunity.

Eggwhite Proteins: Evidence of Immune System Enhancement

Egg white part is rich in bioactive compounds with antimicrobial activity (42, 43), besides immunoprotective proteins, which include ovalbumin, ovotransferrin, ovomucin, lysozyme, and avidin that account for 54, 12, 3.5, 3.4, and 0.5% of the egg white proteins by weight, respectively (42). These proteins exert antimicrobial and immunomodulatory effect through direct action on inflammatory pathways (44, 45).

Inflammation is a conventional physiological reaction to infection by pathogens and physical tissue disruption, but it has also been associated with chronic metabolic disorders (23, 46). Inflammation can be regulated by both nutritional and bioactive supplements thereby reducing the disease risk or pathogenesis (24, 25).

Eggs are the best example of dietary components with immunomodulatory effects (26). Studies have shown that egg proteins and its derivative peptides act on various immunomodulatory pathways (**Table 1**). For instance, ovalbumin modified by methylglyoxal stimulates RAW 264.7 macrophages to secrete TNF-α (56). Elsewhere, Rupa et al. (47) reported that heat denatured ovalbumin modulates cytokines production by CD4+ T cells. Heat-denatured ovalbumin also modulates interleukins' (IL) production by downregulating IL-4 and upregulating IL-10, 12, and 17. Ovalbumin-derived peptides corresponding to amino acids in position 77–84 and 126–134 also enhances the phagocytic activity of macrophages (57) and are reported to enhance immunotherapy (50, 53, 58). Ovotransferrin, another egg white protein, has been shown to stimulate the production of IL-6 and metalloproteinase (MMP) in HD 11 chicken macrophages (59). Xie et al. (59) also reported that ovotransferrin stimulates murine macrophages to produce proinflammatory cytokines through the MAPK signaling pathway. Notably, IL-6 is one of the adaptive immunity components. During chronic inflammation, IL-6 activates T cells, enhances the B cells proliferation and upregulates antibody production. The peptides produced when ovomucin is cleaved by alcalase also express anti-inflammatory activity by inhibiting TNF-mediated NF-κB pathway (49). Tanizaki et al. (60) reported that ovomucin glycoproteins can stimulate macrophages through elevated production of IL-1 and hydrogen. Furthermore, egg white's cystatin enhances the nitric oxide (NO) production by IFN-γ-activated macrophages as a result of IL-10 and TNF-α cytokines activity (61). Cystatin also affects gingival fibroblasts' production of IL-6 and 8 cytokines (62). In another study, Sugahara et al. (63) reported lysozyme-mediated production of immunoglobins. Lysozyme-mediated upregulation of IgM synthesis has been shown to be effective in the management of chronic sinusitis bronchitis and sinusitis (55, 64). Elsewhere, Ha et al. (54) reported the immunomodulatory activity of Maillard-type lysozyme-galactomannan conjugate through NO-enhanced cytokines production in macrophages. This immunomodulatory

effect also originates from stimulation of ERK, NF-κB, and JNK pathways. Livetin from the egg yolk has been reported to suppress proinflammatory cytokines, such as IL-1β, 6, and 10 and TNF-α in macrophages thereby inhibiting inflammation (52). Immunoglobulins (G1, G2, M, and A) have been shown to possess immunomodulatory properties in the management of bacterial and viral diseases. Yolkin from egg yolk has also been reported to inhibit free radical generation, thereby inhibiting oxidative stress and pro-inflammatory cytokines, like IL-1β, 6, and 10 and TNF-α in macrophages (55), although at high temperatures, the rate of immunoglobulins aggregation is greatly enhanced thereby locking immunoglobulins in aggregates before complete denaturation.

FERMENTED DAIRY PRODUCTS AS IMMUNE-ENHANCING FOOD

Historically, fermentation has been widely used in food/beverage processing and preservation. Food fermentation improves palatability besides enhancing nutrients' bioavailability. In addition, microbial fermentation can eradicate toxic milk metabolites, such as galactose and lactose, thereby precluding lactose intolerance and galactose accrual (65). This nutritional and health significance results from modulating intestinal microbiome, ultimately enhancing life expectancy. Fermented dairy products have so far been popular in nutritional enhancement and health promotion. For instance, yogurt resulting from synergistic fermentation of lactose in coagulated milk into lactic acid by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* action, led to the production of a plethora of bioactive compounds in fermented milk, while others have not yet been characterized. As a result of fermented dairy foods consumption, live commensal microbiota can enhance the gut's immunological activity and modulate tolerance from foods-derived antigens (66, 67). The synergy between the immune system and gut microbiome is partially understood compared to the bacteria induced by fermentation by-products.

Nutritional Value of Probiotic Yogurt

First, it is essential to highlight that a probiotic yogurt is not only a rich source of proteins and fats but also contains numerous essential nutrients. As much as 8 g fats and 9 g of proteins can be sourced per yogurt serving which is more than enough of the recommended daily protein intake. Yogurt is also rich in micronutrients, such as phosphorus, zinc, calcium (33% recommended daily intake, 1,000 mg), vitamin A (10% recommended daily intake), vitamin B12 (>40% recommended daily intake), and pantothenic acid and riboflavin as per Canada's health food standards. As a fermented dairy product, yogurt is also potentially effective in alleviating gastrointestinal (GI) complications, such as constipation, lactose intolerance, *Helicobacter pylori* infection and inflammatory bowel diseases (IBDs) (68, 69). Studies have also suggested that human immunity can be enhanced by yogurt since it influences the intestinal microbiota's equilibrium thereby stimulating the GI immunity through lactic acid and other bacterial metabolites (70, 71).

Despite this marvelous nutritional significance, a reduced consumption of fermented dairy products is associated with a weakened immunity and elevated risk of infectious diseases. In the COVID-19 perspective, GI complications have been commonly reported in patients whose clinical conditions deteriorates rapidly (72). However, administration of gastric medications need to be approached cautiously on case-by-case basis considering their potential to disrupt the immune response thus interfering with COVID-19 treatments currently under evaluation (73, 74). However, the suggested probiotics' health effects from fermented dietary products can be realized only when cell viability is reliable (75). So, to ensure probiotics survival through both GI tract and food processing/storage, the minimal recommended presentation of bacteria is 10^6-10^7 colony-forming units (CFUs)/mL organism in each food product (76), despite other factors should also be considered when adding probiotic bacteria in fermented food products to ensure the bacteria's survival. Among them, the compatibility of fermented dairy foods with a wide range of probiotics needs their consideration as "the most efficient delivery vehicle" for probiotics (77, 78), while supplementing fermented milk with probiotics represents an exemplary mode of replenishing the gut microbiota (79).

The Role of Probiotics in Enhancing the Immune System

The healthy benefits from fermented foods including yogurt have been recognized for a long time (80). Regular consumption of yogurt replenishes beneficial bacteria and macronutrients that enhance immunity by specifically boosting cell immunity, while downregulating oxidative stress (81, 82). Yogurt fortification with probiotics has been shown to greatly enhance the beneficial effects from a health perspective (83, 84). Typically, probiotic bacteria can survive to extreme conditions in the GI tract and maintain their functional benefits where other bacterial strains are catabolized (85). There are many probiotic microbes each with unique biological features, including therapeutic significance. For instance, some probiotic strains are effective in enhancing GI regularity and improving lactose tolerance, whereas others beyond this review's scope have been shown to prevent acute upper respiratory tract infections and bacterial vaginosis. Probiotics within the GI tract inhibit both pathogenic microbes adherence and proliferation (83, 86). This is important in immune homeostasis, since probiotic supplements rejuvenate immunity by balancing the gut microbiome, ultimately stabilizing TREG and Th17 cells (87, 88). Moreover, it can be hypothesized that probiotics consumption can potentially dampen the cytokine storm in combination with appropriate antiviral regimens. Despite the gut microbiota's susceptibility to viral perturbation, it can be equilibrated by dietary interventions. Specifically, to what concerns to COVID-19, gastroenteritis and respiratory distress are some of the most common symptoms (89). By hosting the bulk of body's lymphoid tissues, the GI tract largely influences the immune function when

TABLE 1 | Egg proteins and peptides with immunomodulatory activity.

Protein	Peptides-making enzymes	Biological activity	Mechanism of action	References
Ovalbumin	Pepsin, chymotrypsin	Immunomodulation	- Stimulate TNF-secretion - Increase IL-12,IL-17 and IL-10 production and decreased IL-4	(47)
Ovotransferrin	Thermolysin, pepsin	Immunomodulation	- Stimulate IL-6 production - Increase proinflammatory cytokines production	(48)
Ovomucin	Alcalase, pronase, papain	Immunomodulation	- Stimulate macrophages activity	(49)
Cystatin		Immunomodulation	- Induce TNF, IL-10 synthesis	(50)
Lysozyme		Immunomodulation, antimicrobial	- Stimulate immunoglobulinsproduction and improve chronic sinusitis and bronchitis - Increase proinflammatory cytokine production	(51)
Livetin	Pepsin, acalase	Immunomodulation	- Suppress proinflammatory cytokines production	(52)
IgG1	Papain, Peroxidase	Immunomodulation antimicrobial	Inhibit bacterial metabolism by blocking enzymes Stimulate immunoglobulins production Suppress viral and toxin production	(52)
IgG2	Papain, Peroxidase	Immunomodulation antimicrobial	Inhibit bacterial metabolism by blocking enzymes Suppress viral and toxin production Stimulate immunoglobulins production	(52)
IgM	Peroxidase	Immunomodulation antimicrobial	Inhibit bacterial metabolism by blocking enzymes Suppress viral and toxin production	(53)
IgA	Peroxidase	Immunomodulation antimicrobial	Inhibit bacterial metabolism by blocking enzymes Suppress viral and toxin production	(54)
Yolkin	vitellogenin	Immunomodulation Antioxidative, Neuroprotective	Immunoglobulins production Increase proinflammatory cytokine production Inhibition of free radical generation	(55)

its integrity is altered (90). To this effect, both pre and probiotics able to equilibrate GI microbiota have been reported to reduce the risk of infection (91).

Probiotics have been shown to neutralize inflammation by inhibiting NK cells activation and equilibrating the GI barrier and gut microbiome. However, the exact mechanisms through which probiotics modulate immunity are not perfectly understood (92, 93). *Lactobacillus casei* Shirota (LcS) is a widely studied probiotic with immunomodulatory effects (94), being able to enhance immune system against complications from post-operative infection (95), cancer (96), allergies (97), and to regulate GI functions (98). This probiotic boosts the beneficial gut's bacteria species, augments NK cell activity while maintaining a proper balance between harmful and beneficial bacteria (99, 100). Furthermore, experimental evidence has shown that LcS promotes IL-12 production by peripheral blood mononuclear cells (PBMC) from healthy humans and enhance NK cells' activity, while heat-inactivated LcS boost the cytokines production, including IL-10, 12, IFN-γ, and TNF-α that enhances CD69 expression in NK cells (94).

Though the synergy between microbiome, fermentation by-products and immune system is not fully known, antigen presentation by dendritic cells is thought to be a key player in this aspect. Thus, dendritic cells are significant immunomodulators by initiating diverse responses to various immune stimulations (101). Dendritic cells from monocytes lineage can also be modulated when they interact with lactic acid bacteria (LAB). This response characterizes infection by bacteria and cell debris. Furthermore, through action of GI fermentative bacteria, other bioactive metabolites with immunomodulatory properties are produced. For example, bioactive products generated by *Bifidobacterium breve* can modulate cytokines production by intestinal epithelial cells. Indeed, *B. breve* triggers milk serum fermentation and forms a supernatant that stimulates both dendritic cells development and activation (101).

The Role of Prebiotic in Enhancing the Immune System

Prebiotic is another additive on fermented dairy products with immune enhancing potential. Prebiotics are non-digestible food supplements that potentially stimulate development of gut domiciled bacteria with host's health benefits (102). For example, the yeast cell walls metabolism generates mannan oligosaccharide (MOS) that contains 30% glucan, 30% mannan, and 12.5% mannoproteins (103, 104). The MOS ability to bind lectin on pathogenic microbes, such as *Salmonella* spp and *Escherichia coli*, minimizes their intestinal proliferation (105, 106).

Another prebiotic yielded as a by-product of fungal/yeast cell wall catabolism is β-glucan, that derives from D-glucose monomers, and are formed via 1–3 β-glycosidic bonds, while the long side chains have a 1–6 glycosidic bond. β-glucans stimulate sentinel cells to produce cytokines and induce lymphocyte multiplication (107). The three types of lymphocytes include NK, T, and B cells. NK and T cells are involved in innate and adaptive immune responses, respectively, whereas B cells are mainly involved in immunoglobulins production. β-glucan can exert its immunomodulatory potential on three lymphocyte categories.

Fructan is another prebiotic derived from plant polysaccharides hydrolyzation or as a by-product of microbial catabolism of plant polysaccharides. The three classes of fructans include levan, inulin and branched groups. Fructan's biological significance is attributed to its β-glycosidic bond that withstands catabolic activities of digestive enzymes whilst augmenting the levels of commensal bacteria including *Lactobacilli* and *Bifidobacteria* spp., whereas pathogenic microbes such as *E. coli* and *Clostridium pefringens* are inhibited (41, 108, 109). Other oligosaccharides with prebiotic potency include xylo-oligosaccharides, chitosan, galactoglucomannan, and galacto-oligosaccharides, with studies revealing that they have immunomodulatory activity following supplementation with fermented dairy products, acting on both cytokines and immunoglobulins production, and promoting phagocytosis, NK and T cells' activity (110, 111). In conclusion, fortification of fermented dairy products with pre- and probiotics could be beneficial in boosting immune system against infections, possibly including SARS-CoV-2.

Experimental Evidences

A study utilizing fecal samples from Polymerase Chain Reaction (PCR) confirmed and hospitalized COVID-19 patients demonstrated presence of more opportunistic pathogens while the commensal microbes in the gut were depleted compared to healthy individuals. The samples from healthy individuals had prevalence of *Eubacterium, Roseburia, Lachnospiraceae*, *Facecalibacterium prausnitzii*. Currently, three registered clinical trials are investigating the effect of pre and probiotics on COVID-19 patients. One of the studies is evaluating the beneficial effects of *Lactobacillus coryniformis* on COVID-19 incidence amongst health care workers with a high risk of SARS-CoV2 (NCT 04366180). Another registered clinical trial (NCT04368351) is focusing on bacteriotherapy effects in the treatment of patients with acute diarrhea, and in preventing intensive care in COVID-19 patients. Lastly, the third clinical trial (NCT04366089) is evaluating the adjuvant uses of oxygen-ozone therapy along with probiotic supplementation in COVID-19 patients. Based on these ideas, both pre and probiotics could be proposed as potential tools to be included in the nutritional treatment of COVID-19 patients. Some of these potentially therapeutic probiotics include *Lactobacillus rhamnosus* and *Bifidobacterium lactis*, that exhibits anti-inflammatory effects while also rejuvenating both innate and adaptive immunity.

CHEESE

Cheese is a milk-derived product, rich in nutrients, such as fats, proteins, essential minerals and vitamins, processed by souring milk or clotting it with renin (112). Cheese processing leads to the formation of a versatile food product with a variety of flavors, textures, and nutritional benefits. Physical features of cheese are influenced by casein content, type, quantity, and their interactional strength, its proximate composition and ripening forms (113).

Nutritional Value of Cheese

Cheese has high nutritive value, being rich in proteins, fat, vitamins, and minerals like calcium (Ca^{2+}) and phosphorous (114). Save for cysteine and methionine, cheese is rich in all essential amino acids enough for recommended human intake (115). Besides being significant in human nutrition, proteins are currently considered as important sources of bioactive peptides. These are amino acid sequences that give to a modified protein its characteristic biological activity. These bioactive peptides exert their actions through downregulating blood pressure, chelating minerals, exerting antimicrobial effects, modifying immune system, reducing inflammation, and cholesterol levels (116).

Cheese as an Immunity Enhancing Product

Studies have shown association between low protein levels (especially immunoglobulins) from cheese and an enhanced risk of infection (114). These nutritional supplements are important in regulating inflammation and oxidative stress responses both known to influence immunity (117). The nutritional impact on immune response is a key aspect in the formulation of anti-inflammatory dietary index (117). For example, dietary supplements rich in omega-3 fatty acids are known to have excellent antioxidant and anti-inflammatory effects, and also a good immunomodulatory potency (118). Elsewhere, experimental protein feeding below 0.8 g/kg body weight in mice, predisposed the animals to severe influenza infection, due to insufficient antibody response, persistence lung viremia, and elevated inflammation with fatal outcomes (119), whereas cheese-derived dietary products rich in calories and saturated fats inhibits inflammation and lipogenesis (120). Thus, the dietary incorporation cheese can reduce post-prandial lipogenesis and inflammatory activities [60]. Furthermore, it has been stated that protein meals, rich in essential amino acids can modulate post-meal glycemic stimuli and improve satiety, since they have better gastric retention and prolonged GI

transit (121). This underscores the significance of high quality cheese-derived nutrients, such as calories and saturated fats, as anti-inflammatory enhancing diets that can be incorporated in nutritional immune modulation (122).

MILK

Milk is one of the main dietary sources of protein for humans, and is primarily composed of water (87%), lactose (5%), fats (0.3%), proteins (4%), vitamins (0.1%), and minerals (123). Milk protein can be categorized as either insoluble (casein) or soluble (whey), with latter constituting 20% of milk proteins, while insoluble proteins constitute the remaining fraction (80%) (124). Both protein categories can sufficiently meet the human's amino acid requirements, besides being digestible and bioavailable. In newborns, milk forms the primary source of bioactive molecules, acting on immune system enhancement against bacteria and viruses and promoting the development of both GI system and bones. The antiviral activity of milk has been attributed to bioactive components with immunomodulatory and anti-inflammatory potential, such as casein, whey proteins and associated peptides (41).

Dietetic Benefits of Milk

Milk is rich in nutritional components essential for a healthy growth and human development. Minerals, such as calcium is vital in regulating high blood pressure besides being crucial in teeth and bones formation, whereas selenium is a key antioxidant that boosts immune system. The active biological compounds present in milk, including essential amino acids and fatty acids makes it superior to meat in terms of biological significance and value. A good example of these fatty acids is omega-3 which has been implicated in inhibiting some types of cancer and CVD. Caseins, mainly classified as alpha, beta and kappa, act by chelating minerals, such as phosphorus and calcium thereby transporting them as a coagulum in the gut (125).

Recent studies have proposed a correlation between beta casein protein components and human health (126, 127). Bovine milk is an ideal protein source containing all essential amino acids, and high levels of branched chain amino acids, thereby considered as a reference baseline for quantifying other proteins with huge nutritive value (3). Branched chain amino acids, particularly leucine, have been indicated in enhancing synthesis of muscle proteins. Whey protein is also rich in methionine and sulfur-containing amino acids, crucial for glutathione formation, to whom great antioxidant and immunomodulatory effects have been attributed, besides being effective against cancer cells (4, 99).

Casein Proteins

Casein is a heterogenous protein family, mainly composed of calcium-phosphate micelle complexes (97), classified into alpha-, beta-, gamma-, and kappa-casein (128, 129). Casein and its derivative peptides are known to modulate immune responses, ultimately enhancing the antiviral activity while mitigating sepsis by immune downregulation (126, 130). Caseins also activate B and T-cell mediated responses, thereby linking innate to adaptive immunity. Several casein-derived peptides are also known to have human health benefits by stimulating CV, digestive, immune and central nervous (CNS) systems. For example, some peptides have shown good antioxidant (131), cytoprotective, immunomodulatory (126), antithrombotic and anti-hypertensive effects (131). An example of a peptide that interacts with the CNS is β-casomorphine that exert analgesic effects by acting like opioids (132). Studies have also demonstrated peptides' gut interference via enhanced mucin production, thereby inhibiting the pathogens adherence while enhancing the GI retention which has been linked to a better weight management, through regulated food intake (133).

Whey Proteins

Whey proteins are specific high-energy crude nutrients with various textures and thicknesses. They often accompany other palatable foods provided to hospitalized patients to improve protein-calorie intake. In addition, they have been associated with immunomodulatory benefits besides complementing antiviral regimens in patients under medication (134, 135).

Whey proteins found in milk are soluble heat labile globular structured compounds stabilized by intermolecular disulfide linkages. Being a heterogenous protein, it is composed of polymorphic proteins including beta-lactoglobulin and alpha-lactalbumin that confers the characteristic foaming and gelation properties (124, 136). Proteose, peptone, immunoglobulin, and glycomacropeptide found in whey have also shown *in vitro* and *in vivo* bioactivities (137). Other whey constituents include lactoferrin, bovine serum albumin (BSA), lactoperoxidase, non-protein components, such as vitamins, fats, lactose, and minerals (138, 139). Lactoferrin is an important immunomodulator with interesting antimicrobial and antioxidant effects. Animal and human experiments have even shown that oral lactoferrin administration is effective against infections, cancer and inflammation, thereby making it an appropriate food additive (140).

Immune System Enhancement by Whey Proteins

Experimental evidence has demonstrated that whey proteins have huge immune response enhancing properties. For instance, whey was able to elevate mucosal antibody response against cholera toxin and ovalbumin when fed to mice for 12 weeks compared to control on a regular diet (141). In another study, bovine whey proteins consumption (for 5–8 weeks) by mice led to a marked raise on foot pad delayed-type hypersensitivity responses and *in vitro* concanavalin A-induced spleen cell proliferation (141). The helper T cell (L3T4+) populations were also elevated in mice receiving undenatured whey protein (25 g) for 4 weeks compared to the control group fed an isocaloric casein diet (142). Not only the L3T4+ cells population were elevated but also the helper (L3T4+) to suppressor T-cells (Lyt-2+) ratio too. In comparison to casein and soy protein rich diets, whey protein-diet has been linked to an elevated count of CD4+, and C8+ lymphocytes, total white blood cells and increased IFN-γ production by concanavalin A-stimulated spleen in experimental mice (143). Whey proteins also increase the glutathione levels in plasma and enhance the NK cells activity in patients with chronic hepatitis B (144). Moreover, whey proteins' antiviral activity has been

reported against HIV. Further, β-LF strongly inhibits reverse transcriptase and mildly inhibits integrase and protease, whereas β-LG and α-LA inhibits integrase and protease but not reverse transcriptase during the early infection stages (145). Elsewhere, whey derivatives, such as β-lactoglobulin and α-lactalbumin have been shown to bind and deter attachment of rotavirus to the host's cells receptors (146). In addition, lactoperoxidase enzyme from whey when combined with thiocyanate and hydrogen peroxide substrates also exhibits antiviral activity against herpes, HIV and echovirus (147, 148). Moreover, when orally administered, lactoperoxidase can mediate pneumonia disruption in mice experimentally infected with influenza by suppressing inflammation in lung cells (149).

CONCLUSION

SARS-CoV-2 infection has triggered a serious global devastation in human being's well-being and health maintenance, and a huge impact in healthcare delivery systems. Elderly individuals are particularly vulnerable to such infection, as both physiological and immunological performance declines with age, ultimately raising the risk of refractory infections and gets serious ill. Hence, implementation of infection control programs is difficult for them. This review mostly aimed to clarify the value of systemic dietary distortions in COVID-19 patients, as immunity is impaired by a poor nutritional status. For that purpose, here the different types of food that enhance humans' immune response, such as egg-derived proteins, milk, and fermented dairy products containing different probiotics were addressed and discussed. Eggs, yogurt, cheese, and milk are rich sources of essential macro and micronutrients, that possess excellent immunomodulatory, anti-inflammatory, antioxidant and antiviral activities, being thus essential for the maintenance of a healthy growth and development.

AUTHOR CONTRIBUTIONS

GB, DA, AA, RN, MW, MS, MNA, BA, NC-M, NK, HH, AG, and J-MS wrote and carefully revised the paper. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

NC-M acknowledges the Portuguese Foundation for Science and Technology under the Horizon 2020 Program (PTDC/PSI-GER/28076/2017). **Figures 1**, **2** were made using Biorender.com.

REFERENCES

1. Weston S, Frieman M. COVID-19: knowns, unknowns, and questions. *mSphere.* (2020) 5:e00203-20. doi: 10.1128/mSphere.00203-20
2. Lake MA. What we know so far: COVID-19 current clinical knowledge and research. *Clin Med.* (2020) 20:124. doi: 10.7861/clinmed.2019-coron
3. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun.* 2020:102433 .doi: 10.1016/j.jaut.2020.102433
4. Phelan AL, Katz R, Gostin LO. The novel coronavirus originating in Wuhan, China: challenges for global health governance. *Jama.* (2020) 323:709–10. doi: 10.1001/jama.2020.1097
5. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet.* (2020) 395:470–3. doi: 10.1016/S0140-6736(20)30185-9
6. Alwarawrah Y, Kiernan K, MacIver NJ. Changes in nutritional status impact immune cell metabolism and function. *Front Immunol.* (2018) 9:1055. doi: 10.3389/fimmu.2018.01055
7. Yaqoob P. Ageing alters the impact of nutrition on immune function. *Proc Nutr Soc.* (2017) 76:347–51. doi: 10.1017/S0029665116000781
8. Lomax AR, Calder PC. Prebiotics, immune function, infection and inflammation: a review of the evidence. *Br J Nutr.* (2008) 101:633–58. doi: 10.1017/S0007114508055608
9. Villani A-C, Sarkizova S, Hacohen N. Systems immunology: learning the rules of the immune system. *Ann Rev Immunol.* (2018) 36:813–42. doi: 10.1146/annurev-immunol-042617-053035
10. Routy J-P, Mehraj V, Cao W. *HIV Immunotherapy Comes of Age: Implications for Prevention, Treatment and Cure*. Abingdon: Taylor & Francis (2016). doi: 10.1586/1744666X.2016.1112269
11. Naidoo J, Page D, Wolchok J. Immune modulation for cancer therapy. *Br J Cancer.* (2014) 111:2214–9. doi: 10.1038/bjc.2014.348
12. Ozkan MC, Tombuloglu M, Sahin F, Saydam G. Evaluation of immunomodulatory drugs in multiple myeloma: single center experience. *Am J Blood Res.* (2015) 5:95.
13. Flores C, Fouquet G, Moura IC, Maciel TT, Hermine O. Lessons to learn from low-dose cyclosporin-A: a new approach for unexpected clinical applications. *Front Immunol.* (2019) 10:588. doi: 10.3389/fimmu.2019.00588
14. Gong Y, Klingenberg SL, Gluud C. D-penicillamine for primary biliary cirrhosis. *Cochr Datab Syst Rev.* (2004) 18:CD004789. doi: 10.1002/14651858.CD004789.pub2
15. Ahlmann M, Hempel G. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. *Cancer Chemother Pharmacol.* (2016) 78:661–71. doi: 10.1007/s00280-016-3152-1
16. Ulrich C, Bichel J, Euvrard S, Guidi B, Proby C, Van De Kerkhof P, et al. Topical immunomodulation under systemic immunosuppression: results of a multicentre, randomized, placebo-controlled safety and efficacy study of imiquimod 5% cream for the treatment of actinic keratoses in kidney, heart, and liver transplant patients. *Br J Dermatol.* (2007) 157:25–31. doi: 10.1111/j.1365-2133.2007.08269.x
17. Turvey SE, Broide DH. Innate immunity. *J Allergy Clin Immunol.* (2010) 125:S24–32. doi: 10.1016/j.jaci.2009.07.016
18. Raval RR, Sharabi AB, Walker AJ, Drake CG, Sharma P. Tumor immunology and cancer immunotherapy: summary of the (2013). SITC primer. *J Immunother Cancer.* (2014) 2:14. doi: 10.1186/2051-1426-2-14
19. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol.* (2014) 5:491. doi: 10.3389/fimmu.2014.00491
20. Lim YT. Vaccine adjuvant materials for cancer immunotherapy and control of infectious disease. *Clin Exp Vaccine Res.* (2015) 4:54–8. doi: 10.7774/cevr.2015.4.1.54
21. Broere F, van Eden W. T cell subsets and T cell-mediated immunity. In: Parnham M, Nijkamp F, Rossi A, editors. *Nijkamp and Parnham's Principles of Immunopharmacology*. Cham: Springer (2019). p. 23–35. doi: 10.1007/978-3-030-10811-3_3
22. Parnham MJ, Nijkamp FP. *Principles of Immunopharmacology*. Basel: Springer (2005). doi: 10.1007/3-7643-7408-X
23. Pan M-H, Lai C-S, Ho C-T. Anti-inflammatory activity of natural dietary flavonoids. *Food Funct.* (2010) 1:15–31. doi: 10.1039/c0fo00103a
24. Zeng C, Zhong P, Zhao Y, Kanchana K, Zhang Y, Khan ZA, et al. Curcumin protects hearts from FFA-induced injury by activating Nrf2 and

inactivating NF-κB both *in vitro* and *in vivo*. *J Mol Cell Cardiol.* (2015) 79:1–12. doi: 10.1016/j.yjmcc.2014.10.002
25. Siriwardhana N, Kalupahana NS, Cekanova M, LeMieux M, Greer B, Moustaid-Moussa N. Modulation of adipose tissue inflammation by bioactive food compounds. *J Nutr Biochem.* (2013) 24:613–23. doi: 10.1016/j.jnutbio.2012.12.013
26. Herron KL, Fernandez ML. Are the current dietary guidelines regarding egg consumption appropriate? *J Nutr.* (2004) 134:187–90. doi: 10.1093/jn/134.1.187
27. Millward DJ, Layman DK, Tomé D, Schaafsma G. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. *Am J Clin Nutr.* (2008) 87:1576S−81S. doi: 10.1093/ajcn/87.5.1576S
28. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr.* (2009) 89:161–8. doi: 10.3945/ajcn.2008.26401
29. CotterillO, Geiger G. Egg product yield trends from shell eggs. *Poult Sci.* (1977) 56:1027–31. doi: 10.3382/ps.0561027
30. Ridgman W. The Avian Egg. Chemistry and Biology, by RW Burley & DV Vadehra. v+ 472 pp. New York: Wiley. (1989) £ 66.70 (hard covers). ISBN 0 471 84995 2. *J Agric Sci.* (1991) 116:169–70. doi: 10.1017/S0021859600076310
31. Li-Chan EC. The chemistry of eggs and egg products. In: Eunice C, Li-Chan Y, Powrie WD, Nakai S, editors. *Egg Science and Technology*. Boca Raton, FL: CRC Press (1995). p. 105–75.
32. Juneja LR, Yamamoto T, Hatta H, Kim M. *Hen Eggs: Their Basic and Applied Science*. Boca Raton, FL: CRC Press (1997).
33. Mine Y. Recent advances in egg protein functionality in the food system. *World's Poult Sci J.* (2002) 58:31–9. doi: 10.1079/WPS20020005
34. Børsheim E, Tipton KD, Wolf SE, Wolfe RR. Essential amino acids and muscle protein recovery from resistance exercise. *Am J Physiol Endocrinol Metab.* (2002) 283:E648–57. doi: 10.1152/ajpendo.00466.2001
35. US Department of Agriculture ARS. *USDA national nutrient database for standard reference, release 28*. Nutrient data laboratory home page (2011).
36. Kovacs-Nolan J, Phillips M, Mine Y. Advances in the value of eggs and egg components for human health. *J Agric Food Chem.* (2005) 53:8421–31. doi: 10.1021/jf050964f
37. Miranda JM, Anton X, Redondo-Valbuena C, Roca-Saavedra P, Rodriguez JA, Lamas A, et al. Egg and egg-derived foods: effects on human health and use as functional foods. *Nutrients.* (2015) 7:706–29. doi: 10.3390/nu7010706
38. Kuang H, Yang F, Zhang Y, Wang T, Chen G. The impact of egg nutrient composition and its consumption on cholesterol homeostasis. *Cholesterol.* (2018) 2018:6303810. doi: 10.1155/2018/6303810
39. Jung S, Kim DH, Son JH, Nam K, Ahn DU, Jo C. The functional property of egg yolk phosvitin as a melanogenesis inhibitor. *Food Chem.* (2012) 135:993–8. doi: 10.1016/j.foodchem.2012.05.113
40. Natoli S, Markovic T, Lim D, Noakes M, Kostner K. Unscrambling the research: eggs, serum cholesterol andcoronary heart disease. *Nutr Diet.* (2007) 64:105–11. doi: 10.1111/j.1747-0080.2007.00093.x
41. Kim G-B, Seo Y, Kim C, Paik I. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poult Sci.* (2011) 90:75–82. doi: 10.3382/ps.2010-00732
42. Kobayashi Y, Rupa P, Kovacs-Nolan J, Turner PV, Matsui T, Mine Y. Oral administration of hen egg white ovotransferrin attenuates the development of colitis induced by dextran sodium sulfate in mice. *J Agric Food Chem.* (2015) 63:1532–9. doi: 10.1021/jf505248n
43. Yokooji T, Hamura K, Matsuo H. Intestinal absorption of lysozyme, an egg-white allergen, in rats: kinetics and effect of NSAIDs. *Biochem Biophys Res Commun.* (2013) 438:61–5. doi: 10.1016/j.bbrc.2013.07.024
44. Yokooji T, Nouma H, Matsuo H. Characterization of ovalbumin absorption pathways in the rat intestine, including the effects of aspirin. *Biol Pharm Bull.* (2014) 37:1359–65. doi: 10.1248/bpb.b14-00290
45. Medzhitov R. Origin and physiological roles of inflammation. *Nature.* (2008) 454:428–35. doi: 10.1038/nature07201
46. Medzhitov R. Recognition of microorganisms and activation of the immune response. *Nature.* (2007) 449:819–26. doi: 10.1038/nature06246
47. Rupa P, Schnarr L, Mine Y. Effect of heat denaturation of egg white proteins ovalbumin and ovomucoid on CD4+ T cell cytokine production and human mast cell histamine production. *J Funct Foods.* (2015) 18:28–34. doi: 10.1016/j.jff.2015.06.030
48. Lee JH, Ahn DU, Paik H-D. *In vitro* immune-enhancing activity of ovotransferrin from egg white via MAPK signaling pathways in RAW 264.7 macrophages. *Korean J Food Sci Anim Resour.* (2018) 38:1226. doi: 10.5851/kosfa.2018.e56
49. Sun X, Chakrabarti S, Fang J, Yin Y, Wu J. Low-molecular-weight fractions of Alcalase hydrolyzed egg ovomucin extract exert anti-inflammatory activity in human dermal fibroblasts through the inhibition of tumor necrosis factor–mediated nuclear factor κB pathway. *Nutr Res.* (2016) 36:648–57. doi: 10.1016/j.nutres.2016.03.006
50. Vidovic D, Graddis T, Chen F, Slagle P, Diegel M, Stepan L, et al. Antitumor vaccination with HER-2-derived recombinant antigens. *Int J Cancer.* (2002) 102:660–4. doi: 10.1002/ijc.10785
51. Pellegrini A, Thomas U, Wild P, Schraner E, Von Fellenberg R. Effect of lysozyme or modified lysozyme fragments on DNA and RNA synthesis and membrane permeability of Escherichia coli. *Microbiol Res.* (2000) 155:69–77. doi: 10.1016/S0944-5013(00)80040-3
52. Meram C, Wu J. Anti-inflammatory effects of egg yolk livetins (α, β, and γ-livetin) fraction and its enzymatic hydrolysates in lipopolysaccharide-induced RAW 264.7 macrophages. *Food Res Int.* (2017) 100:449–59. doi: 10.1016/j.foodres.2017.07.032
53. He X, Tsang TC, Luo P, Zhang T, Harris DT. Enhanced tumor immunogenicitythrough coupling cytokine expression with antigen presentation. *Cancer Gene Ther.* (2003) 10:669–77. doi: 10.1038/sj.cgt.7700618
54. Ha Y-m, Chun S-H, Hong S-t, Koo Y-c, Choi H-d, Lee K-w. Immune enhancing effect of a Maillard-type lysozyme-galactomannan conjugate via signaling pathways. *Int J Biol Macromol.* (2013) 60:399–404 .doi: 10.1016/j.ijbiomac.2013.06.007
55. Sava G. Pharmacological aspects and therapeutic applications of lysozymes. *EXS.* (1996) 75:433–49. doi: 10.1007/978-3-0348-9225-4_22
56. Fan X, Subramaniam R, Weiss M, Monnier V. Methylglyoxal–bovine serum albumin stimulates tumor necrosis factor alpha secretion in RAW 264.7 cells through activation of mitogen-activating protein kinase, nuclear factor κB and intracellular reactive oxygen species formation. *Arch Biochem Biophys.* (2003) 409:274–86. doi: 10.1016/S0003-9861(02)00599-4
57. Mine Y, D'Silva I. Bioactive components in egg white. In: Mine Y, editor. *Egg Bioscience and Biotechnology*. New York, NY: John Wiley & Sons (2008). p. 141–84. doi: 10.1002/9780470181249.ch4
58. Goldberg J, Shrikant P, Mescher MF. *In vivo* augmentation of tumor-specific CTL responses by class I/peptide antigen complexes on microspheres (large multivalent immunogen). *J Immunol.* (2003) 170:228–35. doi: 10.4049/jimmunol.170.1.228
59. Xie H, Huff GR, Huff WE, Balog JM, Rath NC. Effects of ovotransferrin on chicken macrophages and heterophil-granulocytes. *Dev Comp Immunol.* (2002) 26:805–15. doi: 10.1016/S0145-305X(02)00028-9
60. Tanizaki H, Tanaka H, Iwata H, Kato A. Activation of macrophages by sulfated glycopeptides in ovomucin, yolk membrane, and chalazae in chicken eggs. *Biosci Biotechnol Biochem.* (1997) 61:1883–9. doi: 10.1271/bbb.61.1883
61. Verdot L, Lalmanach G, Vercruysse V, Hoebeke J, Gauthier F, Vray B. Chicken cystatin stimulates nitric oxide release from interferon-γ-activated mouse peritoneal macrophages via cytokine synthesis. *Eur J Biochem.* (1999) 266:1111–7. doi: 10.1046/j.1432-1327.1999.00964.x
62. Kato T, Imatani T, Miura T, Minaguchi K, Saitoh E, Okuda K. Cytokine-inducing activity of family 2 cystatins. *Biol Chem.* (2000) 381:1143–7. doi: 10.1515/BC.2000.141
63. Sugahara T, Murakami F, Yamada Y, Sasaki T. The mode of actions of lysozyme as an immunoglobulin production stimulating factor. *Biochim Biophys Acta.* (2000) 1475:27–34. doi: 10.1016/S0304-4165(00)00041-6
64. Asakura K, Kojima T, Shirasaki H, Kataura A. Evaluation of the effects of antigen specific immunotherapy on chronic sinusitis in children with allergy. *Auris Nasus Larynx.* (1990) 17:33–8. doi: 10.1016/S0385-8146(12)80018-6
65. Shiby V, Mishra H. Fermented milks and milk products as functional foods—a review. *Crit Rev Food Sci Nutr.* (2013) 53:482–96. doi: 10.1080/10408398.2010.547398

66. Brandtzaeg P. Current understanding of gastrointestinal immunoregulation and its relation to food allergy. *Ann N Y Acad Sci.* (2002) 964:13–45. doi: 10.1111/j.1749-6632.2002.tb04131.x
67. Rhee K-J, Sethupathi P, Driks A, Lanning DK, Knight KL. Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. *J Immunol.* (2004) 172:1118–24. doi: 10.4049/jimmunol.172.2.1118
68. Oskar A, Meydani S, Russell R. Yogurt and gut function. *Am J Clin Nutr.* (2004) 80:245–56. doi: 10.1093/ajcn/80.2.245
69. Kerry RG, Patra JK, Gouda S, Park Y, Shin H-S, Das G. Benefaction of probiotics for human health: a review. *J Food Drug Anal.* (2018) 26:927–39. doi: 10.1016/j.jfda.2018.01.002
70. Meydani SN, Ha W-K. Immunologic effects of yogurt. *Am J Clin Nutr.* (2000) 71:861–72. doi: 10.1093/ajcn/71.4.861
71. HooperLV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science.* (2012) 336:1268–73. doi: 10.1126/science.1223490
72. Pan L, Mu M, Yang P, Sun Y, Wang R, Yan J, et al. Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study. *Am J Gastroenterol.* (2020) 115:620. doi: 10.14309/ajg.0000000000000620
73. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, et al. Clinical characteristics of coronavirus disease 2019 in China. *New Eng J Med.* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
74. Wang T, Du Z, Zhu F, Cao Z, An Y, Gao Y, et al. Comorbidities and multi-organ injuries in the treatment of COVID-19. *Lancet.* (2020) 395:e52. doi: 10.1016/S0140-6736(20)30558-4
75. Galdeano CM, Perdigon G. Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. *J Appl Microbiol.* (2004) 97:673–81. doi: 10.1111/j.1365-2672.2004.02353.x
76. Talwalkar A, Kailasapathy K. A review of oxygen toxicity in probiotic yogurts: influence on the survival of probiotic bacteria and protective techniques. *Comp Rev Food Sci Food Safety.* (2004) 3:117–24. doi: 10.1111/j.1541-4337.2004.tb00061.x
77. Hosono A, Otani H, Yasui H, Watanuki M. Impact of fermented milk on human health: cholesterol-lowering and immunomodulatory properties of fermented milk. *Anim Sci J.* (2002) 73:241–56. doi: 10.1046/j.1344-3941.2002.00034.x
78. Boza-Méndez E, López-Calvo R, Cortés-Muñoz M. Innovative dairy products development using probiotics: challenges and limitations. *In Tech Costarica.* (2012) 10:213–36. doi: 10.5772/50104
79. Lourens-Hattingh A, Viljoen BC. Yogurt as probiotic carrier food. *Int Dairy J.* (2001) 11:1–17. doi: 10.1016/S0958-6946(01)00036-X
80. Oyewole OB. Lactic fermented foods in Africa and their benefits. *Food Control.* (1997) 8:289–97. doi: 10.1016/S0956-7135(97)00075-3
81. Meyer AL, Micksche M, Herbacek I, Elmadfa I. Daily intake of probiotic as well as conventional yogurt has a stimulating effect on cellular immunity in young healthy women. *Ann Nutr Metab.* (2006) 50:282–9. doi: 10.1159/000091687
82. Sanders ME. Use of probiotics and yogurts in maintenance of health. *J Clin Gastroenterol.* (2008) 42:S71–4. doi: 10.1097/MCG.0b013e3181621e87
83. Anukam KC, Osazuwa EO, Osadolor HB, Bruce AW, Reid G. Yogurt containing probiotic *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 helps resolve moderate diarrhea and increases CD4 count in HIV/AIDS patients. *J Clin Gastroenterol.* (2008) 42:239–43. doi: 10.1097/MCG.0b013e31802c7465
84. Reid G. The importance of guidelines in the development and application of probiotics. *Curr Pharm Des.* (2005) 11:11–6. doi: 10.2174/138161205338282395
85. Tuomola E, Crittenden R, Playne M, Isolauri E, Salminen S. Quality assurance criteria for probiotic bacteria. *Am J Clin Nutr.* (2001) 73:393–8. doi: 10.1093/ajcn/73.2.393s
86. Canani RB, Cirillo P, Terrin G, Cesarano L, Spagnuolo MI, De Vincenzo A, et al. Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. *Bmj.* (2007) 335:340. doi: 10.1136/bmj.39272.581736.55
87. Wu D, Yang XO. TH17 responses in cytokine storm of COVID-19: an emerging target of JAK2 inhibitor Fedratinib. *J Microbiol Immunol Infect.* (2020) 53:368–70. doi: 10.1016/j.jmii.2020.03.005
88. De Lorenzo A, Costacurta M, Merra G, Gualtieri P, Cioccoloni G, Marchetti M, et al. Can psychobiotics intake modulate psychological profile and body composition of women affected by normal weight obese syndrome and obesity? A double blind randomized clinical trial. *J Transl Med.* (2017) 15:135. doi: 10.1186/s12967-017-1236-2
89. Ferrey AJ, Choi G, Hanna RM, Chang Y, Tantisattamo E, Ivaturi K, et al. A case of novel coronavirus disease 19 in a chronic hemodialysis patient presenting with gastroenteritis and developing severe pulmonary disease. *Am J Nephrol.* (2020) 51:337–42. doi: 10.1159/000507417
90. Dandekar S. Pathogenesis of HIV in the gastrointestinal tract. *Curr HIV/AIDS Rep.* (2007) 4:10–5. doi: 10.1007/s11904-007-0002-0
91. Yang H, Sun Y, Cai R, Chen Y, Gu B. The impact of dietary fiber and probiotics in infectious diseases. *Microb Pathog.* (2020) 140:103931. doi: 10.1016/j.micpath.2019.103931
92. Gori A, Rizzardini G, Van't Land B, Amor KB, Van Schaik J, Torti C, et al. Specific prebiotics modulate gut microbiota and immune activation in HAART-naive HIV-infected adults: results of the "COPA" pilot randomized trial. *Mucosal Immunol.* (2011) 4:554–63. doi: 10.1038/mi.2011.15
93. Kekkonen RA, Lummela N, Karjalainen H, Latvala S, Tynkkynen S, Järvenpää S, et al. Probiotic intervention has strain-specific anti-inflammatory effects in healthy adults. *World J Gastroenterol.* (2008) 14:2029. doi: 10.3748/wjg.14.2029
94. Takeda K, Suzuki T, Shimada SI, Shida K, Nanno M, Okumura K. Interleukin-12 is involved in the enhancement of human natural killer cell activity by Lactobacillus casei Shirota. *Clin Exp Immunol.* (2006) 146:109–15. doi: 10.1111/j.1365-2249.2006.03165.x
95. Sugawara G, Nagino M, Nishio H, Ebata T, Takagi K, Asahara T, et al. Perioperative synbiotic treatment to prevent postoperative infectious complications in biliary cancer surgery: a randomized controlledtrial. *Ann Surg.* (2006) 244:706. doi: 10.1097/01.sla.0000219039.20924.88
96. Aso Y, Akaza H, Kotake T, Tsukamoto T, Imai K, Naito S, et al. Preventive effect of a Lactobacillus casei preparation on the recurrence of superficial bladder cancer in a double-blind trial. *Eur Urol.* (1995) 27:104–9. doi: 10.1159/000475138
97. Tamura M, Shikina T, Morihana T, Hayama M, Kajimoto O, Sakamoto A, et al. Effects of probiotics on allergic rhinitis induced by Japanese cedar pollen: randomized double-blind, placebo-controlled clinical trial. *Int Arch Allergy Immunol.* (2007) 143:75–82. doi: 10.1159/000098318
98. Shioiri T, Yahagi K, Nakayama S, Asahara T, Yuki N, Kawakami K, et al. The effects of a synbiotic fermented milk beverage containing Lactobacillus casei strain Shirota and transgalactosylated oligosaccharides on defecation frequency, intestinal microflora, organic acid concentrations, and putrefactive metabolites of sub-optimal health state volunteers: a randomized placebo-controlled cross-over study. *Biosci Microflora.* (2006) 25:137–46. doi: 10.12938/bifidus.25.137
99. Matsumoto K, Takada T, Shimizu K, Moriyama K, Kawakami K, Hirano K, et al. Effects of a probiotic fermented milk beverage containing Lactobacillus casei strain Shirota on defecation frequency, intestinal microbiota, and the intestinal environment of healthy individuals with soft stools. *J Biosci Bioeng.* (2010) 110:547–52. doi: 10.1016/j.jbiosc.2010.05.016
100. Dong H, Rowland I, Thomas LV, Yaqoob P. Immunomodulatory effects of a probiotic drink containing Lactobacillus casei Shirota in healthy older volunteers. *Eur J Nutr.* (2013) 52:1853–63. doi: 10.1007/s00394-012-0487-1
101. Granier A, Goulet O, Hoarau C. Fermentation products: immunological effects on human and animal models. *Pediatr Res.* (2013) 74:238–44. doi: 10.1038/pr.2013.76
102. Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, et al. Prebiotic effects: metabolic and health benefits. *Br J Nutr.* (2010) 104:S1–63. doi: 10.1017/S0007114510003363
103. Baurhoo B, Ferket P, Zhao X. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. *Poult Sci.* (2009) 88:2262–72. doi: 10.3382/ps.2008-00562

104. Yang Y, Iji P, Choct M. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World Poult Sci J.* (2009) 65:97–114. doi: 10.1017/S0043933909000087
105. Ofek I, Beachey EH. Mannose binding and epithelial cell adherence of Escherichia coli. *Infect Immun.* (1978) 22:247–54. doi: 10.1128/iai.22.1.247-254.1978
106. Spring P, Wenk C, Dawson K, Newman K. The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. *Poult Sci.* (2000) 79:205–11. doi: 10.1093/ps/79.2.205
107. Swiatkiewicz S, Arczewska-Włosek A, Józefiak D. Immunomodulatory efficacy of yeast cell products in poultry: a current review. *World Poult Sci J.* (2014) 70:57–68. doi: 10.1017/S0043933914000051
108. Ricke S. Potential of fructooligosaccharide prebiotics in alternative and nonconventionalpoultry production systems. *Poult Sci.* (2015) 94:1411–8. doi: 10.3382/ps/pev049
109. Xu Z, Hu C, Xia M, Zhan X, Wang M. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poult Sci.* (2003) 82:1030–6. doi: 10.1093/ps/82.6.1030
110. Nagao F, Nakayama M, Muto T, Okumura K. Effects of a fermented milk drink containing Lactobacillus casei strain Shirota on the immune system in healthy human subjects. *Biosci Biotechnol Biochem.* (2000) 64:2706–8. doi: 10.1271/bbb.64.2706
111. Forssten SD, Lahtinen SJ, Ouwehand AC. The intestinal microbiota and probiotics. In: Malago J, Koninkx J, Marinsek-Logar R, editors. *Probiotic Bacteria and Enteric Infections.* Dordrecht: Springer (2011). p. 41–63 .doi: 10.1007/978-94-007-0386-5_2
112. Kress-Rogers E, Brimelow CJ. *Instrumentation and Sensors for the Food Industry*. Sawston: Woodhead Publishing (2001). doi: 10.1201/9781439833049
113. Banville V, Morin P, Pouliot Y, Britten M. Physical properties of pizza Mozzarella cheese manufactured under different cheese-making conditions. *J Dairy Sci.* (2013) 96:4804–15. doi: 10.3168/jds.2012-6314
114. Badawi O. *Dairy products as source of some bacterial zoonosis*. PH. D. thesis. Fac. Vet. Med., Cairo University (1996).
115. Tome D, Bos C, Mariotti F, Gaudichon C. Protein quality and FAO/WHO recommendations. *Sci des Alim*. (2002) 22:393–405 .doi: 10.3166/sda.22.393-405
116. Bachmann H-P, Bütikofer U, Sieber R. Über dasVorkommen von bioaktiven Peptiden in Käse. *Mitt Lebensm Hyg*. (2003) 94:136–54.
117. Gabriele M, Pucci L. Diet bioactive compounds: implications for oxidative stress and inflammation in the vascular system. *Endocr Metab Immune Disord Drug Targets.* (2017) 17:264–75. doi: 10.2174/1871530317666170921142055
118. Calder PC. Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. *Mol Nutr Food Res.* (2008) 52:885–97. doi: 10.1002/mnfr.200700289
119. Taylor AK, Cao W, Vora KP, Cruz JDL, Shieh W-J, Zaki SR, et al. Protein energy malnutrition decreases immunity and increases susceptibility to influenza infection in mice. *J Infect Dis.* (2013) 207:501–10. doi: 10.1093/infdis/jis527
120. Jakulj F, Zernicke K, Bacon SL, Van Wielingen LE, Key BL, West SG, et al. A high-fat meal increases cardiovascular reactivity to psychological stress in healthy young adults. *J Nutr.* (2007) 137:935–9. doi: 10.1093/jn/137.4.935
121. Arora SK, McFarlane SI. The case for low carbohydrate diets in diabetes management. *Nutr Metab.* (2005) 2:1–9. doi: 10.1186/1743-7075-2-16
122. O'Keefe JH, Gheewala NM, O'Keefe JO. Dietary strategies for improving post-prandial glucose, lipids, inflammation, and cardiovascular health. *J Am Coll Cardiol.* (2008) 51:249–55. doi: 10.1016/j.jacc.2007.10.016
123. Lindmark-Månsson H, Fondén R, Pettersson H-E. Composition of Swedish dairy milk. *Int Dairy J.* (2003) 13:409–25. doi: 10.1016/S0958-6946(03)00032-3
124. Walzem R, Dillard C, German JB. Whey components: millennia of evolution create functionalities for mammalian nutrition: what we know and what we may be overlooking. *Crit Rev Food Sci Nutr.* (2002) 42:353–75. doi: 10.1080/10408690290825574
125. Holt C, Carver J, Ecroyd H, Thorn D. Invited review: caseins and the casein micelle: their biological functions, structures, and behavior in foods. *J Dairy Sci.* (2013) 96:6127–46. doi: 10.3168/jds.2013-6831
126. Mishra S, Joshi B. Genetic and non-genetic factors affecting lactation milk constituents and yield traits in Holstein Friesian× Karan crossbred cows. *Indian J Dairy Sci.* (2009) 57:69–72.
127. Sodhi SS, Mehra M, Jain A, Trehan P. Effect of non-genetic factors on the composition of milk of Murrah buffaloes. *Indian Vet J.* (2008) 85:950–52.
128. Saxelin M, Korpela R, Mäyrä-Mäkinen A. Introduction: classifying functional dairy products. In: Mattila-Sandholm T, Saarela M, editors. *Functional Dairy Products*. Boca Raton, FL: CRC Press (2003). p. 1–16 .doi: 10.1533/9781855736917.1
129. Pihlanto A. Whey proteins and peptides. *Nutrafoods.* (2011) 10:29–42. doi: 10.1007/BF03223386
130. Tellez A, Corredig M, Brovko LY, Griffiths MW. Characterization of immune-active peptides obtained from milk fermented by Lactobacillus helveticus. *J Dairy Res.* (2010) 77:129–36. doi: 10.1017/S002202990999046X
131. Zimecki M, Kruzel ML. Milk-derived proteins and peptides of potential therapeutic and nutritive value. *J Exp Ther Oncol*. (2007) 6:89–106.
132. Xia T, Li N, Nel AE. Potential health impact of nanoparticles. *Annu Rev Public Health.* (2009) 30:137–50. doi: 10.1146/annurev.publhealth.031308.100155
133. De Noni I, Cattaneo S. Occurrence of β-casomorphins 5 and 7 in commercial dairy products and in their digests following *in vitro* simulated gastro-intestinal digestion. *Food Chem.* (2010) 119:560–6. doi: 10.1016/j.foodchem.2009.06.058
134. Ng TB, Cheung RCF, Wong JH, Wang Y, Ip DTM, Wan DCC, et al. Antiviral activities of whey proteins. *Appl Microbiol Biotechnol.* (2015) 99:6997–7008. doi: 10.1007/s00253-015-6818-4
135. Olsen MF, Abdissa A, Kæstel P, Tesfaye M, Yilma D, Girma T, et al. Effects of nutritional supplementation for HIV patients starting antiretroviral treatment: randomised controlled trial in Ethiopia. *Bmj.* (2014) 348:g3187. doi: 10.1136/bmj.g3187
136. Séverin S, Wenshui X. Milk biologically active components as nutraceuticals. *Crit Rev Food Sci Nutr.* (2005) 45:645–56. doi: 10.1080/10408690490911756
137. Hall W, Millward D, Long S, Morgan L. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *Br J Nutr.* (2003) 89:239–48. doi: 10.1079/BJN2002760
138. Keri Marshall N. Therapeutic applications of whey protein. *Alternat Med Rev.* (2004) 9:136–56.
139. Krissansen GW. Emerging health properties of whey proteins and their clinical implications. *J Am Coll Nutr.* (2007) 26:713–23. doi: 10.1080/07315724.2007.10719652
140. El-Loly MM, Mahfouz MB. Lactoferrin in relation to biological functions and applications: a review. *Int J Dairy Sci.* (2011) 6:79–111. doi: 10.3923/ijds.2011.79.111
141. Wong CW, Watson DL. Immunomodulatory effects of dietary whey proteins in mice. *J Dairy Res.* (1995) 62:359–68. doi: 10.1017/S0022029900031058
142. Baruchel S, Bounous G, Falutz J, Gold P. Whey proteins as a food supplement in HIV-seropositive individuals. *Clin Invest Med.* (1993) 16: 204–9.
143. Ford JT, Wong CW, Colditz IG. Effects of dietary protein types on immune responses and levels of infection with Eimeria vermiformis in mice. *Immunol Cell Biol.* (2001) 79:23–8. doi: 10.1046/j.1440-1711.2001.00788.x
144. Watanabe A, Okada K, Shimizu Y, Wakabayashi H, HiguchiK, Niiya K, et al. Nutritional intervention of chronic hepatitis by whey protein (non-heated). *J Med*. (2000) 31:283–302.
145. Ng T, Lam T, Au T, Ye X, Wan C. Inhibition of human immunodeficiency virus type 1 reverse transcriptase, protease andintegrase by bovine milk proteins. *Life Sci.* (2001) 69:2217–23. doi: 10.1016/S0024-3205(01)01311-X
146. Superti F, Ammendolia M, Valenti P, Seganti L. Antirotaviral activity of milk proteins: lactoferrin prevents rotavirus infection in the enterocyte-like cell line HT-29. *Med Microbiol Immunol.* (1997) 186:83–91. doi: 10.1007/s004300050049

147. Pourtois M, Binet C, Van Tieghem N, Courtois P, Vandenabbeele A, Thiry L. Inhibition of HIV infectivity by lactoperoxidase-produced hypothiocyanite. *J Biol Buccale.* (1990) 18:251–3.
148. Mikola H, Waris M, Tenovuo J. Inhibition of herpes simplex virus type 1, respiratory syncytial virus and echovirus type 11 by peroxidase-generated hypothiocyanite. *Antiviral Res.* (1995) 26:161–71. doi: 10.1016/0166-3542(94)00073-H
149. Shin K, Wakabayashi H, Yamauchi K, Teraguchi S, Tamura Y, Kurokawa M, et al. Effects of orally administered bovine lactoferrin and lactoperoxidase on influenza virus infection in mice. *J Med Microbiol.* (2005) 54:717–23. doi: 10.1099/jmm.0.46018-0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Batiha, Alqarni, Awad, Algammal, Nyamota, Wahed, Shah, Amin, Adetuyi, Hetta, Cruz-Martins, Koirala, Ghosh, Echeverría, Pagnossa and Sabatier. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership