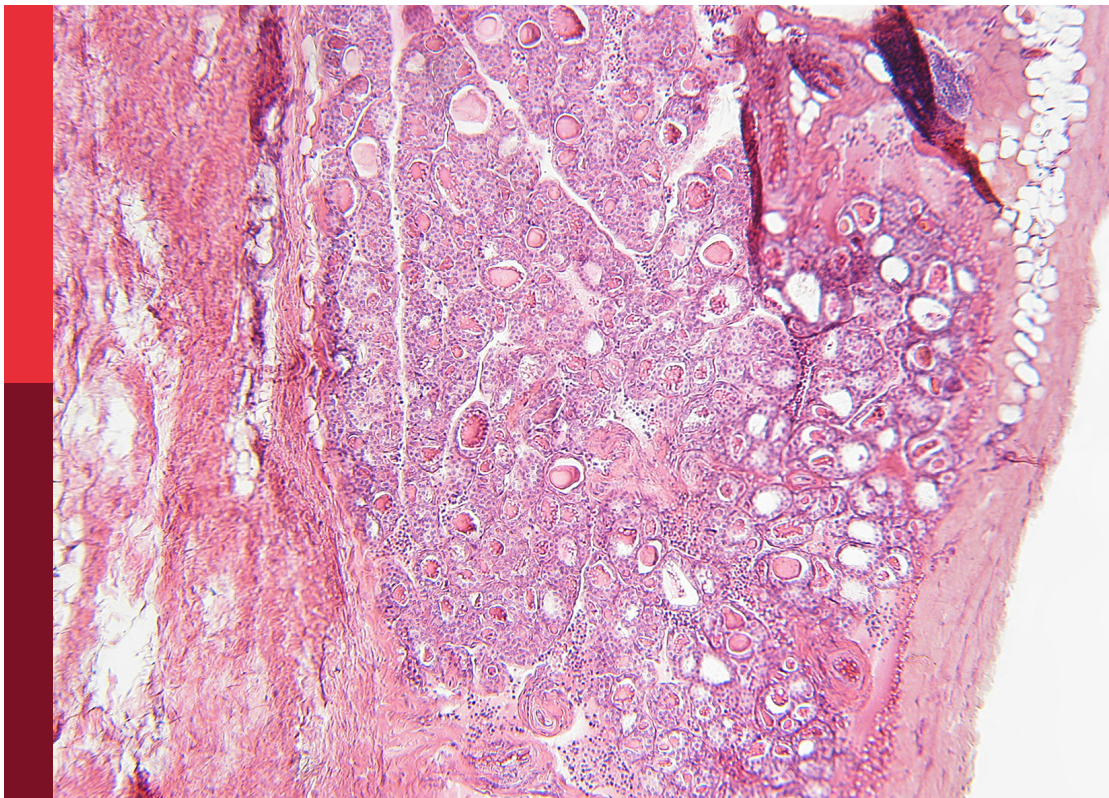
frontiers | Research Topics

Endocrine and metabolic consequences of childhood obesity, volume II

Edited by
Artur Mazur, Elpis Vlachopapadopoulou, Malgorzata Wojcik, Grzegorz Telega, Dénes Molnár and Aneta Monika Gawlik

Published in
Frontiers in Endocrinology



ISSN 1664-8714
ISBN 978-2-8325-3207-2
DOI 10.3389/978-2-8325-3207-2

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

Endocrine and metabolic consequences of childhood obesity, volume II

Topic editors

Artur Mazur — University of Rzeszow, Poland
Elpis Vlachopapadopoulou — Panagiotis & Aglaia Kyriakou Children's Hospital, Greece
Malgorzata Wojcik — Jagiellonian University Medical College, Poland
Grzegorz Telega — Medical College of Wisconsin, United States
Dénes Molnár — University of Pécs, Hungary
Aneta Monika Gawlik — Medical University of Silesia, Poland

Citation

Mazur, A., Vlachopapadopoulou, E., Wojcik, M., Telega, G., Molnár, D., Gawlik, A. M., eds. (2023). *Endocrine and metabolic consequences of childhood obesity, volume II*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3207-2

Table of contents

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 21 July 2023
DOI 10.3389/fendo.2023.1239914

OPEN ACCESS

EDITED AND REVIEWED BY
Katherine Samaras,
St Vincent's Hospital Sydney, Australia

*CORRESPONDENCE
Artur Mazur
drmazur@poczta.onet.pl

RECEIVED 14 June 2023
ACCEPTED 19 June 2023
PUBLISHED 21 July 2023

CITATION
Mazur A, Molnár D, Gawlik AM, Telega G, Vlachopapadopoulou E and Wojcik M (2023) Editorial: Endocrine and metabolic consequences of childhood obesity, volume II. *Front. Endocrinol.* 14:1239914. doi: 10.3389/fendo.2023.1239914

Editorial: Endocrine and metabolic consequences of childhood obesity, volume II

Artur Mazur[1]*, Dénes Molnár[2,3], Aneta Monika Gawlik[4], Grzegorz Telega[5], Elpis Vlachopapadopoulou[6] and Malgorzata Wojcik[7]

[1]Department of Pediatrics, Pediatric Endocrinology and Diabetes, Medical Faculty, University of Rzeszów, Rzeszów, Poland, [2]Department of Pediatrics, Medical School, University of Pécs, Pécs, Hungary, [3]National Laboratory for Human Reproduction, Szentágothai Research Centre, University of Pécs, Pécs, Hungary, [4]Department of Pediatrics and Pediatric Endocrinology, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland, [5]Division of Gastroenterology, Hepatology and Nutrition, Children's Hospital of Wisconsin, Medical College of Wisconsin, Milwaukee, WI, United States, [6]Department of Endocrinology, Panagiotis & Aglaia Kyriakou Children's Hospital, Athens, Greece, [7]Department of Pediatric and Adolescent Endocrinology, Chair of Pediatrics, Pediatric Institute, Jagellonian University Medical College, Krakow, Poland

KEYWORDS

children, obesity, hypertension, CVD, insulin resistance

Editorial on the Research Topic
Endocrine and metabolic consequences of childhood obesity, volume II

Obesity – a pandemic of the twenty-first century – is one of the greatest public health problems worldwide. Overweight and obesity affect nearly one in five children in the world and one in three in Europe (1). Recent estimates suggest that overweight and obesity is the fourth most common risk factor for noncommunicable diseases in Europe, following hypertension, dietary risk factors and tobacco use. Moreover, obesity during adolescence increases the risk for cardiovascular disease and premature death during adulthood, independently of the persistence of obesity in adulthood (2, 3). However, improvement of weight control and normalization of BMI in adolescence diminishes the risk of developing DM2 in adulthood (3). Certain comorbidities such as type 2 diabetes mellitus (T2DM) and metabolic associated fatty liver disease, until recently considered "adult diseases", are now frequently encountered in children with obesity (2, 4). Evaluation and monitoring for these comorbidities are important components of health care for children the growing impact of childhood obesity on the development of short- and long-term endocrine and metabolic complications.

No single cause is responsible for increased incidence of childhood obesity. It cannot be blamed on genetics factors or environment factors alone. In this Research Topic our contributors explore the mechanisms behind, linking intrauterine, postnatal, and early childhood metabolic environment to obesity and its complications (2, 5, 6).

Nakhleh et al. revealed that class 1 obesity in children's and adolescents (BMI ≥ 110% of the 95th percentile) was associated with higher prevalence and clustering of cardiometabolic risk factors.

Rajamoorthi et al. highlighted the role of the environmental factors, including the globalization of the western diet and unhealthy lifestyle choices. In an elegant review they argued that starting from conception type and timing such exposures come into play impacting on the overall risk of obesity and future adverse health outcomes.

An important new observation was reported by Seget et al. as they documented that the prevalence of obesity is increasing among in children with diabetes mellitus type 1 (T1DM) and may influence the glycemic control.

On the other hand, Pixner et al. investigated LACA and its mediators (amino acids and glucagon), focusing on the relationship between glucose and the LACA in adult and pediatric subjects.

Kącka et al. introduced novel markers of metabolic complications in obese T1DM and non-diabetic subjects.

Analysis of the taste preferences and sensitivity of mothers and their children in the relation to excessive body weight of children is presented by Sobek and Dąbrowski in article "*Lifestyle intervention changes are crucial in the prevention and treatment of childhood obesity*".

Strączek et al. found that one-year dietary education resulted in significant improvements in body weight, waist, and hip circumference, WHtR and selected measured carbohydrate and lipid metabolism parameters with the exception of total cholesterol. The one-year dietary intervention did not have the same effect on the change in dietary habits in children and in their mothers.

The assessment of childhood obesity comorbidities and risk of its complication is challenging and difficult. de Lamas et al. concluded that controlling obesity and cardiometabolic risk factors, especially insulin resistance and blood pressure in children during the prepubertal stage appears to be effective in prevention of pubertal metabolic syndrome.

Artemniak-Wojtowicz et al. experimentally proved that weight reduction leads to significant decrease of circulating Th17 cells and improvement of lipid parameters. This significant reduction of proinflammatory Th17 cells is a promising finding suggesting that obesity-induced inflammation in children could be reversible.

One of the key problems in the development of obesity complications is the liver involvement. Liver abnormalities - collectively known as metabolic associated fatty liver disease is becoming a more prevalent clinical problem, in obese children and adolescents. Maruszczak et al. described determinants of hyperglucagonemia in Pediatric Non-Alcoholic Fatty Liver Disease. Brunnert et al. revealed usefulness of the liver stiffness measurement in the evaluation of liver involvement in obese adolescents. Furdela et al. revealed that triglyceride glucose index, pediatric NAFLD fibrosis index, and triglyceride to high-density lipoprotein cholesterol ratio are a valuable combination of predictive markers of metabolically unhealthy phenotype in Ukrainian overweight/obese boys.

Obesity can also associate with complications of calcium-phosphorus and bone metabolism regulation (5). That was investigated by Erazmus et al. in the article "*Decreased level of soluble Receptor Activator of Nuclear Factor-κβ Ligand (sRANKL) in overweight and obese children*".

Krajewska et al. confirmed that vitamin D has positive effect on metabolic profile in overweight and obese children, but the relationship between vitamin D and chemerin is not clear.

Zembura and Matusik found that sarcopenic obesity is highly prevalent in children and adolescents and is associated with various adverse health outcomes including significant association with cardiometabolic outcomes, severity of non-alcoholic fatty liver disease (NAFLD), inflammation, and mental health. Findings of this review highlight the need for the development of a consensus regarding definition, standardized evaluation methods, and age and gender thresholds for sarcopenic obesity for different ethnicities in the pediatric population.

Many factors influencing the development of obesity and its complications are still unknown. Future studies are needed to elucidate many questions and concerns raised by our contributors. Nevertheless, we do hope that readers will find our Research Topic informative and inspiring.

Author contributions

AM, MW - writing draft of manuscript. AG, GT, EV - review. DM - final correction and approval.

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

References

1. World Health Organization and Regional Office for Europe. *WHO European regional obesity report 2022.* World Health Organization. Regional Office for Europe (2022). Available at: https://apps.who.int/iris/handle/10665/353747.

2. Mazur A, Zachurzok A, Baran J, Dereń K, Łuszczki E, Weres A, et al. Childhood obesity: position statement of Polish Society of Pediatrics, Polish Society for Pediatric Obesity, Polish Society of Pediatric Endocrinology and Diabetes, The College of Family

Physicians in Poland and Polish Association for Study on Obesity. *Nutrients* (2022) 14 (18):3806. doi: 10.3390/nu14183806

3. Bjerregaard LG, Jensen BW, Ängquist L, Osler M, Sørensen TIA, Baker JL. Change in overweight from childhood to early adulthood and risk of type 2 diabetes. *N Engl J Med* (2018) 378(14):1302–12. doi: 10.1056/NEJMoa1713231

4. Drozdz D, Alvarez-Pitti J, Wójcik M, Borghi C, Gabbianelli R, Mazur A, et al. Obesity and cardiometabolic risk factors: from childhood to adulthood. *Nutrients* (2021) 13(11):4176. doi: 10.3390/nu13114176

5. Gawlik A, Salonen A, Jian C, Yanover C, Antosz A, Shmoish M, et al. Personalized approach to childhood obesity: lessons from gut microbiota and omics studies. Narrative review and insights from the 29th European childhood obesity congress. *Pediatr Obes* (2021) 16(10):e12835. doi: 10.1111/ijpo.12835

6. Wolters M, Marron M, Foraita R, Charalampos H, De Henauw S, Eiben G, et al. Longitudinal associations between vitamin D status and cardiometabolic risk markers among children and adolescents. *J Clin Endocrinol Metab* (2023), dgad310. doi: 10.1210/clinem/dgad310

frontiers | Frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 01 June 2022
doi: 10.3389/fendo.2022.914740

Sarcopenic Obesity in Children and Adolescents: A Systematic Review

Marcela Zembura * *and Paweł Matusik*

Department of Pediatrics, Pediatric Obesity and Metabolic Bone Diseases, Chair of Pediatrics and Pediatric Endocrinology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland

Sarcopenic obesity (SO) is defined as co-occurrence of increased fat mass and sarcopenia and may predict adverse health outcomes in the pediatric population. However, the prevalence of SO and its association with adverse health outcomes have not been well defined in children and adolescents. We systematically reviewed data on the SO definition, prevalence, and adverse outcomes in the pediatric population. A total of 18 articles retrieved from PubMed or Web of Science databases were included. Overall, there was a wide heterogeneity in the methods and thresholds used to define SO. The prevalence of SO ranged from 5.66% to 69.7% in girls, with a range between 7.2% and 81.3% in boys. Of the 8 studies that evaluated outcomes related to SO, all showed a significant association of SO with cardiometabolic outcomes, non-alcoholic fatty liver disease (NAFLD) severity, inflammation, and mental health. In conclusion, this review found that SO is highly prevalent in children and adolescents and is associated with various adverse health outcomes. Findings of this review highlight the need for the development of a consensus regarding definition, standardized evaluation methods, and age and gender thresholds for SO for different ethnicities in the pediatric population. Further studies are needed to understand the relationship between obesity and sarcopenia and SO impact on adverse health outcomes in children and adolescents.

Keywords: sarcopenia, obesity, muscle mass, muscle strength, children, adolescents

OPEN ACCESS

Edited by:
Malgorzata Wojcik, Jagiellonian University Medical College, Poland

Reviewed by:
Elpis Vlachopapadopoulou, Panagiotis & Aglaia Kyriakou Children's Hospital, Greece
Julio Álvarez, Hospital General Universitario De Valencia, Spain

***Correspondence:**
Marcela Zembura
marcela.zembura@gmail.com

Specialty section:
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

Received: *07 April 2022*
Accepted: *02 May 2022*
Published: *01 June 2022*

Citation:
Zembura M and Matusik P (2022) Sarcopenic Obesity in Children and Adolescents: A Systematic Review. Front. Endocrinol. 13:914740. doi: 10.3389/fendo.2022.914740

1 INTRODUCTION

According to the World Health Organization (WHO), over 340 million children and adolescents aged 5–19 years were overweight or obese in 2016 (1). Despite the implementation of numerous obesity prevention programs, the prevalence of overweight and obesity among children and adolescents is rising on each of the continents. The growing prevalence of childhood obesity is associated with noncommunicable comorbidities affecting almost every system in the body, including insulin resistance (IR), type 2 diabetes mellitus (T2DM) (2), elevated blood pressure (3), dyslipidemia (4), non-alcoholic fatty liver disease (NAFLD) (5), obstructive sleep apnea (OSA) (6), and psychosocial sphere (7).

The term "sarcopenia" (Greek "sarx" or flesh + "penia" or loss) was first introduced in 1989 by Irwin Rosenberg as the term to describe the decrease of muscle mass in the population of the elderly (8, 9). Since then, major changes occurred in the definition of sarcopenia and led to the development of The European Working Group on Sarcopenia in Older People (EWGSOP), which proposed a new definition of *sarcopenia* involving the assessment of muscle function. The EWGSOP

recommended using the presence of both low muscle mass (LMM) and low muscle function (strength or performance) for the diagnosis of sarcopenia (10). However, according to EWGSOP2 revised consensus from 2019, muscle strength is now the primary parameter of sarcopenia. The occurrence of sarcopenia is probable when low muscle strength is observed. To confirm sarcopenia diagnosis, low muscle quantity or quality must be detected. When low muscle strength, low muscle quantity/quality, and low physical performance co-occur, sarcopenia is regarded as severe (11).

"Sarcopenic obesity (SO) is an emerging clinical entity characterized by excessive fat mass in the presence of reduced muscle mass (12)". The co-occurrence of sarcopenia and obesity indicates a synergistically amplified risk of adverse health outcomes (13).

Sarcopenia and SO were once considered as afflictions affecting only the elderly, regarding the changes in body composition with aging involving muscle mass decline after the fourth decade (14), reduced resting metabolic rates (15), and reduced metabolic adaptation (16). A reduction in energy expenditure is not in line with a decrease in appetite that can lead to the development of obesity (16). Correlation of this factors leads to the development of SO. Furthermore, the sedentary lifestyle of elderly people further exacerbates changes in metabolism and body composition (16).

However, sarcopenia is now also linked to the pediatric population, as sarcopenia was found to be a risk factor of insulin resistance and higher metabolic risk in children and adolescents (17, 18). There is no established consensus regarding SO definition, diagnostic methods, and age- and gender-specific cutoff points in children and adolescents.

Currently, a wide range of various techniques are used to estimate muscle mass. Computed tomography (CT) and magnetic resonance imaging (MRI) are considered gold standards for estimating muscle mass because of their accuracy (10). However, few factors limit the usage of imaging techniques for routine clinical practice, including high cost, limited access to equipment, radiation exposure, and contraindications for scanning (19). The use of gold standard methods in the pediatric population has been limited mostly to patients with end-organ failure and children with solid/hematologic malignancies. Moreover, there is no consensus regarding landmarks (L2–L5) and muscle type/number used in studies concerning the pediatric population; most commonly used are the measures of psoas muscle area or psoas muscle index measured at L3 on CT, although some studies used L2, L4, or L5; MRI; paraspinous muscle area; or intramuscular adipose tissue area (20). Dual-energy X-ray absorptiometry (DXA) is now considered to be the most widely utilized method for muscle mass quantification because this method is quick, simple, low-cost, more available than CT/MRI, and is associated with minimal radiation exposure (10, 19, 21). The usage of bioelectrical impedance analysis (BIA) is inexpensive, readily reproducible, and appropriate for both ambulatory and bedridden patients (10). It has been found that BIA results under standard conditions correlate well with MRI predictions (22). However, susceptibility to patient hydration status is considered to be the main disadvantage of both DXA and BIA (20). Nevertheless, usage of these methods may be clinically warranted to assess for sarcopenia when CT and MRI are unavailable (23). Considering the fact that muscle mass is influenced by body size, absolute skeletal muscle (SM) mass (SMM) indicators should be adjusted for body size using height squared [appendicular skeletal muscle mass (ASM)/height^2], weight (ASM/weight), or body mass index (BMI) (ASM/BMI) (24). Both total/partial body potassium per fat-free soft tissue and anthropometric measurements are not routinely used in clinical practice (10). There are a few studies providing reference values for DXA- and BIA-derived muscle mass parameters in children (25–28). Whereas only one study warranted reference values regarding CT-derived parameters of muscle mass in children (29). Assessment of muscle function should always be performed, since muscle strength is not linearly related to muscle mass (30). In older children and adolescents, strength and performance tests utilized in adults such as handgrip test, chair stand test, Timed Up and Go test, 6-min walk test, stair climb power test, and 400-m walk test can be used (10, 11). For younger children, standardized motor function assessment scales can be used in order to evaluate motor performance that may be affected by impaired muscle function. For this purpose, the validated Alberta Infant Motor Scale and the Peabody Developmental Motor Scale can be used (31).

To our knowledge, until now, no systematic reviews regarding solely SO in children and adolescents have been published. In this review, we aim to assess the current state of knowledge about SO among children and adolescents and its prevalence and evaluate outcomes related to SO, answering the question: "What are the diagnostic criteria, prevalence, and outcomes related to sarcopenic obesity in the population of children and adolescents?" in accordance to "participants, interventions, comparisons, and outcomes (PICO)" scheme.

2 MATERIALS AND METHODS

A literature search of articles published in English was completed *via* PubMed and Web of Science databases on October 25, 2021. The following search query was used: (pediatrics or pediatric or childhood or child or children or adolescent or adolescence) AND (obese or obesity or overweight or adiposity or excessive weight) AND (sarcopenia or sarcopenic or muscle loss or sarcopenic obesity). Adequate filter regarding publication language (English) was applied. The search was done without limiting the years of publication. Two independent researchers (MZ, PM) screened the results. The inclusion criteria were studies that assessed the concept of SO among the pediatric population (<21 years) (32). Studies that addressed outcomes related to SO were also included. Studies in which the age of the control group exceeded age criterium and studies that evaluated only one sex were ruled out. Articles were excluded if they were editorials, letters, replies from authors, review articles,

commentaries, case reports, articles conducted on animal models or cell culture, non-English articles, and articles without a full text. Studies performed in a population of children with neuromuscular disorders (e.g., Duchenne muscular dystrophy, spinal muscular atrophy) and autoimmune diseases affecting SM (e.g., dermatomyosis) were excluded considering possible disease impact on body composition. Studies addressing cachexia and frailty were ruled out.

In order to examine the studies' methodological quality, the quality assessment tool adopted from the National Institutes of Health/National Heart, Lung and Blood Institute for observational cohort studies, cross-sectional studies, and case control studies was used (12). After answering the series of questions (14 regarding observational cohort studies and cross-sectional studies, 12 regarding case–control studies), the quality of cohort studies and cross-sectional studies was rated as poor (0–4 points), fair (5–10 points), or good (11–14 points); the quality of case–control studies was rated as poor (0–3 points), fair (4–8 points), or good (9–12 points).

3 RESULTS

The initial search returned 1,241 results. Additional studies were identified by a manual search of bibliographic references of existing reviews. The repeated results of search were ruled out. After screening of abstracts, 137 results were chosen for full-text analysis, of which 18 (25, 33–49) met the inclusion criteria and were included in the study. A flowchart of study inclusion is presented in **Figure 1**. The following information was abstracted from original papers: first author, geographic region, year of publication, time of the study, study design, number, sex, age of patients, study population, and data regarding SO definition, prevalence, and relation with outcomes. The characteristics of studies that included healthy populations are shown in **Table 1**, studies regarding overweight/obese children and adolescents are summarized in **Table 2**, and studies concerning other clinical populations are demonstrated in **Table 3**. Of 18 studies included in this review, 8 (25, 33–37, 42, 45) examined the prevalence of SO and 8 (33–35, 37, 43, 44, 47, 48) evaluated outcomes related

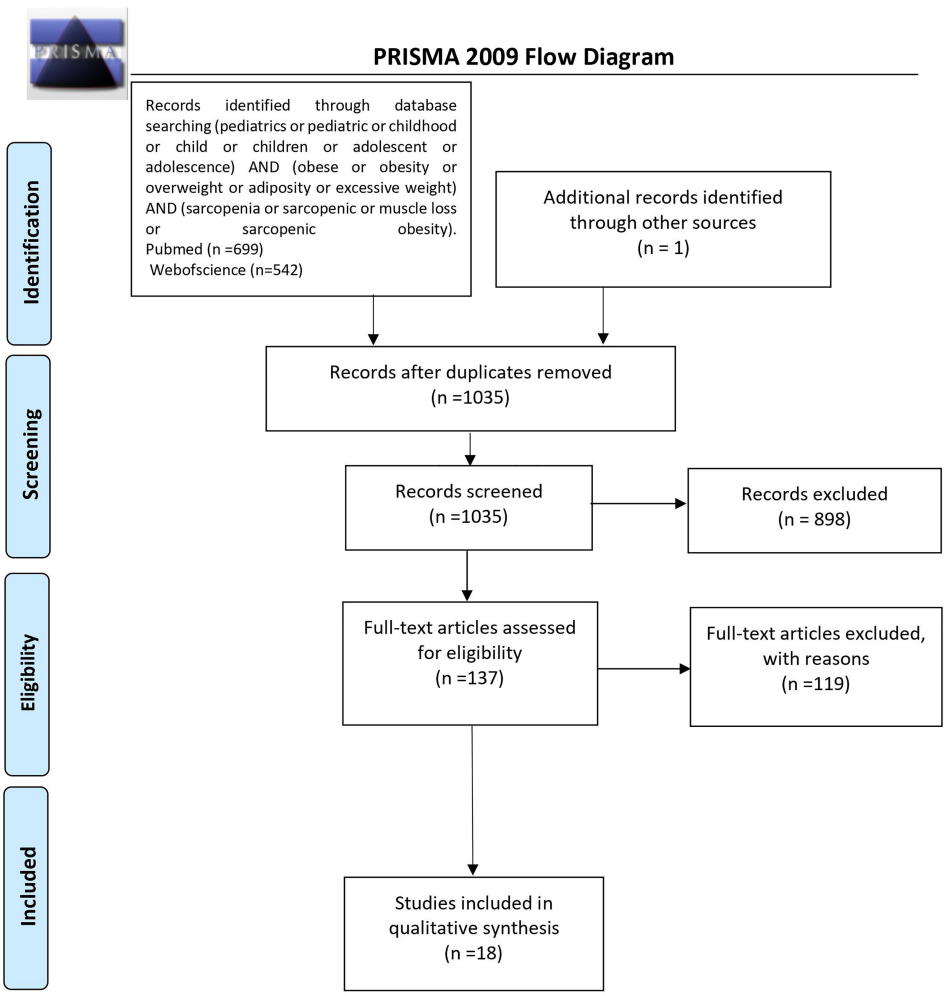


FIGURE 1 | PRISMA protocol for data acquisition. n, number; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses (50).

TABLE 1 | Characteristics of studies regarding healthy population.

Authors	Region	Year published	Time of study	Design	Number	Sex	Age, years	Population	Study quality (NIH)	Method of body composition evaluation	Sarcopenia indicator	Excessive weight indicator	Definition of SO	SO prevalence	Assessment of outcomes related to SO	Control group
Gontarev S et al. (36)	North Macedonia	2020	2017	CS	4021	49.4% male	range: 6-10 mean: 8.6	Healthy children from primary schools	6	BIA, dynamometer	MFR= SMM/BFM, grip-to-BMI ratio= maximal handgrip strength/BMI	NA	mean MFR-2SD of the 3rdBMI quintile/ estimation of cut-off points of grip-to-BMI ratio	boys: 9.2% girls: 5.9% total: 7.5%	NA	no
Steffl M et al. (42)	Czech Republic	2017	2015	CS	730	51.64% male	range: 4-14	Healthy children and adolescents	6	BIA, dynamometer	MFR= SMM/BFM, grip-to-BMI ratio=maximal handgrip strength/BMI	NA	mean MFR-2SD of the 3rdBMI quintile)/ estimation of cut-off points of grip-to-BMI ratio	boys: 7.2% girls: 9.3%	NA	no
Gätjens I et al. (45)	Germany	2021	since 1996	CS	15 392	49.38% male	range: 5-17	Healthy children and adolescents	6	BIA	FM/FFM, FM/FFM^2	age and sex-specific reference percentiles of BMI in children and adolescents according to Kromeyer-Hauschild et al., 2001, BMI>90 th percentile of the study population	FM/FFM >90th percentile/FM/FFM^2>90th percentile	boys: 62.7% girls: 69.7%	NA	no
McCarthy HD et al. (25)	UK	2013	2003-2004	CS	1985	56.22% male	range: 5-18.8	Healthy schoolchildren	6	BIA	MFR= SMMa/FM	highest fifth of BMI-z score for age range and sex	below mean MFR-2 SD of the middle fifth of the BMI range, highest fifth of BMI-z score for age range and sex	boys 5-10y: 8.31% boys 10-18y: 9.67% girls 5-10y: 15.48% girls 10-18y: 5.66%	NA	no
Stefanaki Ch et al. (48)	Italy	2016	2009-2012	CC	2551	lean group 16% females, overweight 95% females	range: 18-21	Healthy lean group, healthy overweight group	6	BIA	SMM	BMI between 25 and 35, fat mass as body weight percentage >25% for males and >32% for females	lower SMM in comparison with healthy lean group	NA	hsCRP, cortisol concentration at 8 a.m. and 8 p.m.	yes-gender and age range matched
Kim K et al. (33)	Republic of Korea	2016	2009-2011	CS	1919	53.36% male	range: 10-18	Healthy non-institutionalized Korean children and adolescents	7	DXA	MFR=ASM/body fat mass	BMI≥85th percentile for sex and age according to Standard Growth Charts of Korean children and adolescents published by the KCDC and Korean Pediatric Society in 2007, highest quintile of BMI	mean MFR-1SD of the 3rdBMI quintile	boys: 32.1% girls: 24.3%	Metabolic syndrome components (BP, glucose level, TG, HDL-C, WC)	no
Moon JH et al. (37)	Republic of Korea	2018	2008-2011	CS	1233	53.69% male	range:12-18	Healthy Korean adolescents	7	DXA	ASM, ASM/Wt (%)	WHtR>0.47 in both sexes	lower 10% of gender-specific ASM/Wt (%), WHtR>0.47 in both sexes	boys: 81.3% girls: 62.6%	Mental health	no
Kim JH et al. (41)	Republic of Korea	2016	2009-2011	CS	1420	52.75% male	range:12-19	Healthy Korean adolescents	7	DXA	ASM/Wt	WC at least 90th percentile for age and sex according to National Cholesterol Education Program-Adult Treatment Panel III Criteria	ASM/Wt below lower quintile for the study population, WC at least 90th percentile	NA	NA	no
Burrows R et al. (43)	Chile	2015	NA	CS	667	52.2% male	range:16-17 mean: 16.8	Healthy Chilean adolescents of middle to low SES	6	DXA	FFMI-estimated according to Wells and Fewtrell	BMI Z-score≥2 according to WHO, WC ≥80 cm in females, WC ≥ 90 cm in males	FFMI as percentage ≤25th percentile in sample (adjusted for sex), BMI Z-score≥2/WC ≥80 cm in females, WC ≥ 90 cm in males	NA	Metabolic syndrome components (BP, fasting serum total glucose, TG, HDL-C), insulin, HOMA-IR, cholesterol, adiponectin, hsCRP	no
Burrows R et al. (44)	Chile	2015	NA	CS	667	52.2% male	range:16-17 mean: 16.8	Healthy Chilean adolescents of middle to low SES	7	DXA	FFMI-estimated according to Wells and Fewtrell	BMI Z-score≥2 according to WHO, WC ≥80 cm in females, WC ≥ 90 cm in males	FFMI as percentage of BMI ≤25th percentile in sample (adjusted for sex),BMI Z-score≥2/WC ≥80 cm in females, WC ≥ 90 cm in males	NA	Metabolic syndrome components (BP, fasting serum total glucose, TG, HDL-C), insulin, HOMA-IR, cholesterol, adiponectin, hsCRP	no
Palacio-Agüero A et al. (39)	Chile	2020	2018	CS	491	51.73% male	range: 10-17 mean: 13.6	Healthy Chilean adolescents	7	dynamometer	RHGS =maximum HGS from dominant hand/BMI	BMI-for-age value over +1 SD according to WHO, WC according to the guidelines from Chilean Ministry of Health	RHGS<25th percentile by sex, BMI-for-age over +1 SD/WC according to the guidelines from Chilean Ministry of Health	NA	NA	no

ASM, appendicular skeletal muscle mass; BFM, body fat mass; BIA, bioelectrical impedance analysis; BMI, body mass index; BP, blood pressure; CC, case–control; CS, cross-sectional; DXA, dual-energy X-ray absorptiometry; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index; HDL-c, high-density lipoprotein cholesterol; HGS, handgrip strength; HOMA-IR, homeostatic model assessment for insulin resistance; hsCRP, high-sensitivity C-reactive protein; MFR, skeletal muscle-to-body fat ratio; NA, not available; RHGS, relative handgrip strength; SES, socioeconomic status; SMM, skeletal muscle mass; SMMa, appendicular skeletal muscle mass; TG, triglyceride; WC, waist circumference; WHtR, waist circumference-to-height ratio; Wt, weight.

TABLE 2 | Characteristics of studies concerning overweight/obese children and adolescents.

Authors	Region	Year published	Time of study	Design	Number	sex	Age, years	Population	Study quality (NIH)	Method of body composition evaluation	Sarcopenia indicator	Excessive weight indicator	Definition of SO	SO prevalence	Assessment of outcomes related to SO	Control group
Videira-Silva A et al. (34)	Portugal	2017	NA	R, CS	240	47.9% male	range: 10-17	Overweight adolescents attending a Pediatric Outpatient Obesity Clinic	8	BIA	%SMM=SMM/body weight MFR=SMM/BFM	BMI≥85th percentile for sex and age	%SMM≤p25 according to reference charts for youth McCarthy H.D. et al.	boys: 33.3% girls: 20,2% total: 26.9%	BP, glucose level, insulin level, HOMA-IR, total cholesterol, TG, HDL-C, LDL-C, CRP	no
Pacifico L et al. (35)	Italy	2020	NA	O, CS	234	56.41% male	range: 6-18	Overweight/obese children and adolescents attending Outpatient Clinics of the Department of Pediatrics	6	DXA	RMM=100x muscle mass/muscle mass +fat mass, ASM/weight index= ASM/weight x100	BMI> 85th percentile for sex and age	tertile 1 of RMM/ tertile 1 of ASM/ weight index	boys: 28.79% girls: 39.22% total: 33.33%	Metabolic syndrome components (WC, BP, glucose, HDL-C, TG),assessment of liver function(ALT, AST, liver US, liver biopsy),insulin, HOMA-IR, total cholesterol	no
Yodoshi T et al. (47)	USA	2020	2009-2018	R, CS	100 histology cohort, 263 liver stiffness cohort, 95 liver PDFF cohort	histology 65% male, liver stiffness 68% male, liver PDFF 77% male	<20	Patients with NAFLD	7	MRI	tPMSA index= $tPMSA/height^2$	BMI≥85th percentile for sex and age, Centers for Disease Control and Prevention growth charts	lower median tPMSA index in comparison with subjects with NAS<5	NA	NAFLD activity score (NAS), liver stiffness, liver fat fraction	no

ALT, alanine aminotransferase; ASM, appendicular skeletal muscle mass; AST, aspartate aminotransferase; BFM, body fat mass; BIA, bioelectrical impedance analysis; BMI, body mass index; BP, blood pressure; CS, cross-sectional; CRP, C-reactive protein; DXA, dual-energy X-ray absorptiometry; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; LDL-c, low-density lipoprotein cholesterol; MFR, skeletal muscle-to-body fat ratio; MRI, magnetic resonance imagining; NA, not available; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; O, observational; PDFF, proton density fat fraction; R, retrospective; RMM, relative muscle mass; SMM, skeletal muscle mass; TG, triglyceride; tPMSA, total psoas muscle surface area; US, ultrasonography; WC, waist circumference.

TABLE 3 | Characteristics of studies which included other clinical populations.

Authors	Region	Year published	Time of study	Design	Number	sex	Age, years	Population	Study quality (NIH)	Method of body composition evaluation	Sarcopenia indicator	Excessive weight indicator	Definition of SO	SO prevalence	Assessment of outcomes related to SO	Control group
Mangus RS et al. (40)	USA	2017	2002-2012	CC	81	35.8% male	≤18	Pediatric end-organ disease patients	7	CT	sarcopenic index= total psoas area/ $height^2$	visceral fat index, subcutaneous fat index	lower sarcopenic index compared to controls, higher visceral fat and subcutaneous fat index compared to controls	NA	NA	yes, 1:1 age- and gender -matched
Mueske NM et al. (46)	USA	2019	2011-2014	P	12	42% male	range: 10-21 mean: 14.4	Pre-adolescents, AYA diagnosed with HR B-ALL or T-cell ALL	9	QCT, DXA	muscle volume/ percent in the lower leg	BMI%≥85th according to Centers for Disease Control and Prevention before treatment initiation, increase of total body fat mass/fat percent during therapy	loss of muscle volume/ muscle percent over time, increase of total body fat mass/fat percent over time	NA	NA	yes- age range matched
Joffe L et al. (49)	USA	2020	2002-2017	R	39	53.8% female	range: 1.33-20 mean: 9.8 median:11	Children, adolescents and young adults with solid tumors	10	CT	SM,RLT	BMI≥85th percentile for sex and age WHO and CDC increase of VAT during therapy	loss of SM and RLT over time, increase of VAT over time	NA	NA	no
Orgel E et al. (38)	USA	2018	NA	P	50	60% male	range: 9.9-19.6 mean: 14.7 median:14.6	Children and adolescents diagnosed with High-Risk B-Precursor ALL or T-cell ALL	7	DXA	Lean muscle mass	BMI≥85th percentile for sex and age according to CDC criteria before treatment initiation, increase of BF% during therapy	loss of lean muscle mass over time, increase of BF% over time	NA	NA	no

ALL, acute lymphoblastic leukemia; AYA, adolescents and young adults; BF, body fat; BMI, body mass index; CC, case–control; CDC, Centers for Disease Control and Prevention; CT, computed tomography; DXA, dual-energy X-ray absorptiometry; NA, not available; P, prospective; QCT, quantitative computed tomography; R, retrospective; RLT, residual lean tissue; SM, skeletal muscle; VAT, visceral adipose tissue; WHO, World Health Organization.

to SO. Seven studies were performed in Europe (25, 34–36, 42, 45, 48), 5 in the United States (38, 40, 46, 47, 49), 3 in Asia (33, 37, 41), and 3 in South America (39, 43, 44). The number of participants ranged from 12 (46) to 15,392 (45). Based on the NIH quality assessment tool, all studies were classified as fair quality. Populations of studies by Kim et al. (33), Moon et al. (37), and Kim and Park (41) might be alike, since all of the studies used data from the Korean National Health and Nutrition Examination Survey (KHANES) conducted in similar years in subjects in the similar age range. However, all of the studies were included in this review, considering the fact that different definitions and cutoff values for SO were used in each of the studies, yielding different SO prevalence. The study by Orgel et al. (38) and the study by Mueske et al. (46) regarded patients newly diagnosed with high-risk B-acute lymphoblastic leukemia (ALL) or T-cell ALL who were prospectively enrolled in a clinical trial studying body composition and bone health. Nevertheless, both studies used different methods to evaluate sarcopenia, and therefore, both of the studies are part of this review. Both studies by Burrows et al. (43, 44) studied adolescents who were part of an iron deficiency anemia preventive trial and a follow-up study beginning in infancy. Although both studies used the same body composition evaluation method and sarcopenia and excessive weight indicators, SO definition differed between the aforementioned studies; therefore, neither of them were excluded from our review. Among the patients included in the 6 studies evaluating the prevalence of SO, the prevalence ranged from 5.66% (25) to 69.7% (45) in girls, with a range between 7.2% (42) and 81.3% (37) in boys.

3.1 Sarcopenic Obesity Evaluation Methods

Overall DXA (n = 8/18) (33, 35, 37, 38, 41, 43, 44, 46) and analysis BIA (n = 6/18) (25, 34, 36, 42, 45, 48) were the most commonly used body composition evaluation methods, followed by other *imaging* techniques (4/18) (40, 46, 47, 49) and assessment of handgrip strength (HGS) using dynamometer (39). Three studies used more than one method to assess SO [BIA along with dynamometer (36, 42) and quantitive computed tomography (QCT) in addition to DXA (46)]. Overall, there was a wide heterogenity in the cutoff values used to define sarcopenia.

3.1.1 Healthy Populations

In total, 11 studies included healthy populations of children and adolescents (25, 33, 36, 37, 39, 41–45, 48). Five studies used BIA to evaluate body composition (25, 36, 42, 45, 48), 5 studies used DXA (33, 37, 41, 43, 44), whereas 1 study was conducted with the use of the handgrip test (39).

3.1.1.1 Bioelectrical Impedance

In the study by McCarthy et al. (25), muscle-to-fat ratio (MFR) was derived by dividing appendicular skeletal muscle mass (SMMa) by fat mass (FM). For each age range, in boys and in girls separately, children were divided into fifths of BMI z-score, and the mean and standard deviation (SD) of MFR within each fifth were calculated. MFR cutoff equating to -2 SD for the middle fifth was introduced for the first time, and SO was considered as a proportion of cases falling below the cutoff in the highest fifth of BMI z-score.

In the study by Stefanaki et al. (48), patients were divided into two groups: healthy lean group and healthy overweight/obese group. Lower SMM in comparison to healthy lean group for each gender separately was used as sarcopenia indicator.

In the study by Gätjens et al. (45), fat mass (FM) and fat-free mass (FFM) were normalized to height^2 to fat mass index (FMI) and fat-free mass index (FFMI). Sarcopenic obese phenotype was defined as >90th percentile of FM/FFM or FM/FFM^2.

Studies by Gontarev et al. (36) and Steffl et al. (42) assessed muscle strength using a dynamometer along with bioelectrical impedance from which MFR was calculated. The purpose of those studies was to determine the relationships between MFR and relative handgrip strength (RHGS) and to determine the ability of HGS relative to BMI (grip-to-BMI) to identify children who are at risk of developing SO. In both studies, a previous methodology used to define sarcopenia in children described by McCarthy et al. (25) and Kim et al. (33) was used. Results indicated that grip-to-BMI ratio is capable of identifying children at risk of SO.

3.1.1.2 Dual-Energy X-Ray Absorptiometry

Studies by Moon et al. (37) and Kim and Park (41) used ASM divided by weight (ASM/Wt%) as a sarcopenia indicator. In the study by Moon et al. (37), LMM was defined as the lower 10% of gender-specific ASM/Wt%. Whereas in the study by Kim et al. (41), LMM was observed if the value for ASM/Wt was below the lower quintile for the study population; LMM cutoff points were calculated for each sex and age.

Two studies by Burrows et al. (43, 44) used FFMI estimated according to Wells and Fewtrell as a sarcopenia indicator. In the study by Burrows et al. (43), FFMI values were expressed as percentage; values ≤25th percentile in the study sample, adjusted for sex, were defined as relative sarcopenia. While in the study by Burrows et al. (44), FFMI values were expressed as the percentage of BMI; values ≤25th percentile in study sample, adjusted for sex, were considered sarcopenia.

In the study by Kim et al. (33), MFR was calculated, each gender was divided into quintiles of BMI z-score, and the mean and SD of MFR were calculated for each quintile; cutoff values were defined using the mean and SD of MFR for the third BMI quintile (cutoff value = mean value -1 SD of MFR for the third BMI quintile).

3.1.1.3 Handgrip Test

In the study by Palacio-Agüero et al. (39), RHGS was calculated by dividing maximum HGS from the dominant hand by BMI. Low RHGS was defined as <25th percentile by sex.

3.1.2 Overweight/Obese Populations

Overall, 3 studies involved overweight/obese children and adolescents (34, 35, 47).

In the study by Videira-Silva and Fonseca (34), %SMM ≤25 percentile based on reference charts for youth by McCarthy et al. (25) was used as a cutoff value for sarcopenia; BIA was used to assess the concept of SO.

In the study by Pacifico et al. (35), DXA was used to assess body composition, population was stratified into tertiles of relative muscle mass (RMM) and ASM/weight index, and children in the lowest tertiles were considered sarcopenic.

In the study by Yodoshi et al. (47), abdominal MRI at the level of the second to third lumbar vertebrae allowing the determination of total psoas muscle surface area (tPMSA) of patients with either histologically confirmed NAFLD with negative workup for other liver disease, and an abdominal MRI within 1 year of the liver biopsy, or presumed NAFLD, defined as overweight/obese patients with MRI-determined hepatic steatosis and a negative workup for other liver diseases, was evaluated. Patients with presumed NAFLD were divided into two groups: one consisted of patients who underwent magnetic resonance (MR) elastography to assess liver stiffness, and patients in group 2 had measured liver fat fraction by MRI proton density fat fraction (PDFF). tPMSA was measured using a geometric region of interest measurement tool (Intellispace). tPMSA was corrected for height, generating tPMSA index (mm^2/m^2). Patients with a lower median tPMSA index compared to patients with a lower grade (<5) of NAFLD activity score (NAS) according to non-alcoholic steatohepatitis (NASH) Clinical Research Network were identified as sarcopenic.

3.1.3 Other Populations

The populations of studies by Orgel et al. (38) and Mueske et al. (46) consisted of children with hematologic malignancies (high-risk B-precursor ALL and T-cell ALL).

In the study by Orgel et al. (38), the concept of SO was evaluated with DXA; subjects underwent three serial assessments of body composition (at diagnosis, at the end of the induction phase, and at the end of the delayed intensification phase), and lean muscle mass was measured. SO was defined as loss of lean muscle mass and increase of body fat mass and body fat percent over time.

In the study by Mueske et al. (46), participants underwent imaging using three-dimensional QCT of the tibia and whole-body DXA within 96 h from the start of chemotherapy, again at 28–35 days later (end of induction phase), and 7–9 months from diagnosis following completion of intensive chemotherapy (end of the delayed intensification phase). Tissue volumes for adipose, muscle, and bone were computed along the entire length of both tibias using a custom MATLAB script. Total body fat mass and percentage were obtained using DXA. Loss of muscle volume/muscle percent and increase of total body fat mass/fat percent over time was considered as an SO indicator.

In the study by Joffe et al. (49), chest CT images obtained at two time points, diagnosis and first follow-up disease evaluation (6–14 weeks after initiation of therapy), of children, adolescents, and young adults who underwent treatment for a primary diagnosis of Wilms tumor, Ewing sarcoma, osteosarcoma, or rhabdomyosarcoma were used. Measurement of SM, residual lean mass (RLT), and visceral adipose tissue (VAT) was performed on single-slice images at a select anatomic landmark located in the intervertebral space between the 12th thoracic and first lumbar vertebrae (T12–L1). Image analysis was performed utilizing Slice-O-Matic image analysis software. SO was defined as a decrease in SM and RLT and increase of VAT between two study points.

In the study by Mangus et al. (40), body composition of children on the kidney, liver, or intestine transplant list with end-organ failure and a CT scan within 6 months of actual transplant date were assessed with measurements taken at the level of the L2/L3 intervertebral disc space. The scan was set to the soft tissue image mode, and the FreehandDrawingTool was employed to outline the target structures using Synapse picture archiving and communication system (PACS). Total psoas muscle area was obtained by outlining both the right and left psoas muscles and summing these measurements. Total perinephric fat was calculated by outlining the kidney and vasculature and subtracting this area from the area obtained by outlining Gerota's fascia. The subcutaneous fat area was obtained by subtracting the area of the outlined abdominal cavity (at the outermost fascial layer) from the area obtained by outlining the level just beneath the dermis. The sarcopenic index was obtained by dividing the total psoas area (in mm^2) by the height (in cm) squared, and the visceral and subcutaneous fat measurements were also scaled for height. SO was defined as lower sarcopenic index and higher visceral fat and subcutaneous fat index compared to controls.

3.2 Sarcopenic Obesity Prevalence

Eight studies included in the review assessed the prevalence of SO (25, 33–37, 42, 45); 6 studies included healthy populations (25, 33, 36, 37, 42, 45), whereas 2 studies examined overweight/obese children and adolescents (34, 35).

3.2.1 Healthy Populations

Among the patients included in the studies evaluating healthy populations, the prevalence ranged from 5.66% (25) to 69.7% (45) in girls, with a range between 7.2% (42) and 81.3% (37) in boys. Evaluation of body composition using BIA with usage of mean MFR-2SD of the third BMI quintile as threshold, which was used in 3 studies (25, 36, 42), was associated with the lowest SO prevalence in boys [7.2% (42), 8.31% in boys 5–10 years (25), 9.2% (36), 9.67% in boys 10–18 years (25)] and in girls [5.66% in girls 10–18 years (25), 5.9% (36), 9.3% (42), 15.48% in girls 5–10 years (25)]. This was followed by a study that used DXA and a cutoff value defined as mean value -1 SD of MFR for the third BMI quintile, which yielded 24.3% SO prevalence in girls and 32.1% in boys (33).

Highest prevalence of SO was found in the usage of DXA and the lower 10% of gender-specific ASM/Wt% in boys (81.3%) (37) and using BIA along with >90th percentile of FM/FFM or FM/FFM^2 in girls (69.7%) (45).

3.2.2 Overweight/Obese Populations

Two studies evaluated SO prevalence in overweight/obese children and adolescents, the study that used DXA to assess RMM and ASM and stratified study population into tertiles of RMM and ASM/weight index yielded 28.79% SO prevalence in boys and 39.22% in girls (35), whereas the study by Videira-Silva and Fonseca (34) that used BIA and a cutoff value defined as % SMM ≤25 percentile based on reference charts for youth by

McCarthy et al. (25) yielded 20.2% prevalence of SO in girls and 33.3% in boys.

3.3 Outcomes Related With Sarcopenic Obesity

A total of 8 (33–35, 37, 43, 44, 47, 48) studies assessed outcomes related to SO. Of those, 5 studies evaluated SO relation with cardiometabolic outcomes (33–35, 43, 44), and 2 studies assessed the association between NAFLD severity and presence of SO (35, 47). Two studies evaluated the relation between SO and inflammation (34, 48). The remaining study investigated the relationship between LMM, obesity, and mental health (37).

In 2 of the studies analyzing the occurrence of metabolic syndrome (MetS) (33, 43), MetS diagnosis was based on 2007 International Diabetes Federation (IDF) consensus, whereas in one study (35), MetS diagnosis was based on the presence of at least 3 risk factors: high waist circumference (WC), elevated blood pressure (BP), low high-density lipoprotein cholesterol (HDL-C) levels, hypertriglyceridemia, and glucose impairment. In all studies evaluating associations between sarcopenia, obesity, and MetS, significant associations were observed. Odds ratios (ORs) of MetS risk were found to be significantly increased in sarcopenic obese individuals [OR 8.28, 95% CI 5.6–11.45 (33); sarcopenic boys, OR 21.2, 95% CI 4.18–107.5 (43); obese boys, OR 3.7, 95% CI 1.23–10.8 (43); and sarcopenic girls, OR 3.61, 95% CI 1.10–11.9) (43)]; the prevalence of MetS was also significantly increased in the tertile 1 of RMM ($p < 0.0001$) (35).

Remaining studies that analyzed SO association with cardiometabolic outcomes showed significant associations in terms of insulin resistance (IR) and obesity (OR 6.6, 95% CI 4.1–10.6) (44) and IR and sarcopenia (OR 4.9, 95% CI 3.2–7.5) in bivariate analysis (44), IR and sarcopenia (OR 1.9, 95% CI 1.1–3.6) and IR and obesity (OR 2.4, 95% CI 1.2–4.9) in the fully adjusted model (including family history of type 2 diabetes, physical inactivity, sarcopenia, obesity, low adiponectin) (44), increased insulin ($p < 0.01$) (34), homeostatic model assessment for insulin resistance (HOMA-IR) ($p < 0.05$) (34, 35), total cholesterol ($p < 0.05$) (34), low-density lipoprotein cholesterol (LDL-C) ($p < 0.01$) (34), triglyceride (TG) ($p < 0.05$) (34, 35), decreased HDL-C ($p < 0.05$) (34, 35), and NAFLD ($p < 0.05$) (35).

In 2 studies (35, 47) aiming to assess NAFLD severity in terms of SO, children with a higher severity of NAFLD [NAS/liver PDFF/non-alcoholic steatohepatitis (NASH) occurrence] had lower values of sarcopenia indicator (tPMSA index/RMM/ASM/weight) in comparison with the ones with lower liver disease severity. In the study by Pacifico et al. (35), children with NASH showed significantly lower RMM [mean 55.7% (SD, 6.0) *vs.* 63.4% (6.0); $p < 0.0001$) and ASM/weight index [mean 25.6% (SD, 2.8) *vs.* 28.6% (2.9); $p = 0.006$], whereas in the histology cohort from the study of Yodoshi et al. (47), median tPMSA index was significantly lower in the subjects with NAS ≥5 compared to those with NAS <5 (544 mm^2/m^2 *vs.* 669 mm^2/m^2, $p < 0.001$). In both univariate logistic regression analysis with proportional odds and multivariable analysis (including all demographic, clinical, radiographic variables), higher NAS was significantly associated with lower tPMSA index (OR 0.67, 95% CI 0.52–0.86, $p = 0.002$) and tPMSA index significantly predicted NAS (OR 0.68, 95% CI 0.52–0.91, $p = 0.008$), respectively. Moreover, in the liver PDFF cohort in the multivariable regression model (including tPMSA index, sex, ethnicity, community deprivation index, and T2DM), liver PDFF was significantly associated with tPMSA index ($p = 0.029$) (47).

Both studies (34, 48) aiming to investigate SO relation with inflammation, an assessment of C-reactive protein (CRP)/high-sensitivity C-reactive protein (hsCRP) concentration, showed significant associations. In the study by Videira-Silva and Fonseca (34), a low value of SMM (%SMM ≤p25) was associated with an increased value of inflammation indicator ($p < 0.05$), whereas in the study by Stefanaki et al. (48), SMM was negatively correlated with hsCRP concentration in overweight/obese individuals.

In the study by Moon et al. (37), girls with LMM and obesity were 3.46 times more at risk of developing depression compared with girls with normal muscle mass after controlling for age, waist-to-height ratio, health habits, self-reported obesity, weight loss efforts, and monthly household income (95% CI 1.00–11.97, $p = 0.049$).

4 DISCUSSION

There are a limited number of studies assessing the prevalence of SO and its relation with adverse health outcomes in children and adolescents. Results of our review indicate that SO is highly prevalent in children and adolescents and influences the occurrence of adverse health outcomes (25, 33–37, 42–45, 47, 48).

In our review, the prevalence of SO ranged from 5.66% to 69.7% in girls, with a range between 7.2% and 81.3% in boys. The wide range of SO prevalence is related to different sarcopenia evaluation methods and the variety of used definitions and cutoff points. Furthermore, studies that evaluated SO prevalence included healthy populations and overweight/obese children and adolescents; therefore, comparisons between those populations may lead to discrepancies, as studies regarding overweight/obese participants may yield higher SO prevalence.

Our review found DXA and BIA to be the most prevalent methods of body composition assessment. Evaluation of body composition with usage of mean MFR-2SD of the third BMI quintile as threshold was the most prevalent. All of the studies that assessed SO relation with adverse health outcomes (cardiometabolic outcomes, NAFLD severity, inflammation, mental health) found significant associations. The main difficulty regarding the assessment of sarcopenia in the population of children and adolescents is their growth, which leads to age-related differences. Puberty is also a crucial factor in body composition evaluation. The present review indicates that the occurrence of SO is associated with a higher metabolic risk. Much work needs to be done to understand the impact of SO on metabolic outcomes, as SO may carry a cumulative metabolic risk of both sarcopenia and obesity and could lead to worse metabolic outcomes than obesity alone. Routine assessment of

SM mass and function should be considered in obese children and adolescents in order to distinguish individuals with a higher metabolic risk.

Only 2 studies included in our review assessed both muscle mass and muscle function (36, 42), as recommended by EWGSOP, yielding inadequate values of SO prevalence. The assessment of muscle function is crucial for the assessment of sarcopenia, since muscle strength is not linearly related to muscle mass (30).

SO has been associated with various adverse health outcomes such as disability, metabolic outcomes, depression, increased stress level, cancer treatment outcomes, and mortality in adults (16). Several studies evaluated SO association with metabolic outcomes in adults, indicating worse cardiovascular risk profiles (hyperglycemia, hypertension, dyslipidemia, insulin resistance, lower cardiorespiratory fitness) in individuals affected by SO (51–53). Results of the Korean Longitudinal Study on Health and Aging (KLoSHA) demonstrated a higher risk of IR and MetS in patients with SO than in those with sarcopenia alone or obesity alone (51). According to KHANES, SO was also associated with NAFLD, and the study found that SO increased stepwise from lowest to highest quintile (independent 3.4-fold risk) of serum gamma-glutamyl transferase activity (GGT) in community-dwelling older adults (54). NASH as a cause of cirrhosis was found in multivariable logistic regression analysis as an independent predictor of SO after controlling for age, gender, alcoholic liver disease diagnosis, and hepatocellular carcinoma ($p = 0.014$, 95% CI, 1.44–25.26, OR 6.03) (55). In a study by Schrager et al. (56), low HGS and high waist circumference and/or BMI were significantly associated with elevated levels of IL-6, C-reactive protein, and Interleukin-1 (IL-1). In a study by Hamer et al. (57), the risk of occurrence of depressive symptoms in obese adults with low HGS was 1.79 (95% CI, 1.10–2.89) times greater compared to non-obese individuals with high HGS after multivariate adjustment. These findings of adult studies are in line with results of our review, which demonstrated a significant association between adverse health outcomes (metabolic syndrome, IR, NAFLD) and SO in children and adolescents. Moreover, in two studies included in our review, low SMM demonstrated an association with increased hsCRP/CRP concentration, indicating a relation between inflammation and low SMM in overweight/obese children and adolescents. Results of our review regarding the association between LMM, obesity, and depressive symptoms also match adult studies.

To our knowledge, this is the first systematic review evaluating the prevalence and outcomes of SO in children and adolescents. Our study is not without limitations. There was considerable inconsistency in the criteria used to define SO, and there was a wide heterogeneity in sarcopenia as well as obesity definitions that limited comparisons among studies. Furthermore, the number of study participants varied between the studies that also led to difficulties regarding comparisons. Included studies differed in terms of included populations (healthy populations, overweight/obese participants, other clinical populations) that yielded further difficulties regarding data comparability.

Consensus regarding SO definition and implementation of standardized evaluation methods in children and adolescents should be reached in order to conduct studies assessing the exact prevalence of SO and its impact on outcomes. Furthermore, according to EWSOGP2, low muscle strength is a primary sarcopenia parameter and should always be evaluated next to muscle mass. Further studies providing age and gender thresholds for SO for different ethnicities are needed.

5 CONCLUSIONS

In conclusion, in our review, the prevalence of SO ranged from 984 5.66% to 69.7% in girls, with a range between 7.2% and 81.3% in 985 boys SO. Association between SO and various adverse health outcomes was found. Considering the fact that no consensus regarding SO definition in children and adolescents has been reached and studies included in our review used a wide range of methods and definitions to evaluate the presence of SO, results of our review concerning SO prevalence and adverse health outcomes related to SO might not be adequate. Findings of this review highlight the need for the development of a consensus regarding definition, standardized evaluation methods, and age and gender thresholds for SO for different ethnicities in the pediatric population in order to produce meaningful results and implications for clinical practice. Routine assessment of SM mass and function in obese pediatric patients should be taken into consideration. Moreover, effective treatment strategies for children and adolescents with SO should be developed, as SO might play a role in the occurrence of adverse health 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.

AUTHOR CONTRIBUTIONS

Conceptualization: MZ and PM. Methodology: MZ and PM. Validation: MZ and PM. Data analysis: MZ. Writing—original draft preparation: MZ. Writing—review and editing: PM. Supervision: PM. Project administration: MZ and PM. All authors have read and agreed to the published version of the article.

REFERENCES

1. WHO. *Overweight and Obesity*. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (Accessed 1.12.2021).

2. HEALTHY Study Group, Kaufman FR, Hirst K, Linder B, Baranowski T, Cooper DM, et al. Risk Factors for Type 2 Diabetes in a Sixth- Grade Multiracial Cohort: The sHEALTHY Study. *Diabetes Care* (2009) 32 (5):953–5. doi: 10.2337/dc08-1774

3. Sorof J, Daniels S. Obesity Hypertension in Children: A Problem of Epidemic Proportions. *Hypertension* (2002) 40:441–7. doi: 10.1161/01.HYP.0000032940.33466.12
4. Kumar S, Kelly AS. Review of Childhood Obesity: From Epidemiology, Etiology, and Comorbidities to Clinical Assessment and Treatment. *Mayo Clin Proc* (2017) 92(2):251–65. doi: 10.1016/j.mayocp.2016.09.017
5. Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angulo P. The Natural History of non-Alcoholic Fatty Liver Disease in Children: A Follow-Up Study for Up to 20 Years. *Gut* (2009) 58:1538–44. doi: 10.1136/gut.2008.171280
6. Spilsbury JC, Storfer-Isser A, Rosen CL, Redline S. Remission and Incidence of Obstructive Sleep Apnea From Middle Childhood to Late Adolescence. *Sleep* (2015) 38:23–9. doi: 10.5665/sleep.4318
7. Strauss RS. Childhood Obesity and Self-Esteem. *Pediatrics* (2000) 105:e15. doi: 10.1542/peds.105.1.e15
8. Rosenberg I. Summary Comments: Epidemiological and Methodological Problems in Determining Nutritional Status of Older Persons. *Am J Clin Nutr* (1989) 50:1231–3. doi: 10.1093/ajcn/50.5.1231
9. Rosenberg IH. Sarcopenia: Origins and Clinical Relevance. *J Nutr* (1997) 127:990S–91S. doi: 10.1093/jn/127.5.990S
10. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European Consensus on Definition and Diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* (2010) 39(4):412–23. doi: 10.1093/ageing/afq034
11. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: Revised European Consensus on Definition and Diagnosis [Published Correction Appears in Age Ageing. *Age Ageing* (2019) 48(1):16–31. doi: 10.1093/ageing/afy169
12. *NIH.Study Quality Assessment Tools*. Available at: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools (Accessed 1.12.2021).
13. Koliaki C, Liatis S, Dalamaga M, Kokkinos A. Sarcopenic Obesity: Epidemiologic Evidence, Pathophysiology, and Therapeutic Perspectives. *Curr Obes Rep* (2019) 8(4):458–71. doi: 10.1007/s13679-019-00359-9
14. Sayer AA, Syddall H, Martin H, Patel H, Baylis D, Cooper C. The Developmental Origins of Sarcopenia. *J Nutr Health Aging* (2008) 12(7):427–32. doi: 10.1007/BF02982703
15. Gallagher D, Belmonte D, Deurenberg P, Wang Z, Krasnow N, Pi-Sunyer FX, et al. Organ-Tissue Mass Measurement Allows Modeling of REE and Metabolically Active Tissue Mass. *Am J Physiol* (1998) 275(2):E249–58. doi: 10.1152/ajpendo.1998.275.2.E249
16. Batsis JA, Villareal DT. Sarcopenic Obesity in Older Adults: Aetiology, Epidemiology and Treatment Strategies. *Nat Rev Endocrinol* (2018) 14(9):513–37. doi: 10.1038/s41574-018-0062-9
17. Benson AC, Torode ME, Singh MA. Muscular Strength and Cardiorespiratory Fitness is Associated With Higher Insulin Sensitivity in Children and Adolescents. *Int J Pediatr Obes* (2006) 1:222–31. doi: 10.1080/17477160600962864
18. Steene-Johannessen J, Anderssen SA, Kolle E, Andersen LB. Low Muscle Fitness is Associated With Metabolic Risk in Youth. *Med Sci Sports Exerc* (2009) 41:1361–7. doi: 10.1249/MSS.0b013e31819aaae5
19. Chien MY, Huang TY, Wu YT. Prevalence of Sarcopenia Estimated Using a Bioelectrical Impedance Analysis Prediction Equation in Community-Dwelling Elderly People in Taiwan. *J Am Geriatr Soc* (2008) 56:1710–5. doi: 10.1111/j.1532-5415.2008.01854.x
20. Gilligan LA, Towbin AJ, Dillman JR, Somasundaram E, Trout AT. Quantification of Skeletal Muscle Mass: Sarcopenia as a Marker of Overall Health in Children and Adults. *Pediatr Radiol* (2020) 50:455–64. doi: 10.1007/s00247-019-04562-7
21. Buckinx F, Landi F, Cesari M, Fielding RA, Visser M, Engelke K, et al. Pitfalls in the Measurement of Muscle Mass: A Need for a Reference Standard. *J Cachexia Sarcopenia Muscle* (2018) 9:269–78. doi: 10.1002/jcsm.12268
22. Janssen I, Heymsfield SB, Baumgartner RN, Ross R. Estimation of Skeletal Muscle Mass by Bioelectrical Impedance Analysis. *J Appl Physiol* (2000) 89:465–71. doi: 10.1152/jappl.2000.89.2.465
23. Ooi PH, Hager A, Mazurak VC, Dajani K, Bhargava R, Gilmour SM, et al. Sarcopenia in Chronic Liver Disease: Impact on Outcomes. *Liver Transpl* (2019) 25(9):1422–1438. doi: 10.1002/lt.25591
24. Kim KM, Jang HC, Lim S. Differences Among Skeletal Muscle Mass Indices Derived From Height-, Weight-, and Body Mass Index-Adjusted Models in Assessing Sarcopenia. *Korean J Intern Med* (2016) 31:643–50. doi: 10.3904/kjim.2016.015
25. McCarthy HD, Samani-Radia D, Jebb SA, Prentice AM. Skeletal Muscle Mass Reference Curves for Children and Adolescents. *Pediatr Obes* (2014) 9(4):249–59. doi: 10.1111/j.2047-6310.2013.00168.x
26. Guo B, Wu Q, Gong J, Xiao Z, Tang Y, Shang J, et al. Relationships Between the Lean Mass Index and Bone Mass and Reference Values of Muscular Status in Healthy Chinese Children and Adolescents. *J Bone Miner Metab* (2016) 34:703–13. doi: 10.1007/s00774-015-0725-8
27. Schmidt SC, Bosy-Westphal A, Niessner C, Woll A. Representative Body Composition Percentiles From Bioelectrical Impedance Analyses Among Children and Adolescents. *MoMo Study Clin Nutr* (2019) 38(6):2712–20. doi: 10.1016/j.clnu.2018.11.026
28. Webber CE, Barr RD. Age- and Gender-Dependent Values of Skeletal Muscle Mass in Healthy Children and Adolescents. *J Cachexia Sarcopenia Muscle* (2012) 3:25–9. doi: 10.1007/s13539-011-0042-6
29. Been E, Shefi S, Kalichman L, Bailey JF, Soudack M. Cross-Sectional Area of Lumbar Spinal Muscles and Vertebral Endplates: A Secondary Analysis of 91 Computed Tomography Images of Children Aged 2-20. *J Anat* (2018) 233(3):358–69. doi: 10.1111/joa.12838
30. Merli M. Pediatric Sarcopenia: Exploring a New Concept in Children With Chronic Liver Disease. *J Pediatr* (2020) 96(4):406–8. doi: 10.1016/j.jped.2019.08.001
31. Griffiths A, Toovey R, Morgan PE, Spittle AJ. Psychometric Properties of Gross Motor Assessment Tools for Children: A Systematic Review. *BMJ Open* (2018) 8(10):e021734. doi: 10.1136/bmjopen-2018-021734
32. Hardin AP, Hackell JM. COMMITTEE ON PRACTICE AND AMBULATORY MEDICINE. *Age Limit Pediatr Pediatr* (2017) 140(3):e20172151. doi: 10.1542/peds.2017-2151
33. Kim K, Hong S, Kim EY. Reference Values of Skeletal Muscle Mass for Korean Children and Adolescents Using Data From the Korean National Health and Nutrition Examination Survey 2009-2011. *PloS One* (2016) 11(4):e0153383. doi: 10.1371/journal.pone.0153383
34. Videira-Silva A, Fonseca H. Skeletal Muscle and Metabolic Risk in Overweight Adolescents. An Indicator of Premature Sarcopenic Obesity. *Int J Health Sci* (2017) 7:34–43.
35. Pacifico L, Perla FM, Andreoli G, Grieco R, Pierimarchi P, Chiesa C. Nonalcoholic Fatty Liver Disease Is Associated With Low Skeletal Muscle Mass in Overweight/Obese Youths. *Front Pediatr* (2020) 8:158. doi: 10.3389/fped.2020.00158
36. Gontarev S, Jakimovski M, Georgiev G. Using Relative Handgrip Strength to Identify Children at Risk of Sarcopenic Obesity. *Nutr Hosp* (2020) 34(3):490–6. doi: 10.20960/nh.02977
37. Moon JH, Kong MH, Kim HJ. Low Muscle Mass and Depressed Mood in Korean Adolescents: A Cross-Sectional Analysis of the Fourth and Fifth Korea National Health and Nutrition Examination Surveys. *J Korean Med Sci* (2018) 33(50):e320. doi: 10.3346/jkms.2018.33.e320
38. Orgel E, Mueske NM, Sposto R, Gilsanz V, Freyer DR, Mittelman SD. Limitations of Body Mass Index to Assess Body Composition Due to Sarcopenic Obesity During Leukemia Therapy. *Leuk Lymphoma* (2018) 59(1):138–45. doi: 10.3109/10428194.2015.1136741
39. Palacio-Agüero A, Díaz-Torrente X, Quintiliano Scarpelli Dourado D. Relative Handgrip Strength, Nutritional Status and Abdominal Obesity in Chilean Adolescents. *PloS One* (2020) 15(6):e0234316. doi: 10.1371/journal.pone.0234316
40. Mangus RS, Bush WJ, Miller C, Kubal CA. Severe Sarcopenia and Increased Fat Stores in Pediatric Patients With Liver, Kidney, or Intestine Failure. *J Pediatr Gastroenterol Nutr* (2017) 65(5):579–83. doi: 10.1097/MPG.0000000000001651
41. Kim JH, Park YS. Low Muscle Mass is Associated With Metabolic Syndrome in Korean Adolescents: The Korea National Health and Nutrition Examination Survey 2009-2011. *Nutr Res* (2016) 36(12):1423–8. doi: 10.1016/j.nutres.2016.09.013
42. Steffl M, Chrudimsky J, Tufano JJ. Using Relative Handgrip Strength to Identify Children at Risk of Sarcopenic Obesity. *PloS One* (2017) 12(5):e0177006. doi: 10.1371/journal.pone.0177006
43. Burrows R, Correa-Burrows P, Reyes M, Blanco E, Albala C, Gahagan S. High Cardiometabolic Risk in Healthy Chilean Adolescents: Associations With

Anthropometric, Biological and Lifestyle Factors. *Public Health Nutr* (2016) 19(3):486–93. doi: 10.1017/S1368980015001585
44. Burrows R, Correa-Burrows P, Reyes M, Blanco E, Albala C, Gahagan S. Healthy Chilean Adolescents With HOMA-IR ≥ 2.6 Have Increased Cardiometabolic Risk: Association With Genetic, Biological, and Environmental Factors. *J Diabetes Res(2015)* (2015) 783296. doi: 10.1155/2015/783296
45. Gätjens I, Schmidt SCE, Plachta-Danielzik S, Bosy-Westphal A, Müller MJ. Body Composition Characteristics of a Load-Capacity Model: Age-Dependent and Sex-Specific Percentiles in 5- to 17-Year-Old Children. *Obes Facts* (2021) 14(6):593–603. doi: 10.1159/000518638
46. Mueske NM, Mittelman SD, Wren TAL, Gilsanz V, Orgel E. Myosteatosis in Adolescents and Young Adults Treated for Acute Lymphoblastic Leukemia. *Leuk Lymphoma* (2019) 60(13):3146–53. doi: 10.1080/10428194.2019.1623889
47. Yodoshi T, Orkin S, Arce Clachar AC, Bramlage K, Sun Q, Fei L, et al. Muscle Mass Is Linked to Liver Disease Severity in Pediatric Nonalcoholic Fatty Liver Disease. *J Pediatr* (2020) 223:93–99.e2. doi: 10.1016/j.jpeds.2020.04.046
48. Stefanaki C, Peppa M, Boschiero D, Chrousos GP. Healthy Overweight/Obese Youth: Early Osteosarcopenic Obesity Features. *Eur J Clin Invest* (2016) 46(9):767–78. doi: 10.1111/eci.12659
49. Joffe L, Shen W, Shadid G, Jin Z, Ladas EJ. Skeletal Muscle and Adipose Tissue Changes in the First Phase of Treatment of Pediatric Solid Tumors. *Cancer Med* (2021) 10(1):15–22. doi: 10.1002/cam4.3584
50. Moher D, Liberati A, Tetzlaff J, Altman DG. PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* (2009) 6(7):e1000097
51. Lim S, Kim JH, Yoon JW, Kang SM, Choi SH, Park YJ, et al. Sarcopenic Obesity: Prevalence and Association With Metabolic Syndrome in the Korean Longitudinal Study on Health and Aging (KLoSHA). *Diabetes Care* (2010) 33(7):1652–4. doi: 10.2337/dc10-0107
52. Chung JY, Kang HT, Lee DC, Lee HR, Lee YJ. Body Composition and its Association With Cardiometabolic Risk Factors in the Elderly: A Focus on Sarcopenic Obesity. *Arch Gerontol Geriatr* (2013) 56:270–8. doi: 10.1016/j.archger.2012.09.007
53. Kim TN, Park MS, Kim YJ, Lee EJ, Kim MK, Kim JM, et al. Association of Low Muscle Mass and Combined Low Muscle Mass and Visceral Obesity With Low Cardiorespiratory Fitness. *PloS One* (2014) 9(6):e100118. doi: 10.1371/journal.pone.0100118
54. Hong N, Lee EY, Kim CO. Gamma-Glutamyl Transferase is Associated With Sarcopenia and Sarcopenic Obesity in Community-Dwelling Older Adults: Results From the Fifth Korea National Health and Nutrition Examination Survey, 2010–2011. *Endocr J* (2015) 62:585–92. doi: 10.1507/endocrj.EJ15-0119
55. Carias S, Castellanos AL, Vilchez V, Nair R, Dela Cruz AC, Watkins J, et al. Nonalcoholic Steatohepatitis is Strongly Associated With Sarcopenic Obesity in Patients With Cirrhosis Undergoing Liver Transplant Evaluation. *J Gastroenterol Hepatol* (2016) 31(3):628–33. doi: 10.1111/jgh.13166
56. Schrager MA, Metter EJ, Simonsick E, Ble A, Bandinelli S, Lauretani F, et al. Sarcopenic Obesity and Inflammation in the InCHIANTI Study. *J Appl Physiol (1985)* (2007) 102(3):919–25. doi: 10.1152/japplphysiol.00627.2006
57. Hamer M, Batty GD, Kivimaki M. Sarcopenic obesity and risk of new onset depressive symptoms in older adults: English Longitudinal Study of Ageing. *Int J Obes (Lond)* (2015) 39(12):1717–20. doi: 10.1038/ijo.2015.124

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.

frontiers | Frontiers in Endocrinology

ORIGINAL RESEARCH
published: 13 June 2022
doi: 10.3389/fendo.2022.920340

Vitamin D Effects on Selected Anti-Inflammatory and Pro-Inflammatory Markers of Obesity-Related Chronic Inflammation

Maria Krajewska[1], Ewelina Witkowska-Sędek[1], Małgorzata Rumińska[1], Anna Stelmaszczyk-Emmel[2], Maria Sobol[3], Anna Majcher[1] and Beata Pyrżak[1]*

[1] Department of Paediatrics and Endocrinology, Medical University of Warsaw, Warsaw, Poland, [2] Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Warsaw, Medical University of Warsaw, Warsaw, Poland, [3] Department of Biophysics, Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland

OPEN ACCESS

Edited by:
Artur Mazur, University of Rzeszow, Poland

Reviewed by:
Maria Giżewska, Pomeranian Medical University, Poland
Serhiy Nyankovskyy, Danylo Halytsky Lviv National Medical University, Ukraine
Artemis Doulgeraki, Institute of Child Health, Greece

***Correspondence:**
Maria Krajewska
maria.krajewska@wum.edu.pl

Specialty section:
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

Received: *14 April 2022*
Accepted: *10 May 2022*
Published: *13 June 2022*

Citation:
Krajewska M, Witkowska-Sędek E, Rumińska M, Stelmaszczyk-Emmel A, Sobol M, Majcher A and Pyrżak B (2022) Vitamin D Effects on Selected Anti-Inflammatory and Pro-Inflammatory Markers of Obesity-Related Chronic Inflammation. Front. Endocrinol. 13:920340. doi: 10.3389/fendo.2022.920340

Background: Obesity is related to changes in adipokine secretion, activity of adipose tissue macrophages, helper T cells, and regulatory T cells. It has been confirmed that vitamin D has potent anti-inflammatory properties. It contributes to reduction in pro-inflammatory mediators and an increase in anti-inflammatory cytokines. There is also evidence that vitamin D could decrease C-reactive protein (CRP) and affect selected haematological indices.

Aim of the Study: We aimed to evaluate the effect of vitamin D on interleukin (IL)-10, IL-17, CRP, blood leukocyte profile, and platelet (PLT) count in overweight and obese children before and after six months of vitamin D supplementation.

Material and Methods: The study group consisted of 67 overweight and obese children aged 9.08-17.5 years. The control group included 31 normal weight peers age- and sex-matched. None of the studied children had received vitamin D supplementation before the study. Data were analyzed at baseline and after vitamin D supplementation.

Results: The study group had lower baseline 25(OH)D ($p<0.001$) and higher white blood cell (WBC) ($p=0.014$), granulocyte ($p=0.015$), monocyte ($p=0.009$) and CRP ($p=0.002$) compared to the control group. In the study group, vitamin D levels were related negatively to nutritional status. Leukocyte profile parameters, PLT, CRP, IL-10 or IL-17 were not related to baseline 25(OH)D. Baseline IL-17 levels correlated with monocytes ($R= 0.36$, $p=0.003$) independently on 25(OH)D deficit. In children with vitamin D <15ng/ml, the baseline 25(OH)D was related to CRP ($R=-0.42$, $p=0.017$). After six months of vitamin D supplementation, we noticed a decrease in CRP levels ($p=0.0003$). Serum 25(OH)D correlated with IL-10 in that period ($R=0.27$, $p=0.028$). Moreover, we noticed that IL-10 correlated with monocyte ($R=-0.28$, $p=0.023$). We did not find any significant associations between 25(OH)D and leukocyte profile parameters, PLT, or IL-17. The multivariable

stepwise regression analysis identified IL-10 as the parameter positively associated with 25(OH)D.

Conclusions: Our study confirmed beneficial effects of vitamin D supplementation in overweight and obese paediatric populations. Vitamin D intake seems to exert its anti-inflammatory effect mainly *via* decreasing the CRP level and protecting stabile values of IL-10, rather than its impact on pro-inflammatory factors such as IL-17 and leukocyte profile parameters.

Keywords: obesity, children, interleukin-10, interleukin-17, C-reactive protein, blood leukocyte profile, platelets, vitamin D

INTRODUCTION

Vitamin D deficiency is commonly observed in overweight and obese children and adolescents (1, 2). The inverse associations between 25-hydroxyvitamin D (25(OH)D) serum levels and both fat volume and body mass index (BMI) have been confirmed (3, 4). The main mechanisms involved in the obesity-related hypovitaminosis D include decreased bioavailability of vitamin D due to its fat solubility and sequestration in abdominal fat, reduced intestinal absorption, impaired metabolism, decreased liver 25(OH)D synthesis as a result of hepatic steatosis, and the influence of leptin and interleukin-6 (IL-6) on hepatic vitamin D receptors (VDRs) (2, 3, 5–10). There is also some evidence that inflammation could reduce 25(OH)D levels *via* oxidative stress resulting in the oxidative 25(OH)D catabolism (11, 12). Sedentary lifestyle and lower outdoor physical activity, leading to insufficient sun exposure as well as inappropriate vitamin D dietary intake, also predispose obese individuals to vitamin D deficiency (8, 13). Taking into account the high prevalence of overweight and obesity in people of all age groups, including children and young adults, the role of vitamin D in the pathogenesis of obesity and prevention of obesity-related metabolic disorders is extensively investigated (2, 11, 14–17). Adipose tissue cannot be considered only as an energy reservoir that consists of adipocytes and their precursors. It also contains mesenchymal progenitor/stem cells, endothelial cells, pericytes, T cells, and M2 macrophages known as stromal vascular fraction, which play an important role in the integration of endocrine, metabolic, and inflammatory signals (2, 18). Excess body fat mass is closely related to significant changes in adipokine secretion, accumulation, and activity of adipose tissue macrophages, helper T (Th) cells, and regulatory T (Treg) cells (2, 19–24). Several studies have shown an increase in pro-inflammatory factors [IL-6, IL-8, IL-1β, IL-17, leptin, tumor necrosis factor alpha (TNF-α)] and reduction in adiponectin and anti-inflammatory interleukins (IL-4, IL-10, IL-13) in obese individuals (2, 25, 26). These mediators are involved in mutual interactions between the immune and metabolic systems, contributing to the development of insulin resistance, hyperglycaemia, atherogenic dyslipidaemia, and hypertension which highly increase the risk of atherosclerotic cardiovascular disease and diabetes mellitus type 2, even in the paediatric and young adult populations (25, 27, 28). Moreover, pro-inflammatory cytokines could affect systemic inflammation by enhancing liver production of acute phase markers including fibrinogen and C-reactive protein (CRP) and by activating granulocyte and monocyte progenitor cells (29–32). Higher blood leukocyte, lymphocyte, granulocyte, eosinophil, and monocyte count is well documented in obese individuals (33–36).

Current studies show that vitamin D exerts multiple non-calcaemic effects. Vitamin D receptors have been discovered in many cells and types of tissues, including human subcutaneous adipose tissue, visceral adipose tissue, pancreatic beta-cells, and T cells (2, 37–42). Moreover, the presence of VDRs has been also confirmed in the brain in arcuate and paraventricular nuclei of the hypothalamus, which are responsible for regulation of body weight (41, 42). Lumeng et al. (21) indicate that hypothalamic inflammation impacts metabolism, mainly by reducing the release of insulin from beta cells, impairing insulin peripheral action and also by aggravating hypertension. The main mechanisms of vitamin D action in obesity include the influence on adipose tissue inflammatory process *via* effects on adipokine secretion and on the immune system cells by regulating their proliferation and metabolism leading to inhibition of T cell proliferation and induction of Treg differentiation (17, 38, 43–46). It has been confirmed that the active form of vitamin D (1,25-dihydroxyvitamin D) has potent anti-inflammatory properties resulting in a switch from Th1/Th17 response, which is more inflammatory to Th2/Treg response, which has less inflammatory potential (11, 47–49). This results in decreased secretion of pro-inflammatory mediators such as interferon gamma (IFN-γ), TNF-α, IL-1β, IL-6, IL-8, IL-12, IL-17 and increased production of anti-inflammatory cytokines such as IL-4 and IL-10 (11, 50–54). There is also some evidence that vitamin D could decrease serum CRP levels and the erythrocyte sedimentation rate (55–61). The effect of hypovitaminosis D, as well as the influence of vitamin D supplementation on selected haematological indices has been also investigated but available data are limited (62–65). In addition to the fact that most authors reported the link between vitamin D and red blood cell parameters (66–68), there are also some studies describing the impact of vitamin D on monocyte and platelet (PLT) count (64, 69, 70). Information about those associations in obese children and adolescents is scarce.

In the present study we aimed to evaluate the effect of vitamin D on IL-10, IL-17, CRP, blood leukocyte profile, and PLT count in a group of overweight and obese children before and after six months of vitamin D supplementation.

MATERIAL AND METHODS

This prospective study was conducted in the Department of Paediatrics and Endocrinology of the Medical University of Warsaw, Poland. Design of the study was approved by the Bioethics Committee at the Medical University of Warsaw, Poland (decision number KB/257/2013). The study group consisted of 67 children (15 overweight and 52 obese) aged from 9.08 to 17.5 years with mean body mass index (BMI) 30.9 ± 4.7. The control group included 31 normal weight peers with mean BMI 18.7 ± 2.7 age- and sex-matched. None of the studied children had received vitamin D supplementation within the last 12 months before being including in the study. In both the study and the control groups, blood morphology was evaluated to exclude iron deficiency anaemia due to its possible effect on platelet count. Patients with iron deficiency features in blood morphology were not included in the study. At the time of blood collection, children in both the study and the control group were healthy, without any symptoms of infection and chronic diseases and were not taking any medication. During the study period the participants did not change their diet or the level of physical activity. Vitamin D status, levels of IL-10, IL-17, CRP, blood leukocyte profile, and PLT count were determined at baseline (in the study group and in the control group) and after six months of vitamin D supplementation (in the study group).

The aim of vitamin D supplementation was to achieve the reference serum 25(OH)D levels between 30 and 50 ng/ml after six months of intervention (71). The doses of vitamin D ranged from 2000 to 4000 units per day depending on the serum 25(OH) D levels, which were assessed every month. Systematic evaluation of serum 25(OH)D concentrations allowed us to control compliance and to modify administered vitamin D doses to achieve reference values after six months of the study.

Anthropometric parameters (height, weight, waist and hip circumference) were measured using standardized methods. Based on these measurements, BMI, waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) were calculated. The skinfold thickness (mm) was measured under the triceps brachii muscle and under the inferior scapular angle. Body fat percentage was calculated in the study group and in the control group using the Slaughter formula (72). Additionally, in the study group, the percentage of fat was calculated using a bioimpedance analysis device (Maltron Body FAT Analyzer BF-905). Height and weight were evaluated according to Polish 2010 growth references for school-aged children and adolescents (73). The degree of obesity expressed as BMI standard deviation score (SDS) was calculated using the LMS method to normalize skewness of the distribution of BMI (73, 74). Obesity was defined as BMI SDS ≥ 2, and overweight as BMI SDS ≥ 1 and < 2 (75).

Data of the study group were analyzed in the whole group and in subgroups depending on baseline vitamin D status (serum 25 (OH)D < 15 ng/ml - a subgroup of overweight and obese children with "severe" baseline vitamin D deficiency and serum 25(OH)D ≥ 15 ng/ml - a subgroup of overweight and obese children with "low" baseline vitamin D deficiency).

Biochemical Analyses

Blood samples were collected after overnight fasting and analyzed by standard methods. White blood cells (WBC) and PLT count were obtained by an automated blood cell counter (XN-1000, Sysmex, Germany). The levels of CRP (mg/dl) were measured using a fixed-point immune-rate method on the Vitros 5600 analyzer (Ortho Clinical Diagnostic, New Jersey, USA). Serum 25(OH)D levels (ng/ml) were determined by the immunoassay method using Architect Analyzer (Abbott Diagnostics, Lake Forest, USA). Serum levels of IL-10 (pg/ml) and IL-17 (pg/ml) were evaluated by ELISA (R&D Systems, Minneapolis, USA) using Asys UVM 340 analyzer.

Statistical Analysis

Statistical analysis was performed using Statistica 13.3. Data distribution was checked using the Shapiro–Wilk test. Data were presented as means with standard deviation or the median and interquartile ranges, as appropriate. Comparisons between baseline data of the study group and the control group were made using the T-test for parametric data or using the U Mann-Whitney test for non-parametric data. Analysis of changes of the same parameter at baseline and after six months of vitamin D supplementation were provided using the T-test or the Wilcoxon test, as appropriate. Correlation analysis was performed using the Spearman correlation coefficient. In further analysis, we used multivariable stepwise regression analysis to determine which inflammatory factors (model 1: IL-10, IL-17, CRP, WBC or model 2: IL-10, IL-17, CRP, monocytes) were associated with 25 (OH)D levels (as dependent variable) at baseline and after six months of vitamin D supplementation.

RESULTS

Baseline anthropometric and biochemical characteristics of the study group and the control group are presented in **Table 1**. The study group characterized significantly lower baseline serum 25 (OH)D levels compared to the control group (median 15.9 vs 23.9 ng/ml, $p < 0.001$). We also found significant baseline differences in leukocyte profile parameters and CRP levels between those groups, while baseline PLT count, IL-10, and IL-17 levels did not differ significantly between the study group and the control group. Baseline WBC ($p = 0.014$), granulocyte ($p = 0.015$), monocyte ($p = 0.009$) count and CRP levels ($p = 0.002$) were significantly higher in the group of overweight and obese children and adolescents.

Severe baseline vitamin D deficiency was found in 54% (36 participants) of the study group. Taking into account baseline vitamin D status of the study group we did not find any significant differences in anthropometric and biochemical parameters between subgroups with lower and severe vitamin D deficiency.

TABLE 1 | Baseline anthropometric measurements, haematological, and biochemical parameters in the study group and in the control group.

p value	STUDY GROUP (n = 67)	CONTROL GROUP (n = 31)
Age (years) ns	13.3 ± 2.11	13.8 ± 2.45
Height SDS < 0.001	0.7 ± 1.26	-0.6 ± 1.37
Weight SDS < 0.001	2.3 ± 0.66	-0.3 ± 1.09
WC (cm) <0.001	91.3 ± 10.17	62.3 ± 6.49
HC (cm) < 0.001	106.1 ± 10.48	78.7 ± 8.86
WHR < 0.001	0.9 ± 0.06	0.8 ± 0.04
WHtR < 0.001	0.9 ± 2.68	0.4 ± 0.03
% FAT (skinfolds) < 0.001	36.9 ± 6.12	22.7 ± 6.06
BMI SDS < 0.001	2.3 ± 0.46	-0.2 ± 0.83
25(OH)D (ng/ml) < 0.001	15.9 (12.6 – 20.0)	23.9 (17.7 – 29.9)
WBC (cells × 10^3/μl) 0.014	6.7 (5.5 – 8.1)	5.7 (5.0 – 6.7)
Granulocytes (cells × 10^3/μl) 0.015	3.2 (2.4 – 4.5)	2.8 (1.9 – 3.7)
Monocytes (cells × 10^3/μl) 0.009	0.5 (0.5 – 0.7)	0.4 (0.4 – 0.6)
Lymphocytes (cells × 10^3/μl) ns	2.4 (2.1 – 2.9)	2.2 (1.9 – 2.9)
PLT (cells × 10^3/μl) ns	270 (236 – 302)	260 (231 – 284)
CRP (mg/dl) 0.003	0.5 (0.5 – 0.7)	0.5 (0.5 – 0.5)
IL-10 (pg/ml) ns	1.25 (0.03 – 1.74)	0.65 (0.19 – 1.15)
IL-17 (pg/ml) ns	0.05 (0.0 – 0.13)	0.05 (0.0 – 0.12)

Data are presented as means ± standard deviations score or as median with interquartile range, as appropriate. SDS, standard deviation score; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; % FAT (skinfolds), percentage of body fat estimated from skinfolds; BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; WBC, white blood cells; PLT, platelets; CRP, C-reactive protein; IL-10, interleukin-10; IL-17, interleukin-17; ns, not significant.

Baseline Associations Between Vitamin D Status, Nutritional Status, and Biochemical Parameters in the Study Group and in the Control Group

At baseline, as expected, we found significant associations between vitamin D status and nutritional status, especially in children with excess body fat. In the study group, vitamin D levels were related negatively to body mass SDS (R = -0.27, p = 0.029), BMI SDS (R = -0.27, p = 0.025, **Figure 1**) and hip circumference (R = -0.26, p = 0.039). In the control group those associations were not seen, apart from the negative association between vitamin D status and hip circumference (R = -0.52, p = 0.014). Leukocyte profile parameters, PLT count, CRP, IL-10, and IL-17 levels were not related to baseline vitamin D status in the study group nor in the control group.

Taking into account baseline vitamin D status of the study group (serum 25(OH)D level ≥ 15 ng/ml or < 15 ng/ml), we noticed that in children with severe vitamin D deficiency, 25(OH)D levels were related negatively to CRP levels (R= -0.42, p = 0.017). The distribution of serum 25(OH)D values in this subgroup is presented on two histograms (**Figure 2**. for participants with CRP ≤ 0.5 mg/dl, **Figure 3** for participants with CRP > 0.5 mg/dl).

In further analysis, we also found that in this subgroup nutritional status parameters such as BMI SDS (R = 0.61, p = 0.0003), waist circumference (R = 0.44, p = 0.014), WHR (R = 0.49, p = 0.005), %FAT BIA (R = 0.43, p = 0.017), and WHtR (R = 0.70, p = 0.00003) were significantly positively related to CRP levels.

These relationships were not seen in the subgroup with low baseline 25(OH)D deficiency and in the control group. In a subgroup with low 25(OH)D deficiency we found only positive associations between hip circumference and WBC count (R = 0.47, p = 0.006) and between hip circumference and granulocyte count (R = 0.55, p = 0.001). We also observed that baseline IL-17 levels correlated positively with baseline monocytes (R = 0.36, p = 0.003, **Figure 4**) in the whole study group independently on baseline 25 (OH)D deficiency (R = 0.36, p = 0.032; R = 0.37, p = 0.038,

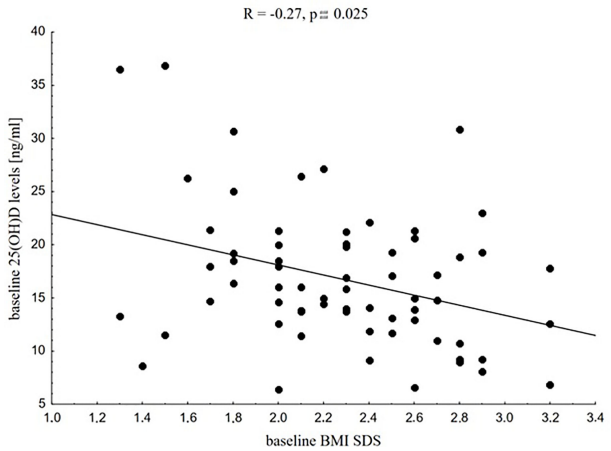


FIGURE 1 | Correlation between baseline 25(OH)D levels and baseline BMI SDS in the whole study group.

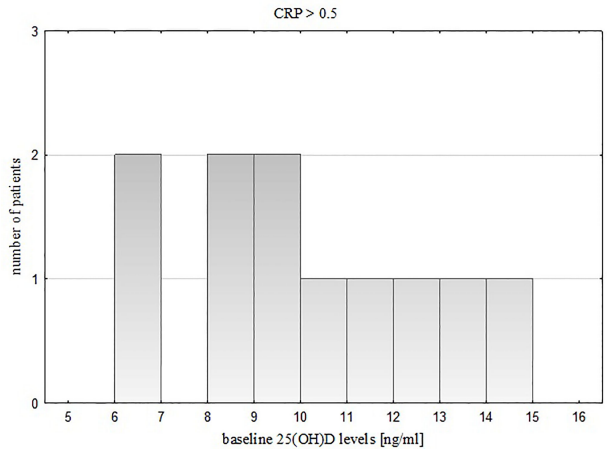


FIGURE 3 | Distribution of vitamin D values in the subgroup with severe 25 (OH)D deficiency and CRP > 0.5.

respectively in subgroups with low and severe baseline vitamin D deficiency).

Effects of Vitamin D Supplementation on Leukocyte Profile Parameters, Platelet Count, CRP, IL-10, and IL-17 Levels in the Study Group

The characteristics of the study group at baseline and after six months of vitamin D supplementation are shown in **Table 2**. After six months of vitamin D supplementation its value increased by an average of 11.3 ± 8.2 ng/ml. Simultaneously, we noticed a significant decrease in CRP levels (p = 0.0003) without any changes in leukocyte profile parameters, PLT count, or IL-10, and IL-17 levels. Serum 25(OH)D levels correlated significantly with IL-10 levels in that period (R = 0.27, p = 0.028). We did not find any significant association between 25(OH)D levels and leukocyte profile parameters, PLT count, or IL-17 levels.

We also noticed that after six months of intervention, IL-10 levels correlated significantly negatively with monocyte count in that period (R = -0.28, p = 0.023).

In further investigation we used multivariable stepwise regression analysis to determine which inflammatory factors are associated with serum 25(OH)D levels (as dependent variable) at baseline and after six months of vitamin D supplementation.

We did not find any significant relationships in multivariable stepwise regression models including baseline 25(OH)D levels and chosen baseline inflammatory parameters as independent variables (model 1: IL-10, IL-17, CRP, WBC; model 2: IL-10, IL-17, CRP, monocytes).

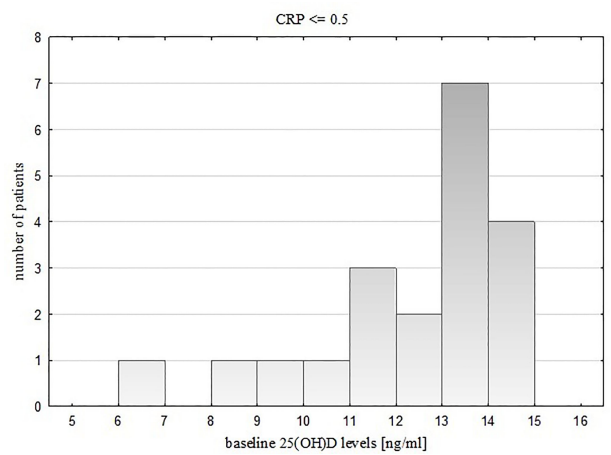


FIGURE 2 | Distribution of vitamin D values in the subgroup with severe 25 (OH)D deficiency and CRP <= 0.5.

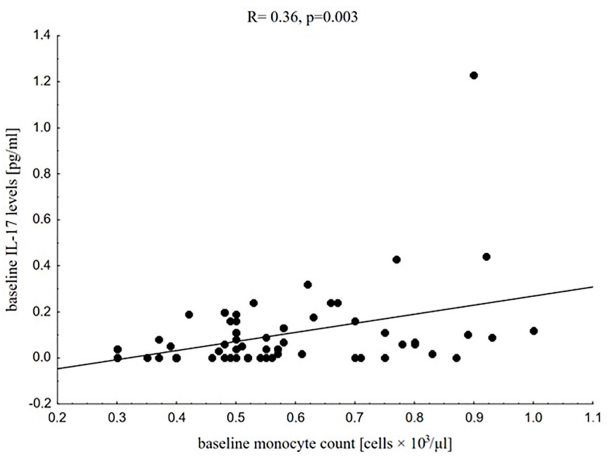


FIGURE 4 | Correlation between baseline IL-17 levels and baseline monocyte count in the whole study group.

TABLE 2 | Comparison between anthropometric measurements, haematological, and biochemical parameters in the study group at baseline and after six months of vitamin D supplementation.

p value	BASELINE	SIX MONTHS
Height SDS ns	0.7 ± 1.26	0.6 ± 1.25
Weight SDS 0.047	2.3 ± 0.66	2.2 ± 0.75
WC (cm) ns	91.3 ± 10.17	90.6 ± 11.06
HC (cm) ns	106.1 ± 10.48	106.4 ± 11.71
WHR ns	0.9 ± 0.06	0.9 ± 0.05
WHtR < 0.001	0.9 ± 2.68	0.5 ± 0.06
% FAT (skinfolds) < 0.001	36.9 ± 6.12	34.2 ± 6.34
% FAT BIA ns	40.6 ± 7.59	38.3 ± 8.93
BMI SDS ns	2.3 ± 0.46	2.2 ± 0.55
25(OH)D (ng/ml) < 0.001	15.9 (12.6 – 20.0)	27.1 (22.9 – 32.6)
WBC (cells × 10^3/μl) ns	6.7 (5.5 – 8.1)	6.2 (5.6 – 7.8)
Granulocytes (cells × 10^3/μl) ns	3.2 (2.4 – 4.5)	3.3 (2.4 – 4.0)
Monocytes (cells × 10^3/μl) ns	0.5 (0.5 – 0.7)	0.5 (0.4 – 0.6)
Lymphocytes (cells × 10^3/μl) ns	2.4 (2.1 – 2.9)	2.3 (2.0 – 2.8)
PLT (cells × 10^3/μl) ns	270 (236 – 302)	261 (232 – 311)
CRP (mg/dl) 0.001	0.5 (0.5 – 0.7)	0.5 (0.5 – 0.6)
IL-10 (pg/ml) ns	1.25 (0.03 – 1.74)	0.78 (0.34 – 1.58)
IL-17 (pg/ml) ns	0.05 (0.0 – 0.13)	0.04 (0.01 – 0.10)

Data are presented as means ± standard deviations score or as median with interquartile range, as appropriate. SDS, standard deviation score; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; %FAT (skinfolds), percentage of body fat estimated from skinfolds; %FAT BIA, percentage of body fat estimated using bioelectrical impedance analysis method; BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; WBC, white blood cells; PLT, platelets; CRP, C-reactive protein; IL-10, interleukin-10; IL-17, interleukin-17; ns, not significant.

After six months of vitamin D supplementation, the multivariable stepwise regression analysis, that included 25 (OH)D levels (as dependent variable) and IL-10, IL-17, CRP, WBC (model 1) or IL-10, IL-17, CRP, monocytes (model 2) as independent variables, identified IL-10 as the parameter significantly positively associated with 25(OH)D levels. Both models were significant with cumulative $R^2 = 0.12$, $p = 0.004$ and the received correlations coefficients were respectively equal $\beta = 0.344 \pm 0.116$.

DISCUSSION

In our study we focused on the relationships between vitamin D status, both baseline and after six months of vitamin D supplementation and selected anti-inflammatory and pro-inflammatory markers. We found significant associations between serum 25(OH)D levels and levels of CRP and IL-10, while leukocyte profile parameters and PLT count, as well as IL-17 levels, seemed not to be vitamin D-dependent. As expected, we confirmed significant negative relationships between 25(OH)D levels and nutritional status parameters. Overweight and obese children from the study group had significantly lower serum baseline 25 (OH)D levels compared to age- and sex-matched healthy peers, despite the lack of vitamin D supplementation in both groups before the initiation of the study. Obesity-dependent hypovitaminosis D has been previously confirmed in many studies in paediatric and adult population (3, 19, 76). Low-grade chronic inflammation characteristics for obese individuals seem to be involved with vitamin D deficiency in this group (11, 12). Analyzing baseline values of selected inflammatory markers in both groups, we noticed that WBC, granulocyte, and monocyte count, as well as CRP levels, were significantly higher in the study group than in the control group. Similar results were presented in our previously published study which considered almost 100 overweight and obese children. Our previous research indicated that WBC and granulocyte count were related to BMI SDS, while monocyte count was related to waist

circumference, which could suggest that visceral adipose tissue has much greater pro-inflammatory potential than subcutaneous tissue, as a source of pro-inflammatory adipokines and cytokines (36).

The main findings of our present study regarded the interactions between vitamin D and serum levels of IL-10 and CRP and could support hypothesis of anti-inflammatory vitamin D properties. Despite this, we did not find any baseline associations between vitamin D status and leukocyte profile parameters, PLT count, CRP, IL-10, and IL-17 levels in the whole study group, and we noticed that in participants with severe baseline vitamin D deficiency (serum 25(OH)D values below 15 ng/ml) 25(OH)D levels were inversely related to CRP values. Moreover, in this subgroup, CRP levels correlated positively with nutritional status parameters. Similar associations were reported by Rodriguez et al. (77) who examined more than one hundred Spanish overweight and obese children from 9 to 12 years of age and reported that low serum 25(OH)D levels were significantly associated with increased high sensitive CRP (hs-CRP). In the study by Bellia et al. (61), based on a cohort of 147 severely obese patients with mean BMI 43.6 ± 4.3 kg/m^2 who were prepared to bariatric surgery, a multivariate regression analysis showed that serum 25 (OH)D was inversely related to hs-CRP levels, even after accounting for age, gender, season of recruitment, BMI, total body fat, and truncal fat mass. The cross-sectional analysis by de Oliveira et al. (78) based on data of 5,870 adult participants from the English Longitudinal Study of Ageing (ELSA) showed an inverse relationship not only between serum levels of 25(OH)D and CRP values but also between 25(OH)D and WBC count. In our analysis WBC count did not depend on vitamin D status but the protocols of our study and the study by de Oliveira et al. (78) were not exactly consistent. The main differences between our study and ELSA included the age of studied groups (children and adolescents in our study vs adult patients 50 years of age and over), the duration of obesity and associated inflammation (possibly much longer in de Oliveira group), differences in the number of participants (67 in our study vs almost 6,000 in ELSA). The study by Palaniswamy et al. (19) based on a cohort of 3,586 individuals with mean BMI 24.8 kg/m2 and mean 25(OH) D levels 50.3 nmol/L also confirmed negative associations between 25(OH)D and hs-CRP levels, which were simultaneously positively associated with BMI. Those findings are strictly in-line with our observations. Conversely, Palaniswamy et al. (19) concluded that their large observational and Mendelian randomization study, which analyzed the associations between 25(OH)D, BMI, and 16 inflammatory biomarkers (including IL-17, IL-1α, IL-1β, IL-4, IL-6, IL-8, TNF-α and hs-CRP), considered together with data from review of randomized controlled trials, did not confirm the beneficial role of vitamin D supplementation in obesity-related inflammation. Similar observations were previously reported by Shea et al. (79) based on data from the Framingham Offspring Study. The lack of association between vitamin D and IL-17 levels is in-line with our findings. Interestingly, we observed baseline positive correlation between IL-17 levels and monocyte count, which was independent from the severity of baseline vitamin D deficiency and could confirm mutual associations between pro-inflammatory interleukins and monocytes enhancing their inflammatory potential.

In our study we also analyzed the impact of vitamin D supplementation on selected markers of inflammation. Our study revealed that six months of vitamin D supplementation led to a significant decrease in CRP levels, influenced IL-10 levels, but did not affect leukocyte profile, PLT count, and IL-17 levels. Interestingly, those effects were observed despite the lack of significant changes in body fat mass and BMI in our study group. Data from animal and human studies regarding the impact of vitamin D supplementation on obesity-related inflammation are contradictory. The rat study by Gomma et al. (80) showed that vitamin D administration in rats that received high fat diet (HFD) led to significant decrease in body weight gain, decrease in serum CRP levels, and significant increase in serum IL-10 levels in comparison with HFD-rats that did not receive vitamin D supplementation. Mirzavandi et al. (81) who investigated the effect of vitamin D intramuscular megadose injections (200 000 IUs at baseline and next at week 4 of intervention) reported significant decrease in CRP levels in vitamin D deficient adults with diabetes mellitus type 2. Similar results, also in patients with diabetes mellitus type 2, were shown by Mousa et al. (55), who provided a systematic review and meta-analysis including twenty trials with a total of 1,270 individuals. The authors reported that vitamin D supplemented patients had lower CRP and TNF-α levels, lower erythrocyte sedimentation rate, and higher leptin levels compared to the control groups. Conversely, a systematic review with meta-analysis by Jamka et al. (82), who assessed changes in 25(OH)D and CRP levels in 1,955 obese and overweight subjects, showed that vitamin D supplementation did not affect CRP levels. Systematic review and meta-analysis of randomized control trials (RCTs) by Mazidi et al. (83) also indicated that vitamin D supplementation had no impact on serum CRP, IL-10, and TNF-α levels but the authors recommend RCTs with longer period of follow-up time (12 months) for future studies to provide explicit results.

Based on our data we noticed that serum 25(OH)D values, achieved after six months of vitamin D supplementation, correlated significantly positively with IL-10 levels in that period. Those relationships found firstly, using the Spearman correlation, was also confirmed in a multivariable stepwise regression analysis taking 25(OH)D levels as dependent variables and IL-10, IL-17, CRP, WBC (model 1), IL-10, IL-17, CRP, and monocytes (model 2) as independent variables. In both models we identified IL-10 levels measured at six months of intervention as the parameter significantly positively associated with 25(OH)D levels. Moreover, we found that IL-10 levels were inversely related to monocyte count also evaluated at six months of vitamin D intake. After six months of vitamin D supplementation, we did not find any relationships between vitamin D status and leukocyte profile parameters, PLT count, or IL-17 levels. The mechanisms of association between vitamin D status and both interleukins and leukocyte profile parameters are not clearly explained and literature data in this field are

insufficient. Hashemi et al. (84), reported that based on a group of multiple sclerosis patients, vitamin D supplementation up-regulates IL-27 and TGF-β1 levels, which in consequence, increases the secretion of anti-inflammatory IL-10 and inhibits pro-inflammatory IL-17 production. The anti-inflammatory role of IL-27 was also reported by other authors (85, 86). However, the precise mechanism of vitamin D-dependent regulation of immune cells function is much more complicated and includes activation of various signalling cascades (2, 17). Most of the presented studies concern the adult population or animals, while the number of studies in the paediatric population is very limited.

In conclusion, our study confirmed beneficial effects of vitamin D supplementation in overweight and obese paediatric population. Vitamin D intake seems to exert its anti-inflammatory effect mainly *via* decreasing of CRP level and protecting stabile values of IL-10, rather than its impact on pro-inflammatory factors such as lL-17 and leukocyte profile parameters.

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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bioethics Committee of Medical University of Warsaw. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MK, EW-S and MR contributed to conception and design of the study. MK and MR organized the database. MK and EW-S prepared the tables. AM performed anthropometric measurements. MK and AS-E took measurements of serum IL-10 and IL-17 levels. MK, MR, EW-S and MS performed statistical analysis. MK and EW-S wrote the manuscript. BP supervised the work. All authors contributed to manuscript revision, read, and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to thank the nurse Małgorzata Przybylska for her contribution in blood sample collection.

REFERENCES

1. Pereira-Santos M, Costa PR, Assis AM, Santos CA, Santos DB. Obesity and Vitamin D Deficiency: A Systematic Review and Meta-Analysis. *Obes Rev* (2015) 16(4):341–9. doi: 10.1111/obr.12239
2. 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 (Lausanne)* (2019) 10:103. doi: 10.3389/fendo.2019.00103
3. Drincic AT, Armas LA, Van Diest EE, Heaney RP. Volumetric Dilution, Rather Than Sequestration Best Explains the Low Vitamin D Status of Obesity. *Obes (Silver Spring)* (2012) 20(7):1444–8. doi: 10.1038/oby.2011.404
4. Vimaleswaran KS, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT, et al. Causal Relationship Between Obesity and Vitamin D Status: Bi-Directional Mendelian Randomization Analysis of Multiple Cohorts. *PLoS Med* (2013) 10(2):e1001383. doi: 10.1371/journal.pmed.1001383
5. Hajhashemy Z, Shahdadian F, Ziaei R, Saneei P. Serum Vitamin D Levels in Relation to Abdominal Obesity: A Systematic Review and Dose-Response Meta-Analysis of Epidemiologic Studies. *Obes Rev* (2021) 22(2):e13134. doi: 10.1111/obr.13134
6. Abbas MA. Physiological Functions of Vitamin D in Adipose Tissue. *J Steroid Biochem Mol Biol* (2017) 165(Pt B):369–81. doi: 10.1016/j.jsbmb.2016.08.004
7. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased Bioavailability of Vitamin D in Obesity. *Am J Clin Nutr* (2000) 72(3):690–3. doi: 10.1093/ajcn/72.3.690
8. Earthman CP, Beckman LM, Masodkar K, Sibley SD. The Link Between Obesity and Low Circulating 25-Hydroxyvitamin D Concentrations: Considerations and Implications. *Int J Obes (Lond)* (2012) 36(3):387–96. doi: 10.1038/ijo.2011.119
9. Saneei P, Salehi-Abargouei A, Esmaillzadeh A. Serum 25-Hydroxy Vitamin D Levels in Relation to Body Mass Index: A Systematic Review and Meta-Analysis. *Obes Rev* (2013) 14(5):393–404. doi: 10.1111/obr.12016
10. Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, et al. Associations Between Serum 25-Hydroxyvitamin D3 Concentrations and Liver Histology in Patients With non-Alcoholic Fatty Liver Disease. *Nutr Metab Cardiovasc Dis* (2007) 17(7):517–24. doi: 10.1016/j.numecd.2006.04.002
11. Cannell JJ, Grant WB, Holick MF. Vitamin D and Inflammation. *Dermatoendocrinol* (2015) 6(1):e983401. doi: 10.4161/19381980.2014.983401
12. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D Status and Ill Health: A Systematic Review. *Lancet Diabetes Endocrinol* (2014) 2(1):76–89. doi: 10.1016/S2213-8587(13)70165-7
13. Brock K, Huang WY, Fraser DR, Ke L, Tseng M, Stolzenberg-Solomon R, et al. Low Vitamin D Status is Associated With Physical Inactivity, Obesity and Low Vitamin D Intake in a Large US Sample of Healthy Middle-Aged Men and Women. *J Steroid Biochem Mol Biol* (2010) 121(1-2):462–6. doi: 10.1016/j.jsbmb.2010.03.091
14. NCD Risk Factor Collaboration (NCD-RisC). 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(10113):2627–42. doi: 10.1016/S0140-6736(17)32129-3
15. Mazur J, Malkowska-Szkutnik A, Dzielska A, Oblacinska A, Bójko M, Izdebski Z, et al. Rozwój Fizyczny I Samoocena Masy Ciała". In: *Zdrowie Uczniów W 2018 Roku Na Tle Nowego Modelu Badań HBSC*. Warszawa: Instytut Matki i Dziecka (2018). 75–6.
16. 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(12):986–1004. doi: 10.1016/S2213-8587(17)30357-1
17. Todosenko N, Vulf M, Yurova K, Khaziakhmatova O, Mikhailova L, Litvinova L. Causal Links Between Hypovitaminosis D and Dysregulation of the T Cell Connection of Immunity Associated With Obesity and Concomitant Pathologies. *Biomedicines* (2021) 9(12):1750. doi: 10.3390/biomedicines9121750
18. Han S, Sun HM, Hwang KC, Kim SW. Adipose-Derived Stromal Vascular Fraction Cells: Update on Clinical Utility and Efficacy. *Crit Rev Eukaryot Gene Expr* (2015) 25(2):145–52. doi: 10.1615/critreveukaryotgeneexpr.2015013057
19. Palaniswamy S, Gill D, De Silva NM, Lowry E, Jokelainen J, Karhu T, et al. Could Vitamin D Reduce Obesity-Associated Inflammation? Observational and Mendelian Randomization Study. *Am J Clin Nutr* (2020) 111(5):1036–47. doi: 10.1093/ajcn/nqaa056
20. Rodríguez-Hernández H, Simental-Mendía LE, Rodríguez-Ramírez G, Reyes-Romero MA. Obesity and Inflammation: Epidemiology, Risk Factors, and

Markers of Inflammation. *Int J Endocrinol* (2013) 2013:678159. doi: 10.1155/2013/678159
21. Lumeng CN, Saltiel AR. Inflammatory Links Between Obesity and Metabolic Disease. *J Clin Invest* (2011) 121(6):2111–7. doi: 10.1172/JCI57132
22. Liu R, Nikolajczyk BS. Tissue Immune Cells Fuel Obesity-Associated Inflammation in Adipose Tissue and Beyond. *Front Immunol* (2019) 10:1587. doi: 10.3389/fimmu.2019.01587
23. Klöting N, Blüher M. Adipocyte Dysfunction, Inflammation and Metabolic Syndrome. *Rev Endocr Metab Disord* (2014) 15(4):277–87. doi: 10.1007/s11154-014-9301-0
24. Zhao RX, He Q, Sha S, Song J, Qin J, Liu P, et al. Increased AHR Transcripts Correlate With Pro-Inflammatory T-Helper Lymphocytes Polarization in Both Metabolically Healthy Obesity and Type 2 Diabetic Patients. *Front Immunol* (2020) 11:1644. doi: 10.3389/fimmu.2020.01644
25. Tilg H, Moschen AR. Adipocytokines: Mediators Linking Adipose Tissue, Inflammation and Immunity. *Nat Rev Immunol* (2006) 6(10):772–83. doi: 10.1038/nri1937
26. Klöting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, Kern M, et al. Insulin-Sensitive Obesity. *Am J Physiol Endocrinol Metab* (2010) 299(3):E506–15. doi: 10.1152/ajpendo.00586.2009
27. Monteiro R, Azevedo I. Chronic Inflammation in Obesity and the Metabolic Syndrome. *Mediators Inflammation* (2010) 2010:289645. doi: 10.1155/2010/289645
28. Rochlani Y, Pothineni NV, Kovelamudi S, Mehta JL. Metabolic Syndrome: Pathophysiology, Management, and Modulation by Natural Compounds. *Ther Adv Cardiovasc Dis* (2017) 11(8):215–25. doi: 10.1177/1753944717711379
29. Wellen KE, Hotamisligil GS. Inflammation, Stress, and Diabetes. *J Clin Invest* (2005) 115(5):1111–9. doi: 10.1172/JCI25102
30. Gabay C, Kushner I. Acute-Phase Proteins and Other Systemic Responses to Inflammation. *N Engl J Med* (1999) 340(6):448–54. doi: 10.1056/NEJM199902113400607Erratumin:NEnglJMed(1999)29;340(17):1376
31. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, Obesity, Stress and Coronary Heart Disease: Is Interleukin-6 the Link? *Atherosclerosis* (2000) 148(2):209–14. doi: 10.1016/s0021-9150(99)00463-3
32. Suwa T, Hogg JC, English D, Van Eeden SF. Interleukin-6 Induces Demargination of Intravascular Neutrophils and Shortens Their Transit in Marrow. *Am J Physiol Heart Circ Physiol* (2000) 279(6):H2954–60. doi: 10.1152/ajpheart.2000.279.6.H2954
33. Yoshimura A, Ohnishi S, Orito C, Kawahara Y, Takasaki H, Takeda H, et al. Association of Peripheral Total and Differential Leukocyte Counts With Obesity-Related Complications in Young Adults. *Obes Facts* (2015) 8(1):1–16. doi: 10.1159/000373881
34. Zaldivar F, McMurray RG, Nemet D, Galassetti P, Mills PJ, Cooper DM. Body Fat and Circulating Leukocytes in Children. *Int J Obes (Lond)* (2006) 30(6):906–11. doi: 10.1038/sj.ijo.0803227
35. Oliveira TM, de Faria FR, de Faria ER, Pereira PF, Franceschini SC, Priore SE. Nutritional Status, Metabolic Changes and White Blood Cells in Adolescents. *Rev Paul Pediatr* (2014) 32(4):351–9. doi: 10.1590/S0103-05822014000400011
36. Rumińska M, Witkowska-Sędek E, Artemniak-Wojtowicz D, Krajewska M, Majcher A, Sobol M, et al. Changes in Leukocyte Profile and C-Reactive Protein Concentration in Overweight and Obese Adolescents After Reduction of Body Weight. *Cent Eur J Immunol* (2019) 44(3):307–15. doi: 10.5114/ceji.2019.89608
37. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-Dihydroxyvitamin D(3) Receptor in the Immune System. *Arch Biochem Biophys* (2000) 374(2):334–8. doi: 10.1006/abbi.1999.1605
38. Bendix M, Dige A, Deleuran B, Dahlerup JF, Jørgensen SP, Bartels LE, et al. Flow Cytometry Detection of Vitamin D Receptor Changes During Vitamin D Treatment in Crohn's Disease. *Clin Exp Immunol* (2015) 181(1):19–28. doi: 10.1111/cei.12613
39. Ding C, Gao D, Wilding J, Trayhurn P, Bing C. Vitamin D Signalling in Adipose Tissue. *Br J Nutr* (2012) 108(11):1915–23. doi: 10.1017/S0007114512003285
40. Wang Y, Zhu J, DeLuca HF. Where Is the Vitamin D Receptor? *Arch Biochem Biophys* (2012) 523(1):123–33. doi: 10.1016/j.abb.2012.04.001
41. Stumpf WE, Sar M, Clark SA, DeLuca HF. Brain Target Sites for 1,25-Dihydroxyvitamin D3. *Science* (1982) 215(4538):1403–5. doi: 10.1126/science.6977846
42. Gascon-Barre M, Huet PM. Apparent [3 H]1,25-Dihydroxyvitamin D3 Uptake by Canine and Rodent Brain. *Am J Physiol* (1983) 244.3:E266–71. doi: 10.1152/ajpendo.1983.244.3.E266
43. El-Hajj Fuleihan G. Can the Sunshine Vitamin Melt the Fat? *Metabolism* (2012) 61(5):603–10. doi: 10.1016/j.metabol.2011.12.006
44. Dupuis ML, Pagano MT, Pierdominici M, Ortona E. The Role of Vitamin D in Autoimmune Diseases: Could Sex Make the Difference? *Biol Sex Differ* (2021) 12(1):12. doi: 10.1186/s13293-021-00358-3
45. Cantorna MT, Snyder L, Lin YD, Yang L. Vitamin D and 1,25(OH)2D Regulation of T Cells. *Nutrients* (2015) 7(4):3011–21. doi: 10.3390/nu7043011
46. Zhou Q, Qin S, Zhang J, Zhon L, Pen Z, Xing T. 1,25(OH)2D3 Induces Regulatory T Cell Differentiation by Influencing the VDR/PLC-γ1/TGF-β1/ Pathway. *Mol Immunol* (2017) 91:156–64. doi: 10.1016/j.molimm.2017.09.006
47. Guo J, Ma Z, Ma Q, Wu Z, Fan P, Zhou X, et al. 1, 25$(OH)_2D_3$ Inhibits Hepatocellular Carcinoma Development Through Reducing Secretion of Inflammatory Cytokines From Immunocytes. *Curr Med Chem* (2013) 20(33):4131–41. doi: 10.2174/09298673113209990248
48. Korf H, Wenes M, Stijlemans B, Takiishi T, Robert S, Miani M, et al. 1,25-Dihydroxyvitamin D3 Curtails the Inflammatory and T Cell Stimulatory Capacity of Macrophages Through an IL-10-Dependent Mechanism. *Immunobiology* (2012) 217(12):1292–300. doi: 10.1016/j.imbio.2012.07.018
49. Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G, Boissier MC. Vitamin D and Inflammation. *Joint Bone Spine* (2010) 77(6):552–7. doi: 10.1016/j.jbspin.2010.09.018
50. Jeffery LE, Henley P, Marium N, Filer A, Sansom DM, Hewison M, et al. Decreased Sensitivity to 1,25-Dihydroxyvitamin D3 in T Cells From the Rheumatoid Joint. *J Autoimmun* (2018) 88:50–60. doi: 10.1016/j.jaut.2017.10.001
51. Soto JR, Anthias C, Madrigal A, Snowden JA. Insights Into the Role of Vitamin D as a Biomarker in Stem Cell Transplantation. *Front Immunol* (2020) 11:966. doi: 10.3389/fimmu.2020.00966
52. Colin EM, Asmawidjaja PS, van Hamburg JP, Mus AM, van Driel M, Hazes JM, et al. 1,25-Dihydroxyvitamin D3 Modulates Th17 Polarization and Interleukin-22 Expression by Memory T Cells From Patients With Early Rheumatoid Arthritis. *Arthritis Rheum* (2010) 62(1):132–42. doi: 10.1002/art.25043
53. Palmer MT, Lee YK, Maynard CL, Oliver JR, Bikle DD, Jetten AM, et al. Lineage-Specific Effects of 1,25-Dihydroxyvitamin D(3) on the Development of Effector CD4 T Cells. *J Biol Chem* (2011) 286(2):997–1004. doi: 10.1074/jbc.M110.163790
54. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. *Physiol Rev* (2016) 96(1):365–408. doi: 10.1152/physrev.00014.2015
55. Mousa A, Naderpoor N, Teede H, Scragg R, de Courten B. Vitamin D Supplementation for Improvement of Chronic Low-Grade Inflammation in Patients With Type 2 Diabetes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutr Rev* (2018) 76(5):380–94. doi: 10.1093/nutrit/nux077
56. Asemi Z, Raygan F, Bahmani F, Rezavandi Z, Talari HR, Rafiee M, et al. The Effects of Vitamin D, K and Calcium Co-Supplementation on Carotid Intima-Media Thickness and Metabolic Status in Overweight Type 2 Diabetic Patients With CHD. *Br J Nutr* (2016) 116(2):286–93. doi: 10.1017/S0007114516001847
57. Farrokhian A, Raygan F, Bahmani F, Talari HR, Esfandiari R, Esmaillzadeh A, et al. Long-Term Vitamin D Supplementation Affects Metabolic Status in Vitamin D-Deficient Type 2 Diabetic Patients With Coronary Artery Disease. *J Nutr* (2017) 147(3):384–9. doi: 10.3945/jn.116.242008
58. Kampmann U, Mosekilde L, Juhl C, Moller N, Christensen B, Rejnmark L, et al. Effects of 12 Weeks High Dose Vitamin D3 Treatment on Insulin Sensitivity, Beta Cell Function, and Metabolic Markers in Patients With Type 2 Diabetes and Vitamin D Insufficiency - A Double-Blind, Randomized, Placebo-Controlled Trial. *Metabolism* (2014) 63(9):1115–24. doi: 10.1016/j.metabol.2014.06.008
59. Sadiya A, Ahmed SM, Carlsson M, Tesfa Y, George M, Ali SH, et al. Vitamin D Supplementation in Obese Type 2 Diabetes Subjects in Ajman, UAE: A Randomized Controlled Double-Blinded Clinical Trial. *Eur J Clin Nutr* (2015) 69(6):707–11. doi: 10.1038/ejcn.2014.251
60. Amer M, Qayyum R. Relation Between Serum 25-Hydroxyvitamin D and C-Reactive Protein in Asymptomatic Adults (From the Continuous National

Health and Nutrition Examination Survey 2001 to 2006). *Am J Cardiol* (2012) 109(2):226–30. doi: 10.1016/j.amjcard.2011.08.032
61. Bellia A, Garcovich C, D'Adamo M, Lombardo M, Tesauro M, Donadel G, et al. Serum 25-Hydroxyvitamin D Levels are Inversely Associated With Systemic Inflammation in Severe Obese Subjects. *Intern Emerg Med* (2013) 8 (1):33–40. doi: 10.1007/s11739-011-0559-x
62. Soliman AT, Eldabbagh M, Elawwa A, Ashour R, Saleem W. The Effect of Vitamin D Therapy on Hematological Indices in Children With Vitamin D Deficiency. *J Trop Pediatr* (2012) 58(6):523–4. doi: 10.1093/tropej/fms020
63. Olmuşçelik O, Sevindik ÖG. Correlation Between Serum Vitamin D Level and Dichotomous Distribution of Hematological Parameters in a Cohort of 12709 Patients. *Turk J Med Sci* (2020) 50(8):1941–50. doi: 10.3906/sag-2008-124
64. Zupo R, Castellana F, Sardone R, Lampignano L, Di Noia C, Savastano S, et al. Hydroxyvitamin D Serum Levels Are Negatively Associated With Platelet Number in a Cohort of Subjects Affected by Overweight and Obesity. *Nutrients* (2020) 12(2):474. doi: 10.3390/nu12020474
65. Bella LM, Fieri I, Tessaro FHG, Nolasco EL, Nunes FPB, Ferreira SS, et al. Vitamin D Modulates Hematological Parameters and Cell Migration Into Peritoneal and Pulmonary Cavities in Alloxan-Diabetic Mice. *BioMed Res Int* (2017) 2017:7651815. doi: 10.1155/2017/7651815
66. Lang E, Jilani K, Bissinger R, Rexhepaj R, Zelenak C, Lupescu A, et al. Vitamin D-Rich Diet in Mice Modulates Erythrocyte Survival. *Kidney Blood Press Res* (2015) 40(4):403–12. doi: 10.1159/000368517
67. Sim JJ, Lac PT, Liu IL, Meguerditchian SO, Kumar VA, Kujubu DA, et al. Vitamin D Deficiency and Anemia: A Cross-Sectional Study. *Ann Hematol* (2010) 89(5):447–52. doi: 10.1007/s00277-009-0850-3
68. Uwaezuoke SN. Vitamin D Deficiency and Anemia Risk in Children: A Review of Emerging Evidence. *Pediatr Health Med Ther* (2017) 8:47–55. doi: 10.2147/PHMT.S129362
69. Mousa H, Islam N, Ganji V, Zughaier SM. Serum 25-Hydroxyvitamin D Is Inversely Associated With Monocyte Percentage to HDL Cholesterol Ratio Among Young Healthy Adults in Qatar. *Nutrients* (2020) 13(1):127. doi: 10.3390/nu13010127
70. Tay HM, Yeap WH, Dalan R, Wong SC, Hou HW. Increased Monocyte-Platelet Aggregates and Monocyte-Endothelial Adhesion in Healthy Individuals With Vitamin D Deficiency. *FASEB J* (2020) 34(8):11133–42. doi: 10.1096/fj.202000822R
71. Rusińska A, Płudowski P, Walczak M, Borszewska-Kornacka MK, Bossowski A, Chlebna-Sokół D, et al. Vitamin D Supplementation Guidelines for General Population and Groups at Risk of Vitamin D Deficiency in Poland-Recommendations of the Polish Society of Pediatric Endocrinology and Diabetes and the Expert Panel With Participation of National Specialist Consultants and Representatives of Scientific Societies-2018 Update. *Front Endocrinol (Lausanne)* (2018) 9:246. doi: 10.3389/fendo.2018.00246
72. Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, et al. Skinfold Equations for Estimation of Body Fatness in Children and Youth. *Hum Biol* (1988) 60(5):709–23.
73. Kułaga Z, Litwin M, Tkaczyk M, Palczewska I, Zajączkowska M, Zwolińska D, et al. Polish 2010 Growth References for School-Aged Children and Adolescents. *Eur J Pediatr* (2011) 170(5):599–609. doi: 10.1007/s00431-010-1329-x
74. Cole TJ. The LMS Method for Constructing Normalized Growth Standards. *Eur J Clin Nutr* (1990) 44(1):45–60.
75. WHO Expert Committee. Physical Status: The Use and Interpretation of Anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* (1995) 854:1–452.
76. Fiamenghi VI, Mello ED. Vitamin D Deficiency in Children and Adolescents With Obesity: A Meta-Analysis. *J Pediatr (Rio J)* (2021) 97(3):273–9. doi: 10.1016/j.jped.2020.08.006
77. Rodríguez-Rodríguez E, Aparicio A, Andrés P, Ortega RM. Moderate Vitamin D Deficiency and Inflammation Related Markers in Overweight/Obese Schoolchildren. *Int J Vitam Nutr Res* (2014) 84(1-2):98–107. doi: 10.1024/0300-9831/a000197
78. de Oliveira C, Biddulph JP, Hirani V, Schneider IJC. Vitamin D and Inflammatory Markers: Cross-Sectional Analyses Using Data From the English Longitudinal Study of Ageing (ELSA). *J Nutr Sci* (2017) 6:e1. doi: 10.1017/jns.2016.37
79. Shea MK, Booth SL, Massaro JM, Jacques PF, D'Agostino RBSr, Dawson-Hughes B, et al. Vitamin K and Vitamin D Status: Associations With Inflammatory Markers in the Framingham Offspring Study. *Am J Epidemiol* (2008) 167(3):313–20. doi: 10.1093/aje/kwm306
80. Gomaa AM, El-Aziz EA. Vitamin D Reduces High-Fat Diet Induced Weight Gain and C-Reactive Protein, Increases Interleukin-10, and Reduces CD86 and Caspase-3. *Pathophysiology* (2017) 24(1):31–7. doi: 10.1016/j.pathophys.2017.01.003
81. Mirzavandi F, Talenezhad N, Razmpoosh E, Nadjarzadeh A, Mozaffari-Khosravi H. The Effect of Intramuscular Megadose of Vitamin D Injections on E-Selectin, CRP and Biochemical Parameters in Vitamin D-Deficient Patients With Type-2 Diabetes Mellitus: A Randomized Controlled Trial. *Complement Ther Med* (2020) 49:102346. doi: 10.1016/j.ctim.2020.102346
82. Jamka M, Woźniewicz M, Walkowiak J, Bogdański P, Jeszka J, Stelmach-Mardas M. The Effect of Vitamin D Supplementation on Selected Inflammatory Biomarkers in Obese and Overweight Subjects: A Systematic Review With Meta-Analysis. *Eur J Nutr* (2016) 55(6):2163–76. doi: 10.1007/s00394-015-1089-5
83. Mazidi M, Rezaie P, Vatanparast H. Impact of Vitamin D Supplementation on C-Reactive Protein; A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *BMC Nutr* (2018) 4:1. doi: 10.1186/s40795-017-0207-6
84. 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
85. Fitzgerald DC, Zhang GX, El-Behi M, Fonseca-Kelly Z, Li H, Yu S, et al. Suppression of Autoimmune Inflammation of the Central Nervous System by Interleukin 10 Secreted by Interleukin 27-Stimulated T Cells. *Nat Immunol* (2007) 8(12):1372–9. doi: 10.1038/ni1540
86. Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, et al. A Dominant Function for Interleukin 27 in Generating Interleukin 10-Producing Anti-Inflammatory T Cells. *Nat Immunol* (2007) 8(12):1380–9. doi: 10.1038/ni1541

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.

frontiers | Frontiers in Endocrinology

ORIGINAL RESEARCH
published: 06 July 2022
doi: 10.3389/fendo.2022.917402

Changes of Peripheral Th17 Cells Subset in Overweight and Obese Children After Body Weight Reduction

Dorota Artemniak-Wojtowicz[1*], *Anna M. Kucharska*[1*], *Anna Stelmaszczyk-Emmel*[2], *Anna Majcher*[1] *and Beata Pyrżak*[1]

[1] Department of Pediatrics and Endocrinology, Medical University of Warsaw, Warsaw, Poland, [2] Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Warsaw, Poland

OPEN ACCESS

Edited by:
Aneta Monika Gawlik, Medical University of Silesia, Poland

Reviewed by:
Joanna M Oswiecimska, Jan Grodek State Vocational Academy in Sanok, Poland
Barbara Glowinska-Olszewska, Medical University of Bialystok, Poland
Suraiya Begum, Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh

***Correspondence:**
Dorota Artemniak-Wojtowicz
dartemniak@wum.edu.pl
Anna M. Kucharska
ankucharska@wum.edu.pl

Specialty section:
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

Received: 11 April 2022
Accepted: 06 June 2022
Published: 06 July 2022

Citation:
Artemniak-Wojtowicz D, Kucharska AM, Stelmaszczyk-Emmel A, Majcher A and Pyrżak B (2022) Changes of Peripheral Th17 Cells Subset in Overweight and Obese Children After Body Weight Reduction. Front. Endocrinol. 13:917402. doi: 10.3389/fendo.2022.917402

Background: Obesity has been a growing problem in young patients leading to serious metabolic complications. There are many studies supporting the idea, that obesity should be considered as a chronic inflammation closely associated with immune system alterations. Th17 subpopulation is strongly involved in this process. The aim of our study was to evaluate circulating Th17 cells in overweight and obese children and explore the relationships between Th17 subset and metabolic parameters.

Methods: We evaluated peripheral Th17 cells in fresh peripheral blood samples from 27 overweight and obese and 15 normal-weight children. Th17 cells were identified by flow cytometry using monoclonal antibody and intracellular IL-17A staining. Th17 cells were defined as $CD3^{+}CD4^{+}CD196^{+}IL\text{-}17A^{ic+}$. The analysis involved anthropometric and metabolic parameters measured at baseline and three months after the change of lifestyle and diet. We evaluated the relationship between metabolic parameters and Th17 cells.

Results: In overweight and obese children we found significantly higher Th17 cells percentage compared to normal weight controls (median 0.097% (0.044 - 0.289) vs 0.041% (0.023 - 0.099), p = 0.048). The percentage of Th17 cells decreased statistically significantly in children who reduced weight after the intervention (0.210% (0.143 - 0.315) vs 0.039% (0.028 - 0.106), p = 0.004). In this group we also noticed statistically significant reduction of TC and LDL-C concentration (p = 0.01, p = 0.04, respectively).

Conclusions: Obesity in children is associated with increased percentage of peripheral Th17 cells. Weight reduction leads to significant decrease of circulating Th17 cells and improvement of lipid parameters. This significant reduction of proinflammatory Th17 cells is a promising finding suggesting that obesity-induced inflammation in children could be relatively easily reversible.

Keywords: children, obesity, metabolic complication, Th17 cells, inflammation

INTRODUCTION

In recent decades obesity has become a global epidemic (1). This is also an increasing problem in younger and younger patients (2), leading to pathological changes. In the light of recent data, adipose tissue (AT) has been considered as an active organ involved in numerous immunological, hormonal and metabolic processes (3). Obesity should be recognized as a factor contributing to the development of generalized low-grade sterile chronic inflammation, closely associated with morphological and functional changes in AT. These alterations include the colonization of AT by increased number of macrophages with its phenotypic switch from M2 to M1, infiltration of mast cells and neutrophils, imbalance between Th17 cells and Treg (4, 5) as well as tissue hypoxia and oxidative stress (6). Just as long-term obesity leads to fixation of structural changes in adipose tissue, as obesity-induced chronic inflammation also may cause difficult to reverse metabolic implications (3), however the mechanisms of this phenomena are still not fully explained. Many studies presented the role of obesity-induced inflammation in the development of such conditions as insulin resistance (IR), type 2 diabetes (T2DM), bronchial asthma, inflammatory bowel disease, rheumatoid arthritis, psoriasis and certain types of cancer (7–9).

Since 2005, when the Th17 lymphocytes subpopulation was described for the first time, it has been attributed to the inflammatory processes (10, 11). Their contribution in the antibacterial and antifungal immunity (7, 12) as well as their influence on the development of autoimmune and allergic disorders has been well documented (13, 14).

Th17 cells are characterized by expression of retinoid acid receptor (RAR)-related orphan receptor C (RORC) – master transcription factor. RORC is essential for the differentiation of naive Th cells into Th17 subset and important in producing Th17 cells' cytokines, whose signature product is interleukin (IL)-17A and IL-17F (15–17).

The Th17 lymphocytes could be the important link between obesity and inflammation (8). An increased number of Th17 cells in the spleen and AT was found in animal models of obesity (8, 18). Likewise, increased Th17 cells frequency was found in peripheral blood in humans with obesity and diabetes mellitus (19).

The aim of our study was to evaluate alterations of peripheral Th17 cells subset in overweight and obese children before and after weight reduction and to explore its potential metabolic implications.

MATERIALS AND METHODS

The study was performed in the Department of Pediatrics and Endocrinology of Medical University of Warsaw, between August 2016 and October 2020, and was approved by the Bioethics Committee at the Medical University of Warsaw. Written informed consent was obtained from parents and simultaneously from patients older than 16 years.

27 children: 7 overweight and 20 obese, between 8 to 18 years of age (mean age 12.76 years, range 8.33 - 17.58) were enrolled to the study: 12 girls and 15 boys. The control group consisted of 15 normal weight children age-matched (7 girls and 8 boys). The study group of children had a median body mass index standard deviation score (BMI SDS) of 2.3 (1.9 - 2.6) in comparison to 0.1 (-0.5 - 0.3) in the control group ($p < 0.005$). Almost in 89% of overweight and obese children, their WHtR met the criteria of abdominal obesity. The patient's anthropometric data are shown in **Table 1**.

Obesity was defined by BMI > +2 SDS based on a nationally representative group of children aged 3-18 years (20). Overweight was defined by BMI between +1 to +1.9 SDS. Secondary obesity due to central nervous system diseases, hormonal or genetic disorders was the criterion of exclusion.

The analysis involved parameters measured at baseline and three months after the change of lifestyle and diet. The baseline visit included a dietary intervention performed by a dietician, who recommended: 5 meals a day at intervals of 2.5-3 h, no sweet drinks, reduction of simple sugars, meals with a low glycemic index. The energy supply was appropriate for sex, age and physical activity. Additionally, a minimum of 60 minutes of physical activity was recommended daily.

A control group consisted of children with normal somatic parameters (BMI < +1SDS) who were age- and sex-matched.

All blood samples were obtained by peripheral venipuncture in the morning after an overnight fasting. In the study group: at baseline and after three months of intervention and in the control group only at baseline.

In all children the allergy, hematological or chronic disease and symptoms of acute infection were excluded by medical history and physical examination.

TABLE 1 | Characteristic of anthropometric measurements in overweight and obese children (study group) and normal weight children (control group).

Variable	Study group (n = 27)	Control group (n = 15)	*p*-value
BMI (kg/m^2)	30.2 (27.2 - 34)	18.6 (16.5 - 20.2)	<0.001
BMI SDS	2.3 (1.9 - 2.6)	0.1 (-0.5 - 0.3)	<0.001
WC (cm)	94.4 ± 13.94	63.91 ± 3.36	<0.001
WC SDS	3.7 (2.6 - 4.4)	-0.3 (-0.7 - 0.1)	<0.001
HC (cm)	106.00 (95.00 - 109.00)	80.00 (74.00 - 87.00)	<0.001
WHR	0.88 ± 0.055	0.80 ± 0.05	<0.001
WHtR	0.56 (0.53 - 0.6)	0.42 (0.40 - 0.44)	<0.001

Data are presented as median values with interquartile range as appropriate or mean ± standard deviation (SD); BMI - body mass index, BMI SDS - body mass index standard deviation score, WC - waist circumference, HC - hip circumference, WHR - waist-to-hip ratio, WHtR - waist-to-height ratio. A $p < 0.05$ was considered significant.

Laboratory Methods

Flow Cytometry

50µl of fresh whole blood was stained with 5 µl of monoclonal antibodies (according to manufacturer's instructions, Becton Dickinson Biosciences): anti-CD3 APC-H7; anti-CD4 PE-Cy7; anti-CD196 APC (CCR6) (Becton Dickinson, Franklin Lakes, NJ, USA). The samples were incubated for 20 minutes in the dark at room temperature. Next, the cells were incubated 15 minutes in 100µl of permeabilization buffer- IntraPrep Permabilization Reagent 1(Immunotech SAS, Beckman Coulter Company, 13276 Marseille Cedex 9, France) in room temperature in the dark. Then, cells were washed in a washing buffer (0.9% NaCl) for 5 minutes, 500 g. Afterwards, cells were incubated 5 minutes in room temperature in the dark with 100µl of permeabilization buffer-IntraPrep Permabilization Reagent 2 (Immunotech SAS, Beckman Coulter Company, 13276 Marseille Cedex 9, France), next washed in washing buffer, centrifuged at 500g for 5 minutes. Subsequently, the cells were stained with 20µl anti-IL-17A PE monoclonal antibody for 20 minutes in room temperature in the dark and washed. Cells were stored in room temperature before analysis. For staining procedures appropriate isotype-matched controls were used.

Flow cytometry was performed on FACS Canto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) using BD FACS Diva 8.0.1 software. Gates were preset and the measurements were performed blinded for sample identity. Th17 cells were defined as $CD3^{+}CD4^{+}CD196^{+}$ expressing intracellularly interleukin-17A ($CD3^{+}CD4^{+}CD196^{+}IL\text{-}17A^{ic+}$). The number of Th 17 cells was expressed as a percentage values.

Representative flow cytometry analyses of Th17 cells, with gating algorithms, are shown in the **Figure 1**.

Biochemical Parameters

Blood samples were analyzed by standard methods.

Concentration of C-reactive protein (CRP) (mg/dl) was determined using a fix-point immune-rate method, on the Vitros 5600 analyzer (Ortho Clinical Diagnostic, Raritan, New Jersey, USA).

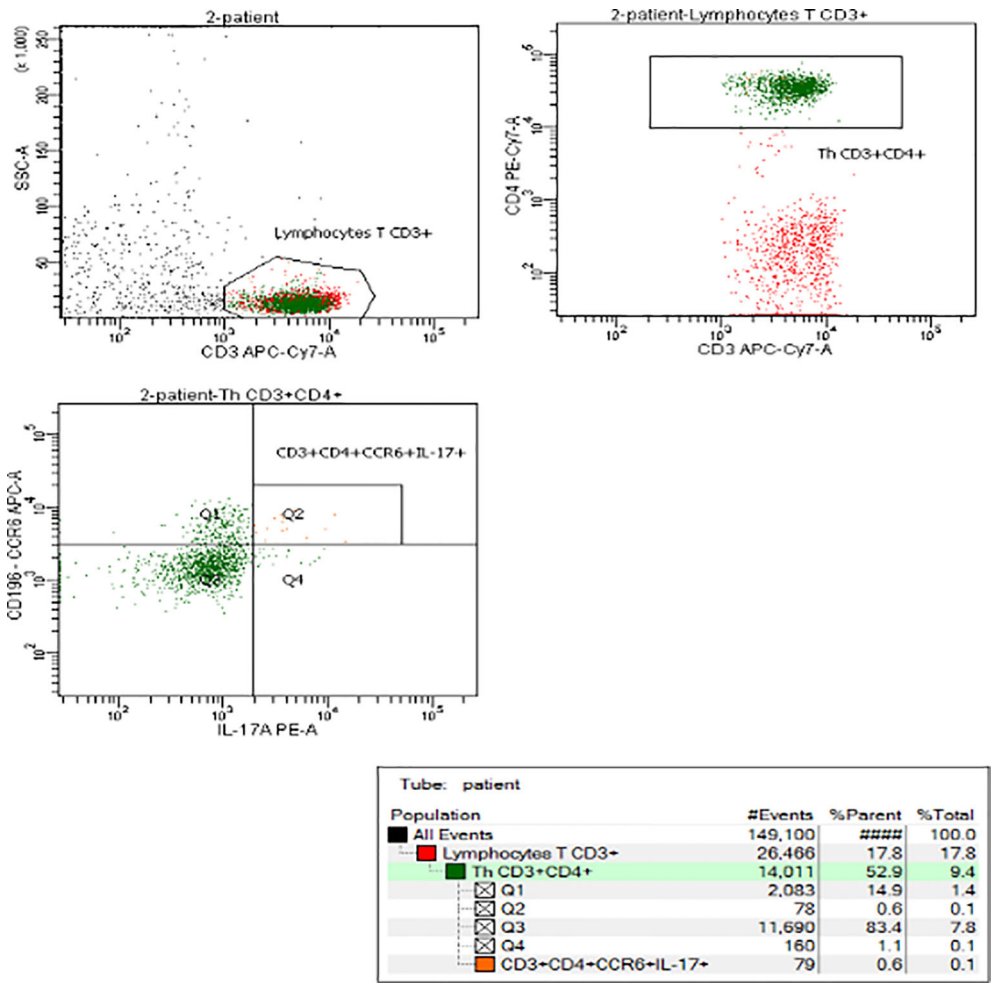


FIGURE 1 | Representative flow cytometry analysis of Th17 cells in obese child.

In obese and overweight's children the Oral Glucose Tolerance Test (OGTT) (1.75 g of glucose/kg body weight, no more than 75 g) with insulin level evaluation was performed after an overnight fasting. Blood samples were taken at 0-, 30-, 60-, 90- and 120-min. Glycated hemoglobin (HbA1c) was also measured. Blood tests in control group included fasting glucose, insulin concentration and HbA1c. The concentration glucose was measured by glucose oxidase colorimetric method using Vitros 5600 analyzer (Ortho Clinical Diagnostic, Raritan, New Jersey, USA). Insulin values were measured in serum by immunoassay using IMMULITE 2000 Xpi Analyzer (Siemens, Erlangen, Germany). HbA1c was measured in whole blood by ion-exchange high-performance liquid chromatography (HPLC) using D-10 Hemoglobin Analyzer (BIO-RAD, California, USA). The values of fasting glucose, insulin and HbA1c were compared to the normal ranges recommended for healthy children. Fasting insulin level ≥ 15 μIU/ml was considered elevated (21). Homeostasis model assessment insulin resistance index (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were calculated based on concentrations of fasting glucose (mg/dl) and fasting insulin (μIU/ml) (22) and have been established as an indicator of IR. The HOMA-IR was calculated as follows: HOMA-IR= [glucose (mmol/l) x insulin (μIU/ml)]/22.5, glucose conversion factor: mmol/l=mg/dl x 0.05551. The QUICKI was calculated as follows: QUICKI=1/[log insulin (μIU/ml) + log glucose (mg/dl). Glucose metabolism and lipid profile parameters were evaluated based on the Polish Diabetes Association' (PTD, 2021) (23) and the Polish Society of Laboratory Diagnostics (PSLD,2020) and the Polish Lipid Association (PoLA,2020) recommendations (24).

Lipid profile: total cholesterol (TC mg/dl), triglycerides (TG, mg/dl), and high-density lipoprotein cholesterol (HDL-C, mg/dl) concentrations were measured by the colorimetric enzymatic method using the Vitros 5600 analyzer (Ortho Clinical Diagnostic, Raritan, New Jersey, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula [LDL-C= TC-(HDL+TG/5)]. The TG: HDL-C ratio were calculated; the value ≥ 3 (25) were consisted as closely correlated with IR.

Aspartate and alanine aminotransferase activity (AST and ALT) was measured by dry chemistry method using Vitros 5600 analyzer (Ortho Clinical Diagnostic, Raritan, New Jersey, USA).

Anthropometry

Body weight (kg) was measured by means of medical scales to the nearest 0,1 kg, height (cm)- using a stadiometer (Holtain Limited) to the nearest 0,1 cm. Waist and hip circumferences were measured by a flexible measuring tape, according to WHO recommendations (26). Waist circumference (cm) was measured midway between the 10^{th} rib and the top of the iliac crest. Height, weight and waist circumferences were normalized for calendar age according to polish national references- the OLAF project (20, 27). Based on these measurements the waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were calculated. WHtR exceeding 0.5 was assumed to define abdominal obesity (28). BMI was calculated: weight (kg) divided by height in square meters (m^2); BMI has been standardized with OLAF norms using the least mean square method (LMS) and converted as BMI in SDS. Anthropometric measurements were taken by one anthropologist, patient was wearing only underwear, standing in the anthropometric position.

Data Analysis

Detailed statistical calculations were performed using SPPS 13.3 software. Shapiro-Wilk test was used to check the normality of distribution. Data were presented as means and standard deviation (SD) for normally distributed data or median with interquartile range (IQR), when the distribution was different from normal. In comparison between overweight and obese children and control group a Student's T-test (parametric data) or U Mann-Whitney test (nonparametric data) was performed, as appropriate. Relationship analysis were performed using the Spearman's rank correlation coefficient test. A p-value < 0.05 was considered as statistically significant.

RESULTS

Flow Cytometry

At Baseline

Increased Th17 Cell Frequency in Obese and Overweight Children

Initially, there was analyzed the subgroup of obese in comparison to overweight children and no statistical difference in Th17 cells frequency was found. In the study group there was statistically significant higher frequency of Th17 cells in the peripheral blood in comparison to normal-weight controls; median value 0.097% *vs* 0.041%, respectively (p = 0.048; **Figure 2**). In the group of all children, the percentage of Th17 lymphocytes correlated positively with BMI (p = 0.048, r = 0.31), BMI SDS (p = 0.032, r = 0.33) and WHtR (p = 0.02, r = 0.37). In overweight and obese group there was statistically significant correlation between Th17 frequency and WHR (p = 0.005, r = 0.54).

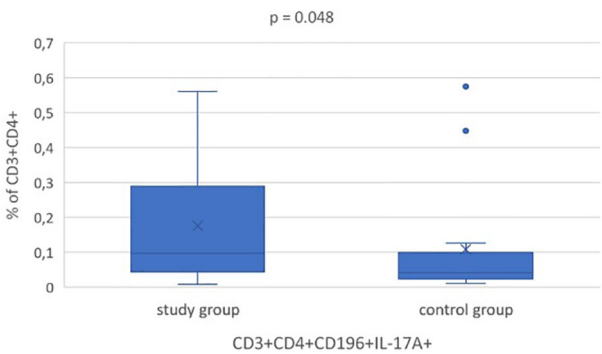


FIGURE 2 | Statistical analysis of Th17 ($CD3^+CD4^+CD196^+IL\text{-}17A^{+ic}$) cells frequency at baseline in overweight and obese children (study group) and normal weight ones (control group). All box plots represent the minimum, the first quartile, the median, the mean, the third quartile and the maximum. Dots represent outliers.

Follow-Up Visit

The Changes of Th17 Cells Percentage After Weight Loss

After three months, 21 overweight and obese children (10 girls and 11 boys) were assessed; 6 children didn't attend the visit. 12 of children lost weight (ΔBMI SDS <0 (-0.4 ≥ ΔBMI SDS ≥ -0.1)), 5 gained weight (ΔBMI SDS >0) and in 4 ones BMI SDS (ΔBMI SDS =0) remained unchanged.

In the group of children who reduced weight (ΔBMI SDS <0, p <0.005) after the intervention, the percentage of Th17 cells decreased statistically significantly: 0.210% vs 0.039%, p = 0.004 (**Figure 3A**) and reached the value similar to the control group (0.041%).

In 9 children, who did not decrease BMI SDS, we did not observe any statistically significant changes in Th17 frequency (p = 0.51; **Figure 3B**).

Biochemical and Metabolic Parameters

At Baseline

Inflammation

CRP concentration was similar in overweight and obese children (0.5 (0.5-0.5)) and controls (0.5 (0.5-0.5)), p = 0.78. In overweight and obese children, we reported a positive correlation between CRP concentration and BMI SDS (p = 0.01, r = 0.47), WC SDS (p = 0.009, r = 0.49) and WHtR (p = 0.001, r = 0.59). In all children, a positive correlation was confirmed between CRP concentration and BMI SDS (p = 0.043, r = 0.3), but there was no correlation between CRP concentration and the percentage of Th17 cells in peripheral blood.

Glucose Metabolism

All children had normal fasting glucose concentration and HbA1c percentage, but among overweight and obese children 19 out of 27 (70%) had elevated fasting insulin concentration (**Figure 4**). In all children it was found a statistically significant correlation between Th17 cells percentage and the parameters of carbohydrate metabolism: fasting insulin (p = 0.01, r = 0.39), HOMA-IR (p = 0.01, r = 0.38), QUICKI (p = 0.015, r = -0.37). In obese and overweight children, the statistically significant positive correlation was found between the frequency of Th17 lymphocytes and the concentration of glucose and insulin 2 h after OGTT (p = 0.017, r = 0.45; p = 0.006, r = 0.52, respectively).

Lipids

14 out of 27 overweight/obese children (52%) had an increased TC concentration, 9 children (33%) LDL-C, 20 ones (74%) TG concentration; 15 children (55.5%) had decreased HDL-C concentration. In all children we found a statistically significant correlation between Th17 cells percentage and concentration of HDL-C (p = 0.046, r = -0.31) and TG (p = 0.027, r = 0.35), as well as TG: HDL-C ratio (p = 0.015; r = 0.38).

Liver

At baseline in overweight and obese children we found increased ALT and AST activity in 41% (11/27) and 22% (7/27), respectively. Aminotransferases' activity correlated positively with CRP concentration (ALT p = 0.018, r = 0.46; AST p = 0.006, r = 0.51). Moreover, we detected positive correlation between AST activity and CRP concentration in normal weight ones (p = 0.04; r = 0.57). In all children we noticed a tendency to correlation between Th17 lymphocytes and ALT activity but without statistical significance (p = 0.07, r = 0.29). The comparison of biochemical parameters at baseline in overweight and obese to normal weight children are reported in **Table 2**.

Additionally, we compared the biochemical parameters in subgroup of overweight and obese children, and we found statistically significant differences only in the value of ALT, HDL-C, fasting insulin and HOMA-IR (**Table 3**).

Follow-Up Visit

As reported in **Table 4**, in overweight/obese children, who reduced BMI after intervention, only TC (p = 0.01) and LDL-C (p = 0.04) improved significantly. There was also

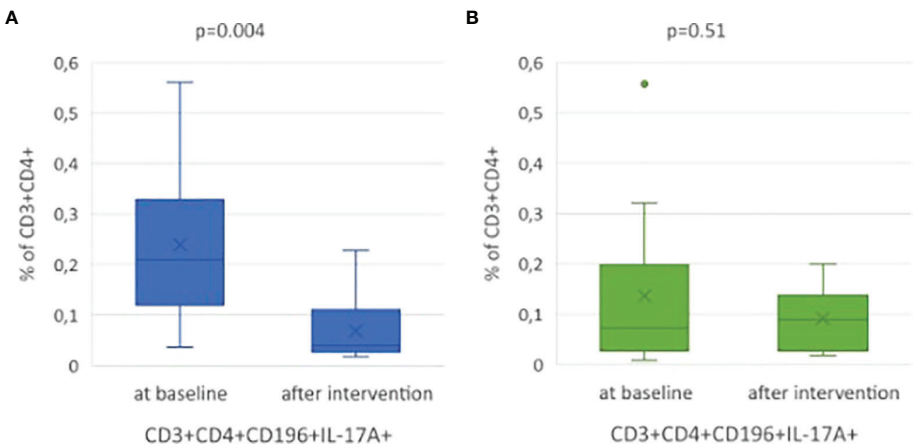


FIGURE 3 | **(A)** Statistical analysis amount of Th17 cells (%) in the study subgroup at baseline and three months later after BMI reduction. **(B)** Statistical analysis of Th17 cells frequency in the study subgroup at baseline and after intervention, without BMI reduction. All box plots represent the minimum, the first quartile, the median, the mean, the third quartile and the maximum. Dots represent outliers.

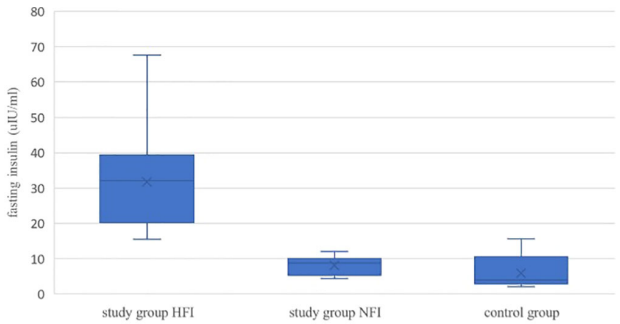


FIGURE 4 | Comparison of fasting insulin concentration in overweight/obese children and controls. Study group HFI v study group NFI $p < 0.001$, study group HFI v control group $p < 0.001$, study group NFI v control group $p = 0.11$. HFI- high fasting insulin, NFI- normal fasting insulin. All box plots represent the minimum, the first quartile, the median, the mean, the third quartile and the maximum. A $p < 0.05$ was considered significant.

decreased insulin level but without statistical significance ($p = 0.27$).

In the group of children without BMI reduction after intervention, no significant changes in biochemical parameters as well as insulin level were observed.

DISCUSSION

Th17 cells, a subset of $CD4^+$ helper T cells, like other immune cells, are recognized by expression of characteristic patterns of master transcription factors, cytokine production profiles and extracellular proteins. In the studies using flow cytometry, there are applied many different staining protocols of Th17 identification (19, 29, 30). In our study, the subset of Th17 cells from human's peripheral blood was defined by the expression of CD196 (CCR6) (31, 32) on the $CD3^+$ $CD4^+$ cell surface and intracellular interleukin (IL)-17A presence ($CD3^+CD4^+CD196^+IL\text{-}17A^{ic+}$).

Th17 cells are a small population of cells and their stimulation, including a.o. ionomycin/PMA, is typically used for their evaluation *in vitro*. The use of these stimulants is a

TABLE 2 | Biochemical parameters in overweight and obese children in comparison to normal weight children.

Variable	Obese children (n = 27)	Normal weight children (n = 15)	p-value
CRP (mg/dl)	0.5 (0.5 - 0.5)	0.5 (0.5 - 0.5)	ns
ALT (U/L)	35 (27 - 47)	18.00 (16.0 - 22.0)	<0.001
AST (U/L)	31 (27 - 38)	30.00 (22.0 - 33.0)	ns
Fasting glucose (mg/dl)	85.96 ± 7.22	83 ± 5.7	ns
Fasting insulin (μIU/ml)	20.4 (10.1 - 36.7)	3.92 (2.89 - 10.6)	<0.001
HbA_1c (%)	5.3 (5.15 - 5.45)	5.3 (5.0 - 5.4)	ns
HOMA-IR	3.8 (2.09 - 7.66)	0.79 (0.58 - 2.2)	<0.001
QUICKI	0.31 ± 0.03	0.39 ± 0.05	<0.001
TC (mg/dl)	161.4 ± 34.76	178.14 ± 24.58	ns
HDL-C (mg/dl)	41.04 ± 9.45	63.28 ± 11.35	<0.001
LDL-C (mg/dl)	93.2 (71.4 - 115.6)	95.9 (81.2 - 121.0)	ns
TG (mg/dl)	145.93 ± 63.5	78.0 ± 20.37	<0.001

Data are presented as mean ± standard deviation (SD) or median values with interquartile range as appropriate; CRP, C-reactive protein; ALT, alanine aminotransferase activity; AST, aspartate aminotransferase activity; HbA1c, glycated haemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; ns, non-significant. A p < 0.05 was considered significant.

TABLE 3 | Biochemical differences between overweight and obese children.

Variable	Overweight children (n = 7)	Obese children (n = 20)	p-value
ALT	27 (24 - 30)	41 (31 - 71)	0.02
HDL-C	47.1 ± 6.8	38.9 ± 9.4	0.04
Fasting insulin	15.5 (6.25 - 20.2)	30.05 (13.75 - 38.8)	0.03
HOMA-IR	3.25 (1.4 - 3.8)	6.08 (3.03 - 8.13)	0.048

Data are presented as mean ± standard deviation (SD) or median values with interquartile range as appropriate; ALT, alanine aminotransferase activity; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance. A p < 0.05 was considered significant.

TABLE 4 | Comparison of biochemical parameters in subgroup of overweight/obese children at baseline and after BMI reduction (n=12).

Variable	At baseline	After BMI reduction	p-value
CRP (mg/dl)	0.5 (0.5 - 0.5)	0.5 (0.5 - 0.5)	0.65
ALT (U/l)	35 (30 - 43.5)	33 (27 - 39)	0.2
AST (U/l)	31.5 (28 - 35.5)	29.5 (26 - 34.5)	0.13
Fasting glucose (mg/dl)	84 ± 7	84 ± 4.6	0.8
Fasting insulin (μIU/ml)	30.1 (20.3 - 36.1)	21.5 (14.4 - 35.3)	0.27
HbA1c (%)	5.35 (5.2 - 5.45)	5.15 (4.8 - 5.5)	0.33
HOMA-IR	6.08 (3.71 - 7.62)	4.6 (2.9 - 7.5)	0.31
QUICKI	0.295 (0.29 - 0.315)	0.305 (0.29 - 0.325)	0.17
TC (mg/dl)	161.6 ± 27.5	148 ± 27	0.01*
HDL-C (mg/dl)	37.8 ± 9.3	38.7 ± 11.4	0.61
LDL-C (mg/dl)	90.9 ± 18	83.5 ± 20	0.04*
TG (mg/dl)	162 (113 - 198)	108 (89 - 169)	0.08
TG/HDL-C ratio	3.78 (2.85 - 5.45)	2.66	0.16

Data are presented as mean ± standard deviation (SD) or median values with interquartile range as appropriate; CRP, C-reactive protein; ALT, alanine aminotransferase activity; AST, aspartate aminotransferase activity; HbA1c, glycated haemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; TC/HDL-C ratio. A $p < 0.05$ was considered significant.*

common method for assessing Th17 lymphocytes activity in flow cytometry but requires incubation for several hours. Therefore, we performed an easier and shortened protocol with reduced cell manipulation *in vitro* that can be used simultaneously in a clinical setting. Our study evaluated the spontaneous intracellular expression of IL-17A in peripheral Th17 cells without stimulation, and yet we obtained a statistically significant difference in the frequency of $CD3^+CD4^+CD196^+IL\text{-}17A^{ic+}$ cells in overweight/obese and normal weight children.

There is growing evidence, that the percentage of Th17 lymphocytes increases in obesity (32). Nevertheless, the mechanism initiating the Th17 immune response in humans with obesity is not fully understood. It seems that dendritic cells with $CD11c^+CD1c^+$ phenotype, present in the AT in mouse and humans, may play an important role in this process (33).

Interestingly, in animals it was found a higher level of $CD4^+IL\text{-}17^+T$ cells in AT of high fat diet (HFD) mice compared to the lean ones (18). Likewise, higher amount Th17 cells were detected in the spleens of HFD mice compared to normal mice (8). The contribution of Th17 cells in AT was also assessed in human (34) and in obese individuals Th17 cells were markedly increased in visceral AT, moreover their number strongly correlated with peripheral blood Th17 subset (35). However, for clinical use, the studies based on the parameters of peripheral blood seem to be more valuable because of its availability. In our opinion, research using less invasive and relatively easily reproducible methods could be more useful, especially in children.

In our study, the statistical significance of the difference of the Th17 frequency was at the borderline ($p = 0.048$). Nevertheless, our results are in line with data of Schindler (36), who in their study observed that the frequency of circulating Th17 cells was significantly increased in overweight children compared to non-overweight controls in the absence of acute or chronic inflammatory diseases. Furthermore, they found a significantly higher expression of RORC- and IL-17A-mRNA transcripts after stimulation in PBMCs from overweight children (36). In the study of Łuczyński et al. it was detected that both children with central obesity and children with long-term diabetes type 1 (DM1) have elevated levels of $CD4^+CD161^+CD196^+IL\text{-}17^+$ cells in the peripheral blood (19).

Calcaterra et al. (37) evaluating the Th17 and Treg lymphocyte balance in obese and normal weight children reported different results. They observed a decreasing trend of circulating Th17 cells in children with obesity compared with normal weight ones, without statistical significance (37). Nevertheless, after dividing these patients into two subgroups: metabolically healthy (MH) or unhealthy (MU), a higher percentage of Th17 cells was observed in the MU group, but this result was not statistically significant (37). However, most studies confirm higher levels of Th17 cells in obese patients (19, 38, 39). Furthermore, the results of our study, as well as Schindler's data (36), detected a positive correlation between the frequency of Th17 cells and BMI. Additionally, we reported for the first time, to our knowledge, that after BMI reduction in obese children Th17 cells frequency statistically significant decreased. We also observed a positive correlation between Th17 cells and WHR or WHtR, which is considered as a sensitive marker of visceral adiposity (40).

The obesity-induced low-grade chronic inflammation is considered to predispose to metabolic disorders. The link between systemic sterile inflammation and increased Th17 cells and development of IR and T2DM was documented in several studies (34, 38, 41, 42). Fabrini et al. (43) showed that the AT from obese insulin-resistant subjects have increased Th17 cell counts compared to the AT of obese ones without signs of IR and neither to non-overweight ones. The study in adults with T2DM and nondiabetic ones revealed that blood from T2DM patients had shown increased circulating Th17 cells and elevated activation of Th17 signature genes (44). Furthermore, a positive correlation was found between BMI and percentage of Th17 cells in the obese T2DM cohort (44). However, there are only few papers evaluating this problem in children (37). In our study, in the group of all children, a statistically significant correlation was found between the frequency of Th17 lymphocytes and fasting insulin, HOMA-IR and QUICKI. Our study showed a statistically significant correlation between the frequency of Th17 cells and the concentration of glucose and

insulin 2h after OGTT in overweight/obese children. These results support the observation, that pro-inflammatory Th17 cells have important contribution in glycemic homeostasis and the development of IR in children. Interestingly, we observed a trend towards an increase in QUICKI simultaneously with a statistically significant decrease in the frequency of Th17 cells after BMI reduction. It is worth noting that the change in the frequency of Th17 cells was observed in our study just after 3 months of the lifestyle changes and relatively small BMI SDS reduction.

In adults 5-10% losing of the initial body weight led to noticeably improve health by reducing obesity-related risk factors (45). This observation may suggest that inflammation and insulin resistance might be relatively easily reversible in children following lifestyle changes. This hypothesis however requires further investigation.

A marker routinely used in the assessment of inflammation is CRP- nonspecific acute-phase reactant that is synthesized in the liver. High-sensitivity CRP (hsCRP) - a more sensitive systemic inflammatory marker that indicates increased risk for metabolic complication of obesity is associated with other proinflammatory factors in plasma e.g., IL-6, Th1 and Th17 lymphocytes (46, 47). In our study, using standard CRP and not hsCRP, we did not find any difference between the plasma concentration in obese and overweight children and normal-weight ones, and no correlation between CRP and the frequency of Th17 cells. Th17 cell frequency assessment seems to be more sensitive than standard plasma CRP concentration, which suggests that it could be a valuable parameter for clinical use.

We realize that our study has some limitations. First of all- a relatively small group of patients who reduced the weight, which may have an impact on the statistical power of results. Secondly, our results show an association rather, than a direct cause-end-effect relationship, between Th17 cells and obesity related complications. However, this is one of the few studies assessing the immunological aspect of childhood obesity and their metabolic complications.

CONCLUSIONS

Obesity in children is associated with increased percentage of peripheral Th17 cells. Weight reduction leads to significant decrease of circulating Th17 cells and improvement of lipid parameters. This significant reduction of proinflammatory Th17 cells is a promising finding suggesting that obesity-induced inflammation in children could be relatively easily reversible.

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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Bioethics Committee at the Medical University of Warsaw (approval number : KB/52/A/2016; KB/61/2016). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

DA-W conceptualized and designed the study, collected data, performed statistical analysis, prepared tables and figures, wrote and edited the manuscript. AK conceptualized and designed the study, interpreted the results, and revised the manuscript. AS-E designed the flow cytometry protocol, performed the flow cytometry analysis, prepared Figure 1 and reviewed the manuscript. AM performed anthropometric measurements, contributed to the writing of the "Anthropometry" part, and reviewed the manuscript. BP reviewed the manuscript. All authors contributed to the article and approved the version submitted.

REFERENCES

1. Jaacks LM, Vandevijvere S, Pan A, McGowan CJ, Wallace C, Imamura F, et al. The Obesity Transition: Stages of the Global Epidemic. *Lancet Diabetes Endocrino* (2019) 3):231–40. doi: 10.1016/S2213-8587(19)30026-9
2. Majcher A, Czerwonogrodzka-Senczyna A, Kądziela K, Rumińska M, Pyrżak B. Development of Obesity From Childhood to Adolescents. *Pediatr Endocrinol Diabetes Metab* (2021) 27(2):70–5. doi: 10.5114/pedm.2021.105297
3. Cinti S. Adipose Organ Development and Remodeling. *Compr Physiol* (2018) 8(4):1357–431. doi: 10.1002/cphy.c170042
4. Wang Q, Wang Y, Xu D. The Roles of T Cells in Obese Adipose Tissue Inflammation. *Adipocyte*. (2021) 10(1):435–45. doi: 10.1080/21623945.2021.1965314
5. Kucharska AM, Pyrżak B, Demkow U. Regulatory T Cells in Obesity. *Adv Exp Med Biol* (2015) 866:35–40. doi: 10.1007/5584_2015_147
6. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased Oxidative Stress in Obesity and its Impact on Metabolic Syndrome. *J Clin Invest* (2004) 114(12):1752–61. doi: 10.1172/JCI21625
7. Kanneganti TD, Dixit VD. Immunological Complications of Obesity. *Nat Immunol* (2012) 13(8):707–12. doi: 10.1038/ni.2343
8. Winer S, Paltser G, Chan Y, Tsui H, Engleman E, Winer D, et al. Obesity Predisposes to Th17 Bias. *Eur J Immunol* (2009) 39(9):2629–35. doi: 10.1002/eji.200838893
9. Stavropoulos-Kalinoglou A, Metsios GS, Koutedakis Y, Kitas GD. Obesity in Rheumatoid Arthritis. *Rheumatol (Oxford)* (2011) 50(3):450–62. doi: 10.1093/rheumatology/keq266
10. Harrington LE, Hatton RD, Mangan PR, Turner R, Murphy TL, Murphy KM, et al. Interleukin 17-Producing CD4+ Effector T Cells Develop *via* a Lineage Distinct From the T Helper Type 1 and 2 Lineages. *Nat Immunol* (2005) 6 (11):1123–32. doi: 10.1038/ni1254
11. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang Y-H, et al. A Distinct Lineage of CD4 T Cells Regulates Tissue Inflammation by Producing Interleukin 17. *Nat Immunol* (2005) 6(11):1133–41. doi: 10.1038/ni1261
12. Todosenko N, Vulf M, Yurova K, Skuratovskaia D, Khaziakhmatova O, Gazatova N, et al. The Pathogenic Subpopulation of Th17 Cells in Obesity. *Curr Pharm Des* (2021) 27(37):3924–38. doi: 10.2174/1381612826666210101154913
13. Ye P, Garvey PB, Zhang P, Nelson S, Bagby G, Summer WR, et al. Interleukin-17 and Lung Host Defense Against Klebsiella Pneumoniae Infection. *Am J Respir Cell Mol Biol* (2001) 25(3):335–40. doi: 10.1165/ajrcmb.25.3.4424

14. Huang W, Na L, Fidel PL, Schwarzenberger P. Requirement of Interleukin-17A for Systemic Anti-Candida Albicans Host Defense in Mice. *J Infect Dis* (2004) 190(3):624–31. doi: 10.1086/422329
15. Morishima Y, Ano S, Ishii Y, Ohtsuka S, Matsuyama M, Kawaguchi M. Th17-Associated Cytokines as a Therapeutic Target for Steroid-Insensitive Asthma. *Clin Dev Immunol* (2013) 2013:609395. doi: 10.1155/2013/609395
16. Newcomb DC, Peebles RSJr. Th17-Mediated Inflammation in Asthma. *Curr Opin Immunol* (2013) 25(6):755–60. doi: 10.1016/j.coi.2013.08.002
17. Manel N, Unutmaz D, Littman DR. The Differentiation of Human T(H)-17 Cells Requires Transforming Growth Factor-Beta and Induction of the Nuclear Receptor RORgammat. *Nat Immunol* (2008) 9(6):641–9. doi: 10.1038/ni.1610
18. Chen Y, Tian J, Tian X, Tang X, Rui K, Tong J, et al. Adipose Tissue Dendritic Cells Enhances Inflammation by Prompting the Generation of Th17 Cells. *PloS One* (2014) 9(3):e92450. doi: 10.1371/journal.pone.0092450
19. Łuczyński W, Grubczak K, Moniuszko M, Głowińska-Olszewska B, Bossowski A. Elevated Levels of Th17 Cells in Children With Central Obesity. *Scand J Clin Lab Invest* (2015) 75(7):595–601. doi: 10.3109/00365513.2015.1066845
20. Kułaga Z, Litwin M, Tkaczyk M, Palczewska I, Zajączkowska M, Zwolińska D, et al. Polish 2010 Growth References for School-Aged Children and Adolescents. *Eur J Pediatr* (2011) 170(5):599–609. doi: 10.1007/s00431-010-1329-x
21. Ten S, Maclaren N. Insulin Resistance Syndrome in Children. *J Clin Endocrinol Metab* (2004) 89(6):2526–39. doi: 10.1210/jc.2004-0276
22. Singh B, Saxena A. Surrogate Markers of Insulin Resistance: A Review. *World J Diabetes* (2010) 1(2):36–47. doi: 10.4239/wjd.v1.i2.36
23. Polish Diabetes Association. Guidelines on the Management of Patients With Diabetes. *Clin Diabetes* (2021) 10(1):68–75. doi: 10.5603/DK.2021.0001
24. Solnica B, Sygitowicz G, Sitkiewicz D, Cybulska B, Jóźwiak J, Odrowąż-Sypniewska G, et al. Guidelines of the Polish Society of Laboratory Diagnostics (PSLD) and the Polish Lipid Association (PoLA) on Laboratory Diagnostics of Lipid Metabolism Disorders. *Arch Med Sci* (2020) 16(2):237–52. doi: 10.5114/aoms.2020.93253
25. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of Metabolic Markers to Identify Overweight Individuals Who are Insulin Resistant. *Ann Intern Med* (2003) 139(10):802–9. doi: 10.7326/0003-4819-139-10-200311180-00007
26. Report of a WHO Expert Committee. Physical Status: The Use and Interpretation of Anthropometry. *World Health Organ Tech Rep Ser* (1995) 854:1–452.
27. Kułaga Z, Litwin M, Zajączkowska MM, Wasilewska A, Morawiec-Knysak A, Różdżyńska A, et al. Comparison of Waist and Hip Circumferences Ranges in Children and Adolescents In Poland 7-18 Years of Age With Cardiovascular Risk Thresholds – Initial Results of OLAF Project (Pl0080). *Standardy Medyczne/Pediatria* (2008) 5:473–85.
28. Tompuri TT, Jääskeläinen J, Lindi V, Laaksonen DE, Eloranta AM, Viitasalo A, et al. Adiposity Criteria in Assessing Increased Cardiometabolic Risk in Prepubertal Children. *Front Endocrinol (Lausanne)* (2019) 10:410. doi: 10.3389/fendo.2019.00410
29. Kleinschek MA, Boniface K, Sadekova S, Grein J, Murphy EE, Turner SP, et al. Circulating and Gut-Resident Human Th17 Cells Express CD161 and Promote Intestinal Inflammation. *J Exp Med* (2009) 206(3):525–34. doi: 10.1084/jem.20081712
30. Mousset CM, Hobo W, Woestenenk R, Preijers F, Dolstra H, van der Waart AB. Comprehensive Phenotyping of T Cells Using Flow Cytometry. *Cytometry A* (2019) 95(6):647–54. doi: 10.1002/cyto.a.23724
31. Annunziato F, Cosmi L, Liotta F, Maggi E, Romagnani S. Main Features of Human T Helper 17 Cells. *Ann N Y Acad Sci* (2013) 1284:66–70. doi: 10.1111/nyas.12075
32. Singh SP, Zhang HH, Foley JF, Hedrick MN, Farber JM. Human T Cells That are Able to Produce IL-17 Express the Chemokine Receptor CCR6. *J Immunol* (2008) 180(1):214–21. doi: 10.4049/jimmunol.180.1.214
33. Bertola A, Ciucci T, Rousseau D, Bourlier V, Duffaut C, Bonnafous S, et al. Identification of Adipose Tissue Dendritic Cells Correlated With Obesity-Associated Insulin-Resistance and Inducing Th17 Responses in Mice and Patients. *Diabetes* (2012) 61(9):2238–47. doi: 10.2337/db11-1274
34. Vega-Cárdenas M, Uresti-Rivera EE, Cortés-García JD, Briones-Espinoza M, Ruíz-Rodríguez VM, Reynaga-Hernández E, et al. Increased Levels of Adipose Tissue-Resident Th17 Cells in Obesity Associated With miR-326. *Immunol Lett* (2019) 211:60–7. doi: 10.1016/j.imlet.2019.05.010
35. 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
36. Schindler TI, Wagner J-J, Goedicke-Fritz S, Rogosch T, Coccejus V, Laudenbach V, et al. TH17 Cell Frequency in Peripheral Blood Is Elevated in Overweight Children Without Chronic Inflammatory Diseases. *Front Immunol* (2017) 8:1543. doi: 10.3389/fimmu.2017.01543
37. Calcaterra V, Croce S, Vinci F, De Silvestri A, Cordaro E, Regalbuto C, et al. Th17 and Treg Balance in Children With Obesity and Metabolically Altered Status. *Front Pediatr* (2020) 8:591012. doi: 10.3389/fped.2020.591012
38. Zhao RX, He Q, Sha S, Song J, Qin J, Liu P, et al. Increased AHR Transcripts Correlate With Pro-Inflammatory T-Helper Lymphocytes Polarization in Both Metabolically Healthy Obesity and Type 2 Diabetic Patients. *Front Immunol* (2020) 11:1644. doi: 10.3389/fimmu.2020.01644
39. Ip B, Cilfone NA, Belkina AC, DeFuria J, Jagannathan-Bogdan M, Zhu M, et al. Th17 Cytokines Differentiate Obesity From Obesity-Associated Type 2 Diabetes and Promote Tnfα Production. *Obes (Silver Spring)* (2016) 24 (1):102–12. doi: 10.1002/oby.21243
40. Caminiti C, Armeno M, Mazza CS. Waist-To-Height Ratio as a Marker of Low-Grade Inflammation in Obese Children and Adolescents. *J Pediatr Endocrinol Metab* (2016) 29(5):543–51. doi: 10.1515/jpem-2014-0526
41. Wang M, Chen F, Wang J, Zeng Z, Yang Q, Shao S. Th17 and Treg Lymphocytes in Obesity and Type 2 Diabetic Patients. *Clin Immunol* (2018) 197:77–85. doi: 10.1016/j.clim.2018.09.005
42. SantaCruz-Calvo S, Bharath L, Pugh G, SantaCruz-Calvo L, Lenin RR, Lutshumba J, et al. Adaptive Immune Cells Shape Obesity-Associated Type 2 Diabetes Mellitus and Less Prominent Comorbidities. *Nat Rev Endocrinol* (2022) 18(1):23–42. doi: 10.1038/s41574-021-00575-1
43. Fabbrini E, Cella M, McCartney SA, Fuchs A, Abumrad NA, Pietka TA, et al. Association Between Specific Adipose Tissue CD4+ T-Cell Populations and Insulin Resistance in Obese Individuals. *Gastroenterology* (2013) 145(2):366–74.e743. doi: 10.1053/j.gastro.2013.04.010
44. Jagannathan-Bogdan M, McDonnell ME, Shin H, Rehman Q, Hasturk H, Apovian CM, et al. Elevated Proinflammatory Cytokine Production by a Skewed T Cell Compartment Requires Monocytes and Promotes Inflammation in Type 2 Diabetes. *J Immunol* (2011) 186(2):1162–72. doi: 10.4049/jimmunol.1002615
45. Yumuk V, Frühbeck G, Oppert JM, Woodward E, Toplak H. An EASO Position Statement on Multidisciplinary Obesity Management in Adults. *Obes Facts* (2014) 7(2):96–101. doi: 10.1159/000362191
46. Ellulu MS, Khaza'ai H, Rahmat A, Patimah I, Abed Y. Obesity can Predict and Promote Systemic Inflammation in Healthy Adults. *Int J Cardiol* (2016) 215:318–24. doi: 10.1016/j.ijcard.2016.04.089
47. Rość D, Adamczyk P, Boinska J, Szafkowski R, Ponikowska I, Stankowska K, et al. CRP, But Not TNF-α or IL-6, Decreases After Weight Loss in Patients With Morbid Obesity Exposed to Intensive Weight Reduction and Balneological Treatment. *J Zhejiang Univ Sci B* (2015) 16(5):404–11. doi: 10.1631/jzus.B1400219

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.

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 19 August 2022
DOI 10.3389/fendo.2022.963467

OPEN ACCESS

EDITED BY
Artur Mazur,
University of Rzeszow, Poland

REVIEWED BY
Barbara Glowinska-Olszewska,
Medical University of Bialystok, Poland
Serhiy Nyankovskyy,
Danylo Halytsky Lviv National Medical University, Ukraine

*CORRESPONDENCE
Małgorzata Rumińska
mruminska@wum.edu.pl

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 07 June 2022
ACCEPTED 18 July 2022
PUBLISHED 19 August 2022

CITATION
Erazmus M, Rumińska M, Witkowska-Sędek E, Kucharska AM, Stelmaszczyk-Emmel A, Majcher A and Pyrżak B (2022) Decreased Level of Soluble Receptor Activator of Nuclear Factor-κβ Ligand (sRANKL) in Overweight and Obese Children.
Front. Endocrinol. 13:963467.
doi: 10.3389/fendo.2022.963467

Decreased level of soluble receptor activator of nuclear factor-κβ ligand (sRANKL) in overweight and obese children

Michał Erazmus[1], Małgorzata Rumińska[1]*, Ewelina Witkowska-Sędek[1], Anna M. Kucharska[1], Anna Stelmaszczyk-Emmel[2], Anna Majcher[1] and Beata Pyrżak[1]

[1]Department of Paediatrics and Endocrinology, Medical University of Warsaw, Warsaw, Poland, [2]Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Warsaw, Poland

Introduction: Childhood obesity contributes to the development of cardiovascular diseases. The molecular pathway – receptor activator of nuclear factor-κβ ligand (RANKL), its receptor RANK and osteoprotegerin (OPG) - takes part not only in bone metabolism but is also involved in the atherosclerosis process. RANKL stimulates osteogenic differentiation and calcification of vascular smooth cells. The associations between the OPG-sRANKL system and various cardiovascular risk factors were displayed.We aimed to evaluate the relationships between serum sRANKL (soluble RANKL) levels and the OPG/sRANKL ratio with cardiometabolic risk factors in overweight and obese children.

Material and methods: The study included 70 children with overweight and obesity (mean age 13.0 ± 2.8) and 35 age-matched normal weight, healthy peers as a control group. In all patients, anthropometric measurements and laboratory tests were performed. Additionally, an oral glucose tolerance test (OGTT) was made only in overweight and obese children. Atherogenic and insulin resistance indices were calculated.

Results: Overweight and obese children had lower sRANKL levels compared to the control group (median 276.95 vs 325.90, p=0.011), and consequently a higher OPG/sRANKL ratio (0.02 vs 0.01, p = 0.013). The studied children in the lowest quartile of sRANKL levels had higher body weight, Body Mass Index, waist circumference and increased glucose and insulin levels 60 minutes after OGTT and higher uric acid values compared to children in the highest quartile. In multivariable linear regression analysis sRANKL negatively correlated only with uric acid (β = - 0.508, p = 0.041). No association was found for the OPG/sRANKL ratio.

10.3389/fendo.2022.963467

Conclusion: Excess fat mass seems to alter the OPG/RANKL ratio mainly by reducing serum sRANKL levels. The correlation between sRANKL and uric acid may suggest a contribution of the OPG-sRANKL system in the cardiometabolic process, but that observation should be confirmed in future studies.

KEYWORDS

sRANKL, OPG/sRANKL ratio, cardiovascular risk factors, obesity, children

Introduction

Overweight and obesity in childhood are associated with increased mortality due to cardiovascular (CV) diseases in adulthood. The atherosclerosis process has long been known to start at an early age and is linked to obesity and disorders related to excess fat mass (1, 2). The combination of classic CV risk factors such as carbohydrate-lipid metabolic abnormalities, insulin resistance, hypertension, as well as altered concentrations of bioactive adipocytokines and adhesion molecules and following immune reactions or chronic inflammation appears to explain at least a part of the relationship of adiposity with endothelial dysfunction (1, 3–5). Nevertheless, the mechanisms linking early fat mass accumulation with the atherosclerosis process are still poorly understood. Recent studies suggested that the cytokine pathway of the OPG/RANKL/RANK (Osteoprotegerin/receptor activator of nuclear factor- kβ ligand/receptor activator of nuclear factor kβ) is implicated in vascular calcification, which is linked with bone metabolism (6, 7). In the bone, RANKL expressed on the surface of osteoblasts (OB) and bone marrow stromal cells (BMSC) initiates differentiation, maturation, and activity of osteoclasts after binding to its receptor (7–11). OPG acts as a soluble decoy receptor, which inhibits osteoclastogenesis after binding with RANKL. OPG is also expressed in endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), whereas RANKL is mainly expressed in infiltrating T cells and activated ECs (12, 13). Several bone structural proteins were identified in calcified vascular tissue in the experimental study (9, 14). It is suggested, that OPG protects against the RANKL-RANK induced bone resorption and vascular calcification. So, the relative balance between OPG and RANKL may affect bone metabolism but also the immune and vascular system. It seems that the ratio of these factors better reflects CV risk (15–17).

Clinical studies conducted mainly in adults supported the association between the OPG-RANKL system and CV pathology. The higher serum levels of OPG correlated with multiple cardiometabolic risk factors as well as with advanced atherosclerosis, CV incidents, and mortality or morbidity (18–20). Several studies show an association of the serum soluble RANKL (sRANKL) level with Body Mass Index (BMI), lipids profile, Homeostatic Model Assessment- Insulin Resistance (HOMA-IR), diabetes, blood pressure, C-reactive protein (CRP) (19, 21–24), carotid intima-media thickness (cIMT) (25) and CV events (26, 27).

In the previous study (28) investigating the relationships between the serum OPG concentrations and chosen cardiometabolic risk factors in overweight and obese children and adolescents, we found that children with excess fat mass had decreased concentration of sRANKL and consequently increased the OPG/sRANKL ratio compare to their normal-weight peers. OPG levels in blood serum were comparable between these groups. Therefore in the present study, we focused on the analysis of the serum sRANKL and the OPG/sRANKL ratio in the same group of patients. Given the above-mentioned clinical studies in adults, we hypothesized, that sRANKL or the OPG/sRANKL ratio correlates with typical cardiometabolic risk factors. Research on this topic in the pediatric population is limited.

Material and methods

The study was carried out at the Department of Pediatrics and Endocrinology Medical University of Warsaw. The protocol was approved by the Bioethical Committee of the Medical University of Warsaw in accordance with the Declaration of Helsinki.

The study group consisted of 70 children and adolescents (36 boys, 34 girls) with overweight (n = 17) and obesity (n = 53), the ages of 7.0 to 17.8 years. Overweight and obesity were defined according to the value of z-score BMI for age and sex: z-score BMI ≥ 1 was considered overweight, z-score BMI ≥ 2 obese (29). Overweight and obese in the group of children enrolled in this study were caused by incorrect eating habits. Exclusion criteria were genetic and endocrine causes of excess fat mass, as well as severe chronic diseases such as diabetes, hypertension, and hepatic or renal disturbances. The history of taking any medications, including vitamin D was negative.

The control group consisted of 35 healthy, age- and sex-matched children and adolescents (21 boys, 14 girls). Their physical parameters were within the normal range.

Anthropometric measurements

In all participants, physical examinations were performed. Height (cm), weight (kg), waist and hip circumferences (cm), and the thickness of skinfolds under the triceps brachii muscle and under the inferior scapular angle (mm) were measured by a qualified anthropologist. Based on the obtained results, BMI, waist-to-hip ratio (WHR), waist-to-height ratio (WHtR) as well as the percentage of fat mass (%BFM) from the sum of 2 skinfolds using the Slaughter formula were calculated (30). The degree of excess fat was expressed as z-score BMI (SDS BMI, standard deviation score), calculated using the LMS (lambda, mu, sigma) method to normalize the data for the age and sex using polish reference values (31, 32).

Laboratory tests

In all patients, blood samples were taken after night fasting. Both OPG (pmol/l) and the total sRANKL (pmol/l) concentrations in blood serum were determined by an enzyme immunoassay - ELISA test (DRG Instruments GmbH, Germany). Lipids profile: total cholesterol (TC, mg/dl), high-density lipoprotein cholesterol (HDL-C, mg/dl), and triglyceride (TG, mg/dl) were measured using the colorimetric enzymatic method using a VITROS 5600 Chemistry Analyzer (Ortho-Clinical Diagnostics, New Jersey, USA). Low-density lipoprotein cholesterol concentration (LDL-C, mg/dl) was calculated using the Friedewald formula (33). Fasting glucose (mg/dl) and insulin (μIU/ml) concentrations and additionally, oral glucose tolerance test (OGTT, oral glucose load of 1.75 g/kg body weight up to the maximum of 75 g) only in the group of overweight and obese children were measured by glucose oxidase colorimetric method using VITROS 5600 Chemistry Analyzer and immunoassay method using IMMULITE 2000 Xpi Analyzer (Siemens, Erlangen, Germany), respectively. The concentrations of glycosylated hemoglobin (HbA1c, %) were determined by ion-exchange high-performance liquid chromatography (HPLC) using D-10 Hemoglobin Analyzer (BIO-RAD). Subsequent blood tests: uric acid (UA, mg/dl), calcium (Ca, mg/dl), phosphorus (P, mg/dl) levels, and total alkaline phosphatase (ALP, U/L) activity were measured by the dry chemistry method using VITROS 5600 Chemistry Analyzer). On the same analyzer, CRP (mg/dl) concentrations were measured using the fixed-point immune-rate method. The serum concentration of intact parathyroid hormone (PTH, pg/ml) and the 25-hydroxyvitamin D (25(OH)D, ng/ml) were measured by immunoassay method using an IMMULITE 2000 Xpi Analyzer and Architect Analyzer (Abbott Diagnostics; Abbott Park, IL), respectively.

The obtained serum results were used to calculate the insulin resistance (HOMA-IR, QUICKI - Quantitative Insulin Sensitivity Check Index, Matsuda index) and atherogenic (non-HDL, TG/HDL-C ratio) indices (34–37).

In overweight and obese children calcium, phosphorus, and creatinine excretion were measured in 24-hour urine samples by dry chemistry system using VITROS 5600 Chemistry Analyzer and were converted to mg/kg/24 hours. Tubular Reabsorption of Phosphate (TRP) was calculated (http://www.scymed.com/en/smnxps/pshpd274.htm).

Statistical analysis

Statistical calculations were performed using the SPPS 13.3 software. To check the normality of data distribution the Shapiro-Wilk test was used. The data with normal distribution were presented as mean and standard deviation (SD), the data with non–normal distribution as median with interquartile range (IQR). The study group was compared to the control group by using a Student's T-test or U Mann-Whitney test. Moreover, we compared the distribution of anthropometric and biochemical parameters in overweight and obese children after stratification according to the sRANKL quartiles and the OPG/sRANKL quartiles. For the comparison of more than three groups, One-Way Analysis of Variance (ANOVA) or a Kruskal-Wallis test were used, as appropriate. To provide detailed information regarding the differences among various combinations of groups stratified according to quartiles Tukey *post-hoc* tests with Bonferroni corrections were made for the ANOVA test and the Dunn *post-hoc* tests with Bonferroni corrections for the Kruskal-Wallis test. The association between two ranked variables was measured by using the Spearman correlation coefficient test. To evaluate independent relationships between sRANKL and the OPG/sRANKL ratio, which were considered dependent variables, and selected anthropometric and biochemical parameters, which were considered independent variables, the multiple linear analysis was used. The p-value < 0.05 was considered statistically significant.

Results

The circulating sRANKL levels in children with overweight and obesity were significantly lower in comparison to the control group (median (IQR) = 261.36 (168.66); 283.28 (238.55); 325.90 (247.30), $p = 0.019$; $p = 0.029$, respectively). The sRANKL concentrations did not differ between overweight and obese children ($p = 0.473$). The median (IQR) of the OPG concentrations were comparable between children with overweight (3.84 (2.23)) and obesity (3.47 (1.28)) and their normal peers (3.74 (1.58)). So, the overweight and obese children were taken together (consider as the study group) for further analysis.

The comparison of anthropometric measurements and cardiometabolic parameters in serum blood as well as calcium-phosphorus metabolism parameters in blood and in urine in the study group and in the control group we presented in Table 1.

As expected, the overweight and obese children had atherogenic lipid profiles, higher insulin resistance status, and increased UA concentrations than their normal peers. Moreover, the studied participants had lower concentrations of 25(OH)D and higher ALP activity. The calcium and phosphorus concentration in blood serum and urine did not differ significantly between the study and control groups (Table 1).

Correlation of sRANKL and the OPG/sRANKL ratio with anthropometric and biochemical parameters in normal weight, overweight and obese children taken together to analysis

In the Spearman correlation coefficient analysis, sRANKL in normal weight, overweight and obese children negatively correlated with body weight (R = - 0.255, p = 0.009), BMI (R = - 0.240, p = 0.014), waist circumference (WC, R = - 0.333, p = 0.003), fasting glucose (R = - 0.197, p = 0.047), UA (R = - 0.388, p = 0.002), and HbA1c (R = - 0.312, p = 0.012). For the OPG/sRANKL ratio we observed association with WC (R = 0.239, p = 0.040), UA (R = 0.326, p = 0.010), and HbA1c (R = 0.425, p = < 0.001).

Anthropometric and biochemical parameters after stratification according to sRANKL and the OPG/sRANKL ratio quartiles in overweight and obese children

The distribution of chosen anthropometric and biochemical parameters after stratification according to the sRANKL quartiles in overweight and obese children is presented in Table 2.

We found that children with the lowest concentrations of sRANKL (first quartile) had higher values of body weight (p = 0.014), BMI (p = 0.029), WC (p = 0.015), as well as increased concentrations of glucose and insulin in 60 minutes of the OGTT (p = 0.014, p = 0.008, respectively) as compared to children with the highest value of sRANKL (fourth quartile). Moreover, we observed, that together with increased quartiles of sRANKL, the concentrations of UA decreased and the differences between the first quartile (Q1), second quartile (Q2), third quartile (Q3), and fourth quartile (Q4) were statistically significant (Q1 vs Q3: p = 0.018, Q1 vs Q4: p = 0.014, Q2 vs Q3: p = 0.053, Q2 vs Q4: p = 0.030).

After dividing the overweight and obese children for subgroups stratification according to the OPG/sRANKL ratio (presented in Table 3) we observed that together with increased quartiles of the OPG/sRANKL ratio, HbA1c levels increased (Q1 vs Q2: p = 0.015, Q1 vs Q3: p = 0.032, Q1 vs Q4: p = 0.015). Similar to sRANKL, children with the lowest values of the OPG/sRANKL ratio (Q1) had smaller WC compared to children in the fourth quartile (Q1 vs Q4 p = 0.043) and lower concentration of glucose in 60 minutes of OGTT and UA compared to children in third and fourth quartiles (for glucose: Q1 vs Q3: p = 0.050, Q1 vs Q4: p = 0.036, for UA: Q1 vs Q3: p = 0.046). Moreover, we observed, that LDL-C and TC levels statistical differ between Q2 and Q3 (p = 0.009 and p = 0.016, respectively) and Q2 and Q4 (p = 0.051, p = 0.044).

Correlation of sRANKL and the OPG/sRANKL ratio with anthropometric and biochemical parameters in overweight and obese children and adolescents

In the Spearman correlation coefficient analysis, serum sRANKL in overweight and obese children were inversely related to fasting glucose and glucose in 60 and 90 minutes of the OGTT (R = - 0.258, p = 0.032; R = - 0.389, p = 0.001; R = - 0.309, p = 0.014, respectively) and insulin in 60 minutes of the OGTT (R = - 0.308, p = 0.014), as well as with UA (R = - 0.387, p = 0.000). The OPG/sRANKL ratio positively correlated with glucose in 60 and 90 minutes of the OGTT (R = 0.356, p = 0.004; R = 0.262, p = 0.041, respectively), insulin in 60 minutes of the OGTT (R = 0.258, p = 0.041), UA (R = 0.326, p = 0.029) and HbA1c (R = 0.3576, p = 0.009). We did not find any association of both sRANKL and the OPG/sRANKL ratio with calcium-phosphorus metabolism parameters.

In multivariable linear regression analysis, where sRANKL was the dependent variable and BMI SDS, WC, HbA1c, HOMA-IR, TC, HDL-C, and LDL-C were the independent variables, sRANKL in overweight and obese children correlated only with UA (β = - 0.508, p = 0.041, 95%CI: -187.74 - -4.12) (Figure 1). In a similar model, which was performed for the OPG/sRANKL ratio as the dependent variable, we did not find any association.

Correlation of UA with anthropometric and biochemical parameters in overweight and obese children and adolescents

In the Spearman correlation coefficient analysis serum UA in overweight and obese children positively correlated with body weight (R = 0.450, p = < 0.001), BMI (R = 0.365, p = 0.006), BMI SDS (R = 0.290, p = 0.033), WC (R = 0.463, p = 0.001), CRP (R = 0.397, p = 0.005), glucose and insulin in 60 minutes of the OGTT (R = 0.306, p = 0.024; R = 0.329, p = 0.015, respectively).

Discussion

The decreased sRANKL level in our overweight and obese children leads to the imbalance between the levels of the

10.3389/fendo.2022.963467

TABLE 1 The comparison of anthropometric measurements, OPG, sRANKL, and biochemical parameters between overweight and obese children and their peer with normal weight.

Variables	Overweight and obese children(n = 70)	Non-obese children(n = 35)	p-value
Anthropometric measurements			
Height (cm)	160.6 ± 14.2	159.8 ± 13.6	Ns
Body weight (kg)	77.0 ± 21.0	50.8 ± 12.7	< 0.001
BMI (kg/m2)	29.4 (5.5)	18.7 (4.6)	< 0.001
BMI SDS	2.1 (0.4)	0.0 (1.1)	< 0.001
WC (cm)	87.9 ± 10.5	64.7 ± 6.2	< 0.001
HC (cm)	103.1 ± 13.0	83.7 ± 9.4	< 0.001
WHR	0.9 ± 0.1	0.8 ± 0.0	< 0.001
WHtR	0.5 ± 0.1	0.4 ± 0.0	< 0.001
% BFM	34.8 (7.4)	26.4 (8.1)	< 0.001
Blood tests			
OPG (pmol/l)	3.61 (1.36)	3.74 (1.58)	ns
sRANKL (pmol/l)	276.00 (188.56)	325.90 (247.30)	0.011
OPG/sRANKL ratio	0.02 (0.02)	0.01 (0.01)	0.013
fasting glucose (mg/dl)	85.85 ± 6.45	83.27 ± 6.97	Ns
fasting insulin (μIU/ml)	13.10 (11.77)	8.49 (7.92)	<0.001
HOMA -IR	2.84 (2.62)	1.71 (1.59)	<0.001
QUICKI	0.33 (0.04)	0.35 (0.06)	<0.001
MATSUDA	2.85 (1.82)	-	
TC (mg/dl)	162.08 ± 26.75	153.09 ± 23.77	Ns
HDL-C (mg/dl)	45.65 ± 11.68	62.28 ± 11.97	< 0.001
LDL-C (mg/dl)	93.36 ± 25.51	78.05 ± 19.64	0.013
TG (mg/dl)	104.00 (34.00)	63.00 (18.00)	< 0.001
TG/HDL-C	2.32 (1.85)	1.00 (0.40)	<0.001
non HDL	116.43 ± 26.27	90.81± 20.34	<0.001
UA (mg/dl)	5.82 ± 1.09	4.34 ± 1.17	0.001
Ca (mg/dl)	10.00 (0.35)	9.90 (0.40)	Ns
P (mg/dl)	4.92 ± 0.78	4.60 ± 1.00	Ns
25(OH)D (ng/ml)	17.80 (10.70)	22.80 (7.40)	0.025
ALP (U/L)	170.00 (138.00)	110.00 (133.00)	0.032
PTH (pg/ml)	21.80 (25.80)	20.60 (20.00)	Ns
CRP (mg/dl)	0.5 (0.05)	0.5 (0.0)	Ns
Urine tests			
Ca (mg/kg/24 h)	0.79 (0.81)	1.68 (0.85)	*0.085*
P (mg/kg/24 h)	9.47 (4.88)	12.79 (12.69)	Ns
TRP (%)	91.51(1.89)	90.67 (5.12)	Ns

Data are presented as mean ± standard deviation (SD) or median values with interquartile range (IQR) as appropriate.
BMI, body mass index; BMI SDS, body mass index standard deviation score; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; % BFM, % of body fat mass; OPG, osteoprotegerin; sRANKL, soluble nuclear factor kappa B ligand; HOMA - IR, Homeostasis model assessment for insulin resistance index; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; TG/HDL-C, triglycerides to high-density lipoprotein cholesterol ratio; non-HDL, non-high-density lipoprotein cholesterol; UA, uric acid; Ca, calcium; P, phosphorus; 25(OH)D, 25-hydroxy vitamin D; ALP, alkaline phosphatase; PTH, parathyroid hormone; CRP, C-reactive protein; TRP, Tubular Reabsorption of Phosphate; ns, nonsignificant.

circulating OPG and sRANKL and consequently increases the OPG/sRANKL ratio. In this study, we analyzed whether there is an association between the sRANKL concentration and the OPG/sRANKL ratio with metabolic disturbances related to excess fat mass.

Data regarding the correlation of the OPG-RANKL system with excess fat mass and cardiometabolic risk factors in children and adolescents are limited and inconsistent. In one study (38) involving healthy children, sRANKL levels slightly decline with age but were not related to gender or BMI SDS. While in another study (39) sRANKL concentrations were higher in boys than in girls and increased with age and body weight percentile. In a total scholar population of 600 children between the ages of 6 and 12 years, higher sRANKL levels were found in those with central obesity determined by the WC and negatively correlated with HDL-C (40). In contrast, in another study, sRANKL levels in groups of obese children did not

TABLE 2 Characteristics of chosen anthropometric and biochemical parameters after stratification according to the sRANKL quartiles in the study group.

Quartiless RANKL(pmol/l)	Quartile 1 (n=17) < 162.84	Quartile 2 (n=18) 162.84 - 275.99	Quartile 3 (n=18) 276.00 - 359.89	Quartile 4 (n=17) ≥ 359.90	p-trend
Variables:					
Body weight (kg)	78.6 ± 15.5	79.4 ± 23.7	80.7 ± 22.3	67.9 ± 20.3*	0.256
BMI (kg/m^2)	29.7 (10.2)	29.8 (12.9)	29.6 (21.7)	28.0 (21.2)*	0.650
WC (cm)	89.8 ± 7.2	87.1 ± 10.0	91.5 ± 12.3	82.7 ± 10.3*	0.129
Glucose 60'in OGTT (mg/dl)	138.1 ± 25.9	139.2 ± 32.1	118.7 ± 32.2	116.9 ± 29.6*$^+$	*0.061*
Insulin 60' in OGTT (μIU/ml)	116.0 (83.3)	96.7 (289.4)	99.0 (281.0)	76.4 (214.0)*	*0.097*
UA (mg/dl)	6.3 ± 1.2	6.0 ± 0.9	5.6 ± 1.3	5.3 ± 0.7*$^+$$^\#$	*0.069*

Data are presented as mean ± standard deviation (SD) or median values with interquartile range (IQR) as appropriate.
The sRANKL, soluble nuclear factor kappa B ligand; BMI, body mass index; WC, waist circumference; OGTT, oral glucose tolerance test; UA, uric acid.
*p < 0.05 found in the comparison of Q1 to Q4.
+p < 0.05 found in the comparison of Q1 to Q3.
#p < 0.05 found in the comparison of Q2 to Q4.
^p < 0.05 found in the comparison of Q2 to Q3.
$p < 0.05 found in the comparison of Q 3 to Q4.

differ compared to the control group and did not correlate with parameters describing nutritional status (41, 42) and with atherogenic and insulin resistance indices (41). Our research has produced different results. We noticed an inverse relationship between the sRANKL concentration and the chosen anthropometric parameters. The subgroup of overweight and obese children with the lowest sRANKL concentration had higher body weight, BMI, and WC than those with the highest sRANKL levels. Abdominal obesity appears to affect the higher OPG/sRANKL ratio, but these dependencies are not consistent directionally across all quartiles. On the other hand, we did not find the differences between sRANKL levels or the OPG/sRANKL ratio and the groups of overweight and obese children, which raises doubts about the effect of adipose tissue on these studied parameters. Conflicted results regarding the association of the sRANKL concentration with fat mass have also been reported in adult studies [positive association with BMI - (21, 22), no relationship - (43, 44)].

In the 3200 Framingham Study adult participants, sRANKL levels displayed inverse association with the multiple CV disease risk factors including diabetes (19). The decreased serum concentration of sRANKL in patients with type 2 diabetes (T2DM) compared to the controls was documented in other studies (20, 23, 45), but not in all (22). Inline, we also found an inverse relationship between sRANKL and glucose concentrations. An increase in the OPG/sRANKL ratio caused an increase in HbA1c concentration. Meanwhile, in children, 13.04 ± 3.53 years of age with type 1 diabetes mellitus (T1DM) both sRANKL and OPG levels were elevated compared to their healthy peers, but these study assessed their relationship with low bone mass (46). In another group of children of similar age and with T1DM both markers did not differ in comparison to the control group, but in those with microalbuminuria, sRANKL negatively correlated with cIMT (25). Clinical trials in adults also yielded different results, both decreased (6, 43) as well as

TABLE 3 Characteristics of chosen anthropometric and biochemical parameters after stratification according to the OPG/sRANKL ratio quartiles in the study group.

Quartiles	Quartile 1 (n=17)	Quartile 2 (n=18)	Quartile 3 (n=18)	Quartile 4 (n=17)	p-trend
OPG/sRANKL	< 0.0084	0.0084-0.0157	0.0158-0.0269	≥ 0.0270	
Variables:	85.7 ± 8.1	87.6 ± 12.6	85.2 ± 12.4	92.1 ± 7.3*	0.250
WC (cm)	117.1 ± 31.2	121.7 ± 26.4	138.2 ± 30.4	140.5 ± 32.5*$^+$	*0.076*
Glucose 60' in OGTT (mg/dl)	157.3 ± 24.3	151.9 ± 19.4	170.6 ± 24.9	170.1 ± 32.0$^\#$^	*0.093*
TC (mg/dl)	89.0 ± 24.5	82.1 ± 21.0	105.2 ± 22.15	100.65 ± 28.2$^\#$^	**0.029**
LDL-C (mg/dl)	5.5 ± 0.6	5.5 ± 1.2	6.4 ± 1.4	6.1 ± 1.0$^+$	*0.067*
UA (mg/dl)	5.1 (1.6)	5.4 (0.7)	5.4 (1.0)	5.5 (1.1)*$^+$	**0.030**
HbA1c (%)					

Data are presented as mean ± standard deviation (SD) or median values with interquartile range (IQR) as appropriate.
The OPG/sRANKL ratio, osteoprotegerin to soluble nuclear factor kappa B ligand ratio; WC, waist circumference; OGTT, oral glucose tolerance test; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; HbA1c, glycosylated hemoglobin.
*p < 0.05 found in the comparison of Q1 to Q4.
+p < 0.05 found in the comparison of Q1 to Q3.
#p < 0.05 found in the comparison of Q2 to Q4.
^p < 0.05 found in the comparison of Q2 to Q3.
&p < 0.05 found in the comparison of Q3 to Q4.

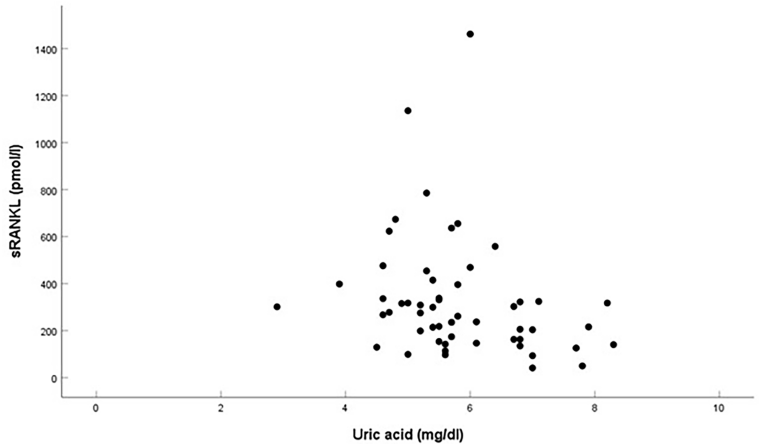


FIGURE 1
Correlation between sRANKL and urid acid in overweight and obese children.

increased (26, 27) sRANKL levels were related to CV diseases. Moreover, Zampetti et al. (47) study showed an association of the OPG/sRANKL ratio with left ventricular hypertrophy and geometric remodeling in overweight in obese boys. Of note, Gaudio et al. (20) supported the role of the increased the OPG/sRANKL ratio as a possible marker of progression of vascular dysfunction. Our overweight and obese children also had an increased OPG/sRANKL ratio. The role of RANKL in the pathogenesis of CV diseases is multifactorial. RANKL enhances chemokine release (monocyte chemoattractant protein-1 (MCP-1)), promotes monocyte/macrophage matrix migration, directly stimulates osteogenic differentiation of VSMC *via* a decrease Matrix Gla Protein (MGP), and indirectly *via* increased BMP-2, as well as increases matrix metalloproteinase activity leads to matrix degeneration (13, 26, 48). Moreover, in the immune and inflammatory pathways promoting atherosclerosis, activated T cells are involved which are a source of RANKL and pro-inflammatory cytokines (such as tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and interleukin - 6 (IL-6)), that up-regulate RANKL expression (6, 12, 49).

New findings from the present study are the detection of the inverse relationship between UA and sRANKL, after adjustment of lipids and insulin resistance variables. In the literature, the association between elevated serum UA with obesity, insulin resistance, glucose and lipids disturbances, metabolic syndrome, hypertension, carotid atherosclerosis, and an increased incidence of CV events in young adults, is well documented (2, 50–54). Excess of UA has paradoxically pro-oxidant effects in the vascular cells, impaired nitric oxide production, increased cytokines (IL 1β, IL 6, TNF α, CRP, MCP-1), and platelet-derived growth factors expression, leading to endothelial dysfunction and VSMC proliferation (55–57). Similar intracellular oxidative stress, together with inflammatory cytokines induced by UA take part in the pathogenesis of osteoporosis (57). Moreover, monosodium urate crystals increased mRNA expression of the RANKL-induced osteoclast formation in an experimental study (58). So, we speculated that the inflammatory process may be the link between obesity, UA, and sRANKL.

The negative correlations between sRANKL and obesity, and some cardiometabolic parameters are difficult to explain. RANKL exists mainly as a transmembrane protein (cellular form). Its soluble form (sRANKL), which was measured in our study, is only a small fraction of the total amount of this cytokine. It is not clear, what is the impact of the bone or vascular microenvironment on the circulating concentration of both OPG and sRANKL. Maybe these factors exert a paracrine action on the local cells and therefore their serum concentrations do not mirror their true interactions (13). Another explanation is that maybe the changes in the vessels or bone metabolism led to a compensatory increase of OPG and the neutralization of sRANKL giving the decreased sRANKL levels in serum (the circulating OPG bound to its ligands RANKL that is not detected using the ELISA test). Moreover, a wide array of factors regulates RANKL production. As we mentioned above, RANKL expression is stimulated by proinflammatory cytokines, linked to low-grade inflammation related to obesity (26). In Puengel et al. study (45) sRANKL positively correlated with adiponectin, leptin receptor, and ghrelin in critically ill patients. Whereas, in OB and the BMSC RANKL expression is up-regulated also by various pro-resorptive stimuli such as PTH, 1,25-dihydroxyvitamin D3, steroids, prostaglandin E2 (11, 12, 57). Despite higher CRP concentration in overweight and obese children compared to their normal peers, this inflammatory marker did not correlate with sRANKL. Except that, PTH concentrations did not differ between groups. Moreover, we have no knowledge of bone metabolism in our

children. Higher total ALP activity was found in our overweight and obese patients, making clinical interpretation difficult without fractionation of these ALP isoforms (59, 60). Serum ALP may be associated with vascular calcification, while bone-specific ALP (BAP) is a marker of bone formation (59). No assessment of BAP and bone mineral density (BMD) is a major limitation of our study.

Conclusion

Excess fat mass seems to alter the OPG/RANKL ratio mainly by reducing serum sRANKL levels. The correlation between sRANKL and UA may suggest the participation of the OPG-sRANKL system in the cardiometabolic process, but that observation should be confirmed in future studies.

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.

Ethics statement

The studies involving human participants were reviewed and approved by Medical University of Warsaw. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

ME wrote the manuscript and collected the literature data. MR designed the study, wrote the manuscript and prepared tables and figures, and collected the literature data. EW-S wrote the manuscript and collected the literature data. AK - critical review of the article. AS-E made the laboratory tests and interpreted the results. AM made the anthropometric parameters and interpreted the results. BP - a critical review of the article. All authors have read and approved the manuscript.

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.

References

1. Berenson GS, Srinivasan SR, Bao W, Newman WPIII, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. the bogalusa heart study. *N Engl J Med* (1998) 338 (23):1650–56. doi: 10.1056/NEJM199806043382302

2. Drozdz D, Alvarez-Pitti J, Wójcik M, Borghi C, Gabbianelli R, Mazur A, et al. Obesity and cardiometabolic risk factors: From childhood to adulthood. *Nutrients* (2021) 13(11):4176. doi: 10.3390/nu13114176

3. Artemniak-Wojtowicz D, Kucharska AM, Pyrżak B. Obesity and chronic inflammation crosslinking. *Cent Eur J Immunol* (2020) 45(4):461–68. doi: 10.5114/ceji.2020.103418

4. Beauloye V, Zech F, Tran Thi Mong H, Clapuyt P, Maes M, Brichard SM. Determinants of early atherosclerosis in obese children and adolescents. *J Clin Endocrinol Metab* (2007) 92(8):3025–32. doi: 10.1210/jc.2007-0619

5. Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Hamid Nasri H. Atherosclerosis: Process, indicators, risk factors and new hopes. *Int J Prev Med* (2014) 5(8):927–46.

6. Bezerra MC, Calomeni GD, Caparbo VF, Gebrim ES, Rocha MS, Pereira RMR. Low bone density and low serum levels of soluble RANK ligand are associated with severe arterial calcification in patients with takayasu arteritis. *Rheumatology* (2005) 44:1503–06. doi: 10.1093/rheumatology/kei045

7. Baud'huin M, Duplomb L, Teletchea S, Lamoureux F, Ruiz-Velasco C, Maillasson M, et al. Osteoprotegerin: multiple partners for multiple functions. *Cytokine Growth Factor Rev* (2013) 24(5):401–9. doi: 10.1016/j.cytogfr.2013.06.001

8. Di Bartolo BA, Cartland SP, Harith HH, Bobryshev YV, Schoppet M, Kavurma MM. TRAIL-deficiency accelerates vascular calcification in atherosclerosis *via* modulation of RANKL. *PloS One* (2013) 8(9):e74211. doi: 10.1371/journal.pone.0074211

9. Papadopouli AE, Klonaris C, Theocharis SE. Role of OPG/RANKL/RANK axis on the vasculature. *Histol Histopathol* (2008) 23:497–506. doi: 10.14670/HH-23.497

10. Boyce BF, Xing L. Biology of RANK, RANKL, and osteoprotegerin. *Arthritis Res Ther* (2007) 9(Suppl 1):S1. doi: 10.1186/ar2165

11. Wright HL, McCarthy HS, Middleton J, Marshall MJ. RANK, RANKL and osteoprotegerin in bone biology and disease. *Curr Rev Musculoskelet Med* (2009) 2 (1):56–64. doi: 10.1007/s12178-009-9046-7

12. Collin-Osdoby P, Rothe L, Anderson F, Nelson M, Maloneyi W. Philip Osdoby. receptor activator of NF-kB and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. *J Biol Chem* (2001) 276(23):P20659–72. doi: 10.1074/jbc.M010153200

13. Ueland T, Yndestad A, Øie E, Florholmen G, Halvorsen B, Frøland SS, et al. Dysregulated Osteoprotegerin/RANK Ligand/RANK axis in clinical and experimental heart failure. *Circulation* (2005) 111(19):2461–8. doi: 10.1161/01.CIR

14. Boström K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL. Bone morphogenetic protein expression in human atherosclerotic lesions. *J Clin Invest* (1993) 91:1800–9. doi: 10.1074/jbc.M010153200

15. Rochette L, Meloux A, Rigal E, Zeller M, Cottin Y, Vergely C. The role of osteoprotegerin and its ligands in vascular function. *Int J Mol Sci* (2019) 20(3):705. doi: 10.3390/ijms20030705

16. Mohammadpour AH, Shamsara J, Nazemi S, Ghadirzadeh S, Shahsavand S, Ramezani M. Evaluation of RANKL/OPG serum concentration ratio as a new biomarker for coronary artery calcification: A pilot study. *Thrombosis* (2012) 2012:306263. doi: 10.1155/2012/306263

17. Forde H, Davenport C, Harper E, Cummins P, Smith D. The role of OPG/RANKL in the pathogenesis of diabetic cardiovascular disease. *Cardiovasc Endocrinol Metab* (2018) 7(2):28–33. doi: 10.1097/XCE.0000000000000144

18. Van Campenhout A, Golledge J. Osteoprotegerin, vascular calcification and atherosclerosis. *Atherosclerosis* (2009) 204(2):321–9. doi: 10.1016/j.atherosclerosis.2008.09.033

19. Lieb W, Gona P, Larson MG, Massaro JM, Lipinska I, Keaney JF, et al. Biomarkers of the osteoprotegerin pathway: clinical correlates, subclinical disease, incident CVD and mortality. *Arteroscler Thromb Vasc Biol* (2010) 30(9):1849–54. doi: 10.1161/ATVBAHA.109.199661

20. Gaudio A, Privitera F, Pulvirenti I, Canzonieri E, Rapisarda R, Fiore CE. Relationships between osteoprotegerin, receptor activator of the nuclear factor kB ligand serum levels and carotid intima-media thickness in patients with type 2 diabetes mellitus. *Panminerva Med* (2014) 56(3):221–5.

21. Semb AG, Ueland T, Aukrust P, Wareham NJ, Luben R, Gullestad L, et al. Osteoprotegerin and soluble receptor activator of nuclear factor-kappaB ligand and risk for coronary events: a nested case-control approach in the prospective EPIC-Norfolk population study 1993-2003. *Arterioscler Thromb Vasc Biol* (2009) 29(6):975–80. doi: 10.1161/ATVBAHA.109.184101

22. Bilgir F, Akan OY, Bayindir AG, Calan M, Bozkaya G, Yuksel A. Relationship between insulin resistance, hs-CRP, and body fat and serum osteoprotegerin/RANKL in prediabetic patients. *Minerva Endocrinol* (2018) 43(1):19–26. doi: 10.23736/S0391-1977.17.02544-5

23. Berezin AE, Kremzer AA, Berezina TA, Yu M. The association of serum osteoprotegerin/sRANKL complex, c-reactive protein and adiponectin with number of circulating endothelial progenitor cells in patients with metabolic syndrome and diabetes mellitus. *J Integr Cardiol* (2015) 1(3):69–75. doi: 10.15751/JIC.1000121

24. Kiechl S, Wittmann J, Giaccari A, Knoflach M, Willeit P, Bozec A, et al. Blockade of receptor activator of nuclear factor-κB (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus. *Nat Med* (2013) 19(3):358–63. doi: 10.1038/nm.3084

25. Karavanaki K, Tsouvalas E, Vakaki M, Soldatou A, Tsentidis CH, Kaparos G, et al. Carotid intima media thickness and associations with serum osteoprotegerin and s-RANKL in children and adolescents with type 1 diabetes mellitus with increased risk for endothelial dysfunction. *J Pediatr Endocrinol Metab* (2018). doi: 10.1515/jpem-2018-0147

26. Kiechl S, Schett G, Schwaiger J, Seppi K, Eder P, Egger G, et al. Soluble receptor activator of nuclear factor-kappa b ligand and risk for cardiovascular disease. *Circulation* (2007) 116(4):385–91. doi: 10.1161/CIRCULATIONAHA.106.686774

27. Wei T, Wang M, Wang M, Gan LY, Li X. Relationship of sRANKL level and vascular calcification score to cardiovascular events in maintenance hemodialysis patients. *Blood Purif* (2009) 28(4):342–5. doi: 10.1159/000232941

28. Rumińska M, Witkowska-Sędek E, Stelmaszczyk-Emmel A, Majcher A, Kucharska A, Pyrżak B. Serum osteoprotegerin and cardiometabolic risk factors in overweight and obese children. *AMS* (2021). doi: 10.5114/aoms/140394

29. WHO Expert Committee. *Physical status: The use and interpretation of anthropometry. WHO technical report series 854*. Geneva: WHO (1995) p. 1–350.

30. Slaughter MH, Lohman TG, Christ CB, Boileau RA. Skinfold equations for estimation of body fitness in children and youth. *Hum Biol* (1998) 60(5):709–23.

31. Cole TJ. The LMS method for constructing normalized growth standard. *Eur J Clin Nutr* (1990) 44(1):45–60.

32. Kułaga Z, Litwin M, Tkaczyk M, Palczewska I, Zajączkowska M, Zwolińska D, et al. Polish 2010 growth references for school-aged children and adolescents. *Eur J Pediatr* (2011) 170:599–609. doi: 10.1007/s00431-010-1329-x

33. Krishnaveni P, Gowda VMN. Assessing the validity of friedewald's formula and anandraja's formula for serum LDL-cholesterol calculation. *J Clin Diagn Res* (2015) 9(12):BC01–4. doi: 10.7860/JCDR/2015/16850.6870

34. Ten S, Maclaren N. Insulin resistance syndrome in children. *J Clin Endocrinol Metab* (2004) 89(6):2526–39. doi: 10.1210/jc.2004-0276

35. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* (2000) 85(7):2402–10. doi: 10.1210/jcem.85.7.6661

36. Krawczyk M, Rumińska M, Witkowska-Sędek E, Majcher A, Pyrżak B. Usefulness of the triglycerides to high-density lipoprotein cholesterol ratio (TG/HDL-c) in prediction of metabolic syndrome in polish obese children and adolescents. *Acta Biochim Pol* (2018) 65(4):605–11. doi: 10.18388/abp.2018_2649

37. Srinivasan SR, Myers L, Berenson GS. Distribution and correlates of non-high density lipoprotein cholesterol in children: The bogalusa heart study. *Pediatrics* (2002) 110(3):e29. doi: 10.1542/peds.110.3.e29

38. Buzi F, Maccarinelli G, Guaragni B, Ruggeri F, Radetti G, Meini A, et al. Serum osteoprotegerin and receptor activator of nuclear factors kB (RANKL) concentrations in normal children and in children with pubertal precocity, turner's syndrome and rheumatoid arthritis. *Clin Endocrinol (Oxf)* (2004) 60(1):87–91. doi: 10.1111/j.1365-2265.2004.01951.x

39. Wasilewska A, Rybi-Szuminska A, Zoch-Zwierz W. Serum osteoprotegrin (OPG) and receptor activator of nuclear factor kappaB (RANKL) in healthy children and adolescents. *J Pediatr Endocrinol Metab* (2009) 22(12):1099–104. doi: 10.1515/jpem.2009.22.12.1099

40. Serrano-Piña R, Trujillo-Güiza ML, Scougall Vilchis RJ, Layton-Tovar CF, Mendieta-Zerón H. sRANKL and its correlation with metabolic syndrome parameters in children. *Int J Paediatr Dent* (2018) 28(6):633–40. doi: 10.1111/ipd.12422

41. Yeşilkaya E, Bideci A, Çamurdan O, Boyraz M, Vurucu S, Cinaz P. Association of osteoprotegerin and rankl levels with insulin resistance in pubertal obese children. *Cent Eur J Med* (2010) 5(2):261–67. doi: 10.2478/s11536-009-0065-y

42. Dimitri P, Wales JK, Bishop N. Adipokines, bone-derived factors and bone turnover in obese children; evidence for altered fat-bone signaling resulting in reduced bone mass. *Bone* (2011) 48(2):189–96. doi: 10.1016/j.bone.2010.09.034

43. Schoppet M, Schaefer JR, Hofbauer LC. Low serum levels of soluble RANK ligand are associated with the presence of coronary artery disease in men. *Circulation* (2003) 107(11):e76. doi: 10.1161/01.cir.0000060815.25798.02

44. Ashley DT, O'Sullivan EP, Davenport C, Devlin N, Crowley RK, McCaffrey N, et al. Similar to adiponectin, serum levels of osteoprotegerin are associated with obesity in healthy subjects. *Metabolism* (2011) 60(7):994–1000. doi: 10.1016/j.metabol.2010.10.001

45. Puengel T, Weber B, Wirtz TH, Buendgens L, Loosen SH, Geisler L, et al. Low serum levels of soluble receptor activator of nuclear factor κ b ligand (sRANKL) are associated with metabolic dysregulation and predict long-term mortality in critically ill patients. *Diagnostics* (2022) 62:12 pages. doi: 10.3390/diagnostics12010062

46. Tsentidis C, Gourgiotis D, Kossiva L, Doulgeraki A, Marmarinos A, Galli-Tsinopoulou A, et al. Higher levels of s-RANKL and osteoprotegerin in children and adolescents with type 1 diabetes mellitus may indicate increased osteoclast signaling and predisposition to lower bone mass: a multivariate cross-sectional analysis. *Osteoporos Int* (2016) 27:1631–43. doi: 10.1007/s00198-015-3422-5

47. Zampetti S, Lucantoni F, Pacifico L, Campagna G, Versacci P, Pierimarchi P, et al. Association of OPG-RANKL ratio with left ventricular hypertrophy and geometric remodeling in male overweight/obese youths. *J Endocrinol Invest* (2019) 42:427–34. doi: 10.1007/s40618-018-0932-y

48. Kawakami R, Nakagami H, Noma T, Ohmori K, Kohno M, Morishita R. RANKL system in vascular and valve calcification with aging. *Inflammation Regeneration* (2016) 36:10. doi: 10.1186/s41232-016-0016-3

49. Sandberg WJ, Yndestad A, Øie E, Smith C, Ueland T, Ovchinnikova O, et al. Enhanced T-cell expression of RANK ligand in acute coronary syndrome: possible role in plaque destabilization. *Arterioscler Thromb Vasc Biol* (2006) 26(4):857–63. doi: 10.1161/01.ATV.0000204334.48195.6a

50. Jørgensen RM, Bøttger B, Vestergaard ET, Kremke B, Bahnsen RF, Nielsen BW, et al. Uric acid is elevated in children with obesity and decreases after weight loss. *Front Pediatr* (2022) 9:814166. doi: 10.3389/fped.2021.814166

51. Luciano R, Shashaj B, Spreghini MR, Del Fattore A, Rustico C, Wietrzykowska Sforza R, et al. Percentiles of serum uric acid and cardiometabolic abnormalities in obese Italian children and adolescents. *Ital J Pediatr* (2017) 43(1):3. doi: 10.1186/s13052-0160-0321-0

52. Pacifico L, Cantisani V, Anania C, Bonaiuto E, Martino F, Pascone R, et al. Serum uric acid and its association with metabolic syndrome and carotid atherosclerosis in obese children. *Eur J Endocrinol* (2009) 160(1):45–52. doi: 10.1530/EJE-08-0618

53. Solis Perez E, González Medina MA, Lopez-Cabanillas Lomeli M, González VT, Villarreal Pérez JZ, Lavalle González FJ, et al. Association between serum uric acid and metabolic syndrome components in prepubertal obese children (Tanner stage I) from nuevo león, Mexico - A preliminary study. *BMC Obes* (2017) 4:25. doi: 10.1186/s40608-017-0160-6

54. Li F, Chen S, Qiu X, Wu J, Tan M, Wang M. Serum uric acid levels and metabolic indices in an obese population: A cross-sectional study. *Diabetes Metab Syndr Obes* (2021) 14:627–35. doi: 10.2147/DMSO.S286299

55. Kanellis J, Kang DH. Uric acid as a mediator of endothelial dysfunction, inflammation, and vascular disease. *Semin Nephrol* (2005) 25(1):39–42. doi: 10.1016/j.semnephrol.2004.09.007

56. Khosla UM, Zharikov S, Finch JL, Nakagawa T, Roncal C, Mu W, et al. Hyperuricemia induces endothelial dysfunction. *Kidney Int* (2006) 67:5. doi: 10.1111/j.1523-1755.2005.00273.x

57. Lin K-M, Lu C-L, Hung K-C, Wu P-C, Pan C-F, Wu C-J, et al. The paradoxical role of uric acid in osteoporosis. *Nutrients* (2019) 11:2111. doi: 10.3390/nu11092111

58. Choe J-Y, Park K-Y, Kim S-K. Monosodium urate in the presence of RANKL promotes osteoclast formation through activation of c-jun n-terminal kinase. *Mediators Inflamm* (2015) 2015:597512. doi: 10.1155/2015/597512

59. Chen H, Li J, Wang Q. Associations between bone-alkaline phosphatase and bone mineral density in adults with and without diabetes. *Med (Baltimore)* (2018) 97(17):e0432. doi: 10.1097/MD.0000000000010432

60. Cheung C-L, Tan KCB, Lam KSM, Cheung BMY. The relationship between glucose metabolism, metabolic syndrome, and bone-specific alkaline phosphatase: a structural equation modeling approach. *J Clin Endocrinol Metab* (2013) 98 (9):3856–63. doi: 10.1210/jc.2013-2024

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 05 September 2022
DOI 10.3389/fendo.2022.1004128

OPEN ACCESS

EDITED BY
Artur Mazur,
University of Rzeszow, Poland

REVIEWED BY
Serhiy Nyankovskyy,
Danylo Halytsky Lviv National Medical University, Ukraine
Sofia Leka,
P&A Kyriakou Childrens Hospital, Greece

*CORRESPONDENCE
Daniel Weghuber
d.weghuber@salk.at

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 26 July 2022
ACCEPTED 08 August 2022
PUBLISHED 05 September 2022

CITATION
Maruszczak K, Radzikowski K, Schütz S, Mangge H, Bergsten P, Forslund A, Manell H, Pixner T, Ahlström H, Kullberg J, Mörwald K and Weghuber D (2022) Determinants of hyperglucagonemia in pediatric non-alcoholic fatty liver disease. *Front. Endocrinol.* 13:1004128. doi: 10.3389/fendo.2022.1004128

Determinants of hyperglucagonemia in pediatric non-alcoholic fatty liver disease

Katharina Maruszczak[1], Konrad Radzikowski[1], Sebastian Schütz[2], Harald Mangge[3], Peter Bergsten[4], Anders Forslund[5], Hannes Manell[5], Thomas Pixner[6], Håkan Ahlström[7], Joel Kullberg[7], Katharina Mörwald[1] and Daniel Weghuber[1]*

[1]Department of Pediatrics, Obesity Research Unit, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria, [2]Department of Mathematics, Paris Lodron University, Salzburg, Austria, [3]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria, [4]Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden, [5]5Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden, [6]Department of Pediatric and Adolescent Medicine, Salzkammergutklinikum Voecklabruck, Voecklabruck, Austria, [7]Department of Radiology, Uppsala University, Uppsala, Sweden & Antaros Medical, BioVenture Hub, Mölndal, Sweden

Objective: Over the years, non-alcoholic fatty liver (NAFLD) disease has progressed to become the most frequent chronic liver disease in children and adolescents. The full pathology is not yet known, but disease progression leads to cirrhosis and hepatocellular carcinoma. Risk factors included hypercaloric diet, obesity, insulin resistance and genetics. Hyperglucagonemia appears to be a pathophysiological consequence of hepatic steatosis, thus, the hypothesis of the study is that hepatic fat accumulation leads to increased insulin resistance and impaired glucagon metabolism leading to hyperglucagonemia in pediatric NAFLD.

Methods: 132 children and adolescents between 10 and 18 years, with varying degrees of obesity, were included in the study. Using Magnetic Resonance Imaging (MRI) average liver fat was determined, and patients were stratified as NAFLD (>5% liver fat content) and non-NAFLD (<5%). All patients underwent a standardized oral glucose tolerance test (OGTT). Additionally, anthropometric parameters (height, weight, BMI, waist circumference, hip circumference) such as lab data including lipid profile (triglycerides, HDL, LDL), liver function parameters (ALT, AST), uric acid, glucose metabolism (fasting insulin and glucagon, HbA1c, glucose 120 min) and indices evaluating insulin resistance (HIRI, SPISE, HOMA-IR, WBISI) were measured.

Results: Children and adolescents with NAFLD had significantly higher fasting glucagon values compared to the non-NAFLD cohort ($p=0.0079$). In the NAFLD cohort univariate analysis of fasting glucagon was associated with BMI-SDS ($p<0.01$), visceral adipose tissue volume (VAT) ($p<0.001$), average liver fat content ($p<0.001$), fasting insulin concentration ($p<0.001$), triglycerides ($p<0.001$) and HDL ($p=0.034$). This correlation equally applied to

10.3389/fendo.2022.1004128

all insulin indices HOMA-IR, WBISI, HIRI (all p<0.001) and SPISE (p<0.002). Multivariate analysis (R^2 adjusted 0.509) for the same subgroup identified HIRI (p=0.003) and VAT volume (p=0.017) as the best predictors for hyperglucagonemia. Average liver fat content is predictive in pediatric overweight and obesity but not NAFLD.

Conclusions: Children and adolescents with NAFLD have significantly higher fasting plasma glucagon values, which were best predicted by hepatic insulin resistance and visceral adipose tissue, but not average liver fat content.

KEYWORDS

glucagon, childhood obesity, NAFLD, pediatrics, hyperglucagonemia

Introduction

Non-alcoholic fatty liver (NAFLD) is associated with obesity, insulin resistance and type 2 diabetes. NAFLD has progressed to become the most frequent chronic liver disease in children and adolescents (1). The estimated prevalence of NAFLD in children is 12.5% (95% CI: 9.2% to 16.7%) in youth with overweight, 36.1% (95% CI: 24.6% to 49.9%) with obesity and 2.3% (95% CI: 1.5% to 3.6%) in children with normal weight, boys thereby exhibiting a higher prevalence than girls (2). NAFLD in adolescents has recently been shown to substantially increase the risk of T2DM in children in general, with as many as one in three children with NAFLD having abnormal glucose metabolism (3–5). Obesity and insulin resistance are known to add up to this development, but beta cell and alpha cell function, respectively, have been shown to alter insulin secretion and cause a hyperglucagonemic state in adults (6–9). The pancreatic hormone glucagon is a key player in blood glucose regulation and the pathophysiology of diabetes (10). Increased fasting levels of glucagon can be found in T2DM patients and in subjects with obesity and normal glucose tolerance (11, 12). Hypoglycemia is one of the major stimulants for glucagon secretion triggering hepatic glucose production (13). NAFLD consequently leads to impaired hepatic glucagon signaling resulting in hyperglucagonemia (13). The concept of the liver-alpha cell axis provides a potential causal explanation for this phenomenon (14, 15). Previously, we demonstrated that hyperglucagonemia is associated with hyperinsulinemia, high plasma free fatty acids (FFAs), high plasma triglycerides, visceral adiposity, and impaired glucose tolerance as early as during childhood (3). To date, there is a lack of studies assessing alpha cell function in pediatric NAFLD. In this study we hypothesize, that hepatic fat accumulation leads to increased insulin resistance and impaired glucagon metabolism followed by hyperglucagonemia in pediatric NAFLD.

Material and methods

Study population and design

The ethics approval for the study was obtained from the ethical committee of Salzburg (Number: 1544/2012) and the Uppsala regional ethical review committee (number 2012/318). The study was carried out according to the Declaration of Helsinki. Written informed consent was achieved from all participants and at least one of their caregivers.

A cross-sectional retrospective study was conducted in two study centers, Uppsala University Hospital, Sweden, and at Paracelsus Medical University Hospital in Salzburg, Austria. Data analysis was based on the material obtained by the BETA JUDO study (BETA cell function in Juvenile Diabetes and Obesity, FP7-HEALTH-2011-two-stage, project number: 279153). In total, 206 patients received MRI scans for body fat composition assessment. The MRI scans (liver fat content, body fat composition, abdominal visceral and subcutaneous fat) as previously described (16) was determined by 1.5 T clinical MRI systems from Philips Medical System (Netherlands). Patients aged 10-18 years with overweight or obesity according to the WHO criteria (BMI-SDS>1.26) and control subjects without overweight or obesity were included. Exclusion criteria were presence of chronic liver disease, known pre- and diabetes, psychiatric disorders, allergies, alcohol intake, consuming steatogenic drugs, endocrine disorders and/or hereditary causes of liver disease. 184 patients (target group) fulfilled the overweight and obesity selection criteria, and 22 patients completed the control group. The target group was divided further into a NAFLD and non-NAFLD (defined by liver fat content ≥5 and ≤ 5 %) group. After matching for BMI-SDS a sub-sample of 132 patients was selected.

10.3389/fendo.2022.1004128

Anthropometric and blood pressure measurements

Height and weight were assessed by standardized and calibrated scales (Seca, Hamburg, Germany) and stadiometers (Uppsala: Ulmer (Busse Design + Engineering GmbH; Elchingen, Germany); Salzburg: Seca). The BMI-SDS was calculated with Microsoft Excel add-in LMS Growth using WHO growth report (17). Waist circumference (cm) was measured midway between the superior border of the iliac crest and lowest rib. Systemic blood pressure was measured using a standardized clinical aneroid sphygmomanometer (Uppsala: CAS 740; CAS Medical Systems, Inc, Branford, Conn; Salzburg: Carescape V100; Dinamap Technology/GE, Vienna, Austria), two measurements were taken, and the means were used for analyses. According to Tanner, patients were categorized into their puberty stages, prepubertal (group 1 = Tanner I), pubertal (group 2 = Tanner II–IV), and postpubertal (group 3 = Tanner V).

Blood sampling and biochemical measurements

All blood sample parameters were drawn after an overnight fast. Following this, all subjects underwent a standard OGTT, as previously described (17). In short, the OGTT was done according to standard procedures. Patients received a glucose solution concentrated 1.75 g glucose/kg body weight (maximum 75g glucose) and blood sampling was performed at time points -5, 5, 10, 15, 30, 60, 90, 180 min. Blood was sampled through a venous catheter.

Glucose, triacylglycerides (TG) and high-density lipoproteins (HDL) were analyzed according to local protocols. In Uppsala, glucose was analyzed using an Architect c8000 instrument (Abbott Diagnostics, Solna, Sweden) and by a Gluco-quant Glucose-Kit (Roche Diagnostics, Mannheim, Germany) in Salzburg. Uppsala quantified TG and HDL using an Architect c800 instrument (Abbott Diagnostics) and in Salzburg an enzymatic photometric test (Modular Analytics System). Additional evaluation of LDL cholesterol was required, which was done with an enzymatic photometric test using Integra Manual by Roche Diagnostics. An enzyme-linked immunosorbent assay (ELISA) (Modular Analytics System, E-Modul by Roche Diagnostics) was used to analyze leptin and adiponectin. HbA1c was measured by reversed-phase chromatography (RP-HPLC). P-Modul, 917; Roche Diagnostics) was used. Validation of analyses was performed between the laboratories in Uppsala and Salzburg using reference blood samples.

Selected samples underwent immediate centrifugation at 2500g for 10 minutes at 4°C, subsequently aliquoted, and frozen at −80°C. Plasma was later used for central analyses of insulin and glucagon in Uppsala for both study centers. Singleplex enzyme-linked immunosorbent assay kits for each analyte were used (Mercodia AB, Uppsala, Sweden). Standardized control samples (Mercodia AB) were used to control for interplate variability.

Assessment of insulin resistance

The following indices were used for the determination of insulin resistance (IR) and insulin sensitivity.

To measure hepatic insulin resistance the homeostatic model assessment (HOMA) (Wallace 2004) and the Hepatic Insulin Resistance Index (HIRI) were used. The HOMA- insulin resistance (IR) was calculated as the product of fasting glucose (mmol/L) and fasting insulin (μU/ml) divided by constant 22.5 (17, 18) and the HIRI measured as the product of the area under the curves (AUCs) of glucose and insulin for the first 30 min of the OGTT (19). Insulin sensitivity was calculated by the Single Point Insulin Sensitivity Estimator (SPISE), the newest biomarker for insulin sensitivity developed by Paulmichl et al (20) and the Matsuda Whole Body Insulin Sensitivity Index (WBISI). The SPISE is calculated by the product of the constant 600 and HDL-cholesterol^{0,185} divided by the product of triglycerides^{0,2} and BMI^{1,33835} and the WBISI (21, 22):

$$\frac{10{,}000}{\sqrt{\frac{(\text{fasting glucose} \times \text{fasting insulin})}{\text{mean glucose} \times \text{mean insulin}}}}$$

Statistical analysis

The data was analyzed descriptively showing results with mean and standard deviation for metric variables and number and percentages for categorical variables for a matched cohort.

NAFLD groups were matched 1:1 according to nearest neighbor algorithm for BMI-SDS. Matching performance was assessed applying Wilcoxon rank sum tests for unpaired samples pre- and post-matching resulting in significant differences before ($p < 0.001$) and non-significant results after matching ($p = 0.383$). Cohort size was reduced from n = 184 (79 vs. 105) to n = 132 (66 vs. 66) due to matching process.

Due to non-normality of the data, groupwise differences in glucagon were examined applying non-parametric Wilcoxon rank sum tests for unpaired samples. Further investigations of dependencies were assessed using univariate regression models. Our standardized multivariate model resulted, including significantly correlating parameters to fasting glucagon, from univariate models. In a second step the multivariate model size was reduced by exclusion of parameters which were not

significantly different in the group differences. In a final step, variables were excluded for multicollinearity based on the variance inflation factor (VIF). The threshold for this exclusion was set to 10 as suggested in the literature (23).

All results are presented along with 95%-Confidence Intervals. Tests are performed at a significance level of 5%. P-values in multivariable models are corrected with Bonferroni-Holm method for multiple testing. No p-value correction for the remaining results. Statistical analysis was done with R (version 4.0.2). Important R-packages: leaps (selection algorithm), MatchIt (matching).

Results

Baseline characteristics

Clinical and anthropometric features of the study population are shown in Table 1. After matching for BMI-SDS the study population included 132 adolescents with overweight and obesity, mean BMI being 31.53 ± 6.86 kg/m2 and mean age 14.09 ± 2.34. Mean BMI between the NAFLD and non-NAFLD groups was similar, however the waist-to-hip ratio showed a significant difference between the groups (NAFLD 0.98 ± 0.08 vs. 0.94 ± 0.08, p = 0.02).

Body fat composition variables presented a significant difference between the mean liver fat content (NAFLD 15.58 ± 10.95% vs. non-NAFLD 3.17 ± 0.97%, p=0.00) and the visceral adipose tissue (VAT) (NAFLD 1753.07 ± 662.31 vs. non-NALFD 1302.27 ± 433.52, p=0.00) in the matched study population (N=132). Of the biochemical parameters of glucose metabolism fasting insulin (NAFLD 129.46 ± 66.40 vs. non NAFLD 104.00 ± 46.58, p=0.08), fasting glucagon (NAFLD 14.20 ± 8.54 vs. non-NAFLD 10.36 ± 3.99, p=0.01) and HOMA-IR (NAFLD 4.41 ± 2.62 vs. non-NAFLD 3.32 ± 1.54, p=0.06) presented significant differences between the groups. Additionally, among the lipid profile markers significant differences between the groups could be observed between the LDL cholesterol (NAFLD 2.58 ± 0.79 vs. 2.40 ± 0.61, p=0.08) and the triglycerides (NAFLD 1.37 ± 0.73 vs. 1.06 ± 0.51, p=0.01). Finally, all enzymatic liver function parameters (ALT, AST, GGT) presented significant differences between the NAFLD and non NAFLD patients (AST: NAFLD 0.64 ± 0.44 vs. non NAFLD 0.45 ± 0.25, p=0.00, ALT: NAFLD 0.80 ± 0.79 vs. non-NAFLD 0.37 ± 0.20, p=0.00, GGT: NAFLD 0.48 ± 0.42 vs. non NAFLD 0.29 ± 0.11, p=0.00). HOMA-IR was the only metabolic index that showed significant differences between the two groups (NAFLD 4.41 ± 2.62 vs. non NAFLD 3.32 ± 1.54, p=0.06).

Fasting glucagon concentrations correlate with metabolic parameters in adolescents with NAFLD and non NAFLD

Figure 1 demonstrates that fasting plasma glucagon levels are significantly different between the NAFLD and non-NAFLD group (p<0.01), N=132.

A univariate analysis for fasting plasma glucagon levels matched for BMI-SDS was constructed according to metabolic variables (glucose metabolism, liver function and lipid profile) such as body fat composition and clinical features. Variables of glucose metabolism (OGTT 120 min p=0.044, fasting insulin p<0.001, SPISE p=0.002, WBISI p=0.001, HIRI p<0.001, HOMA-IR p<0.001), of lipid profile (HDL cholesterol p=0.034, triglycerides p<0.001) and liver function (AST p=0.010, p=0.010, p=0.009), indicated a significant relationship with glucagon (Table 2).

Figure 2 presents a scatterplot of all NAFLD patients stratified by liver fat content quartiles, resulting in the following cut-offs: 25% = 2.91% liver fat content, 50% = 5.11% liver fat content, 75% = 11.73% liver fat content. Looking at the relationship between glucagon and average liver fat within each quartile, a clear positive trend can be observed in quartile 4 (Figure 2).

Predictors of fasting glucagon concentrations in patients with overweight and obesity

A standardized multivariate regression model was constructed based on the glucagon predictors BMI-SDS, average liver fat, fasting insulin, glucose at 120 min, SPISE and ALT in overweight and patients with overweight/obesity (Table 3). Significant drivers of this model (R2 = 0.336) were liver fat content (p=0.044), VAT (p=0.031), fasting insulin levels (p=0.016) and alanine aminotransferase (p=0.030). By contrast BMI-SDS (p=1.00), OGTT glucose 120 min (p=1.00) and SPISE (p=1.00) did not change the significant driving variables.

Predictors of fasting glucagon concentrations in patients with NAFLD

A multivariate regression model to evaluate predictors of glucagon in NAFLD adolescents was applied, with average liver fat being a conditional variable in the models. The model (Table 4, R^2 = 0.509) included VAT and HIRI index. VAT (p=0.017) and the HIRI index (p=0.003) resulted with a predictive effect for hyperglucagonemia in pediatric NAFLD.

10.3389/fendo.2022.1004128

TABLE 1 Descriptive data of all patient (study population, N = 132) and comparison of baseline characteristic difference between NAFLD (n = 66) and non- NAFLD (n = 66) patients.

	Study population (N = 132)	NAFLD (n = 66)	non-NAFLD (n = 66)	p-value
CLINICAL FEATURES				
Age (Years)	14.09 ± 2.34	14.38 ± 2.33	14.03 ± 2.40	0.49
Tanner stage*	I: 10 (7.6%) II-IV: 83 (62.9%) V: 30 (22.7%) n.a.: 9 (6.8%)	I: 7 (10.1%) II-IV: 42 (63.6%) V: 13 (19.6%) n.a.: 4 (6.1%)	I: 3 (4.5%) II-IV: 41 (62.1%) V: 173(25.6%) n.a.: 5 (7.8%)	0.34
BMI (mg/m2)#	31.53 ± 6.86	32.28 ± 4.81	32.05 ± 4.96	0.74
BMI-SDS#	2.57 ± 1.12	2.84 ± 0.51	2.77 ± 0.49	0.38
SBMI (kg/m2)	33.98 ± 5.83	34.93 ± 3.52	34.63 ± 3.51	0.57
Waist circumference (cm)#1	101.95 ± 17.45	105.35 ± 13.25	103.85 ± 12.65	0.77
Hip circumference (cm)	107.81 ± 14.89	107.88 ± 11.57	110.45 ± 12.76	0.16
Waist-to-hip-ratio	0.94 ± 0.09	0.98 ± 0.08	0.94 ± 0.08	0.02
RR systolic (mmHg)#3	120.53 ± 11.57	121.51 ± 12.18	121.28 ± 10.55	0.99
BODY FAT COMPOSITION				
Total body fat (%)	42.50 ± 5.87	41.70 ± 6.42	41.54 ± 4.89	0.87
MRI VAT volume (cm3)#2	1463.46 ± 703.24	1753.07 ± 662.31	1302.27 ± 433.52	0.00
MRI SAT volume (cm3)#2	6332.09 ± 2789.08	6687.25 ± 2060.33	6625.70 ± 2127.87	0.99
MRI liver fat content (%)#	9.38 ± 10.26	15.58 ± 10.95	3.17 ± 0.97	0.00
GLUCOSE METABOLISM				
Fasting glucose (mmol/L)	5.15 ± 1.27	5.25 ± 2.01	5.01 ± 0.60	0.54
OGTT 120 min. glucose (mmol/L)#	6.72 ± 2.10	7.16 ± 2.99	6.41 ± 1.39	0.22
Fasting insulin (pmol/L)#5	111.85 ± 66.79	129.46 ± 66.40	104.00 ± 46.58	0.08
HbA1c (mmol/mol)	35.81 ± 7.08	37.47 ± 11.85	34.77 ± 2.33	0.15
Fasting glucagon (pmol/L)	11.94 ± 6.69	14.20 ± 8.54	10.36 ± 3.99	0.01
METABOLIC INDICES				
SPISE#1	5.47 ± 2.43	4.80 ± 1.24	5.12 ± 1.34	0.22
WBISI#7	5.47 ± 4.33	4.14 ± 2.60	4.89 ± 2.25	0.10
HIRI#8	47861.88 ± 30367.22	52950.30 ± 26984.56	45451.40 ± 21248.58	0.46
HOMA-IR#6	3.75 ± 2.51	4.41 ± 2.62	3.32 ± 1.54	0.06
LIPID PROFILE				
Total cholesterol (mmol/L)	4.12 ± 0.76	4.30 ± 0.87	4.13 ± 0.66	0.24
LDL cholesterol (mmol/L)	2.40 ± 0.69	2.58 ± 0.79	2.40 ± 0.61	0.08
HDL cholesterol (mmol/L)#1	1.25 ± 0.32	1.19 ± 0.24	1.28 ± 0.37	0.32
Triglyceride (mmol/L)#	1.17 ± 0.69	1.37 ± 0.73	1.06 ± 0.51	0.01
LIVER FUNCTION				
AST (µkat/L)#4	0.53 ± 0.31	0.64 ± 0.44	0.45 ± 0.25	0.00
ALT (µkat/L)#	0.55 ± 0.54	0.80 ± 0.79	0.37 ± 0.20	0.00
GGT (µkat/L)#	0.37 ± 0.28	0.48 ± 0.42	0.29 ± 0.11	0.00

Data are expressed a mean ± standard deviation (SD).

$p < 0.05$.

n = 104 for BMI, BMI-SDS, liver fat content, OGTT 120 min. glucose, triglycerides, ALT, GGT; #1n = 103 for waist circumference, HDL-cholesterol, SPISE; #2n= 102 for VAT and SAT volume; #3n = 101 for systolic blood pressure; #4n = 98 for AST; #5n = 75 for fasting insulin; #6n = 73 for HOMA-IR; #7n = 63 for WBISI; #8n = 60 for HIRI.

NAFLD, non-alcoholic fatty liver disease; n.a., not available; BMI, body mass index; BMI-SDS, body mass index standard deviation score; SBMI, smart BMI; RR, blood pressure; HbA1c, hemoglobin A1c; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; OGTT, oral glucose tolerance test; MRI, magnetic resonance imaging; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; SPISE, single point insulin sensitivity estimator; WBISI, whole-body insulin sensitivity index; HOMA-IR, homeostatic model assessment for insulin resistance; HIRI, hepatic insulin resistance index.

*Tanner staging I–V: I, prepubertal; II–IV, pubertal; V = post-pubertal.

10.3389/fendo.2022.1004128

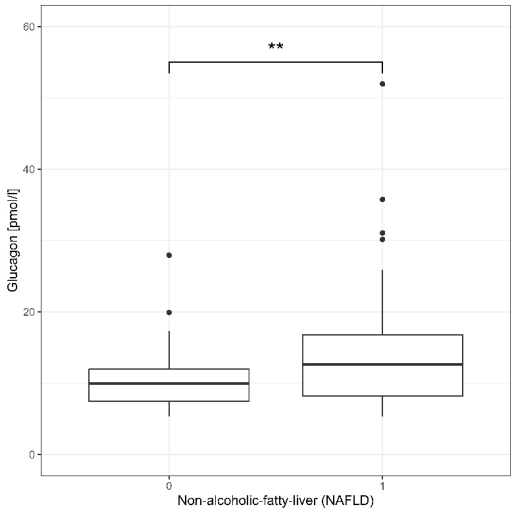


FIGURE 1
Fasting glucagon concentration pmol/L of participant without NAFLD (0, n = 66) and with NAFLD (1, n = 66) are shown a box plot in quartile and outliers. Wilcoxon test yielded a highly significant result p=0.0079 a **p < 0.01.

TABLE 2 Univariate analysi for fasting glucagon (pmol/L) in patient with overweight/obesity (n = 104#) matched for BMI-SDS.

	coefficient	p-value	R2
CLINICAL FEATURES			
BMI (mg/m2)	0.25	0.073	0.022
BMI-SDS	3.50	0.010**	0.054
Waist circumference (cm)	0.09	0.083	0.020
RR systolic (mmHg)	0.05	0.359	-0.002
BODY FAT COMPOSITION			
MRI VAT volume (cm3)	0.01	<0.001***	0.194
MRI SAT volume (cm3)	0.00	0.341	-0.001
MRI liver fat content (%)	0.28	<0.001***	0.144
GLUCOSE METABOLISM			
OGTT 120 min. glucose (mmol/L)	0.54	0.044*	0.030
Fasting insulin (µIU/mL)	0.07	<0.001***	0.248
SPISE	-1.61	0.002**	0.079
WBISI	-1.28	0.001***	0.152
HIRI	0.00	<0.001***	0.234
HOMA-IR	1.72	<0.001***	0.211
LIPID PROFILE			
HDL cholesterol (mmol/L)	-4.51	0.034*	0.034
Triglyceride (mmol/L)	4.34	<0.001***	0.160
LIVER FUNCTION			
AST (µkat/L)	4.55	0.010**	0.057
ALT (µkat/L)	2.61	0.010**	0.055
GGT (µkat/L)	5.45	0.009**	0.056

*p < 0.05, **p < 0.01, ***p < 0.001.
n = 104 for BMI, BMI-SDS, liver fat content, OGTT 120 min. glucose, triglycerides, ALT, GGT; n = 103 for waist circumference, HDL-cholesterol, SPISE; n = 102 for VAT and SAT volume, hsCRP; n = 101 for systolic blood pressure; n = 98 for AST; n = 78 for Il-6, TNF alpha; n = 75 for fasting insulin; n = 73 for HOMA-IR; n = 63 for WBISI; n = 60 for HIRI.
BMI, body mas index; BMI-SDS, body mas index standard deviation score; SBMI, smart BMI; RR, blood pressure; HbA1c, hemoglobin A1c; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; OGTT, oral glucose tolerance test; MRI, magnetic resonance imaging; VAT, visceral adipose tissue; SAT, subcutaneou adipose tissue; DSAT, deep subcutaneou adipose tissue; SSAT, superficial subcutaneou adipose tissue; SPISE, single point insulin sensitivity estimator; WBISI, whole-body insulin sensitivity index; HOMA-IR, homeostatic model assessment for insulin resistance; HIRI, hepatic insulin resistance index.

10.3389/fendo.2022.1004128

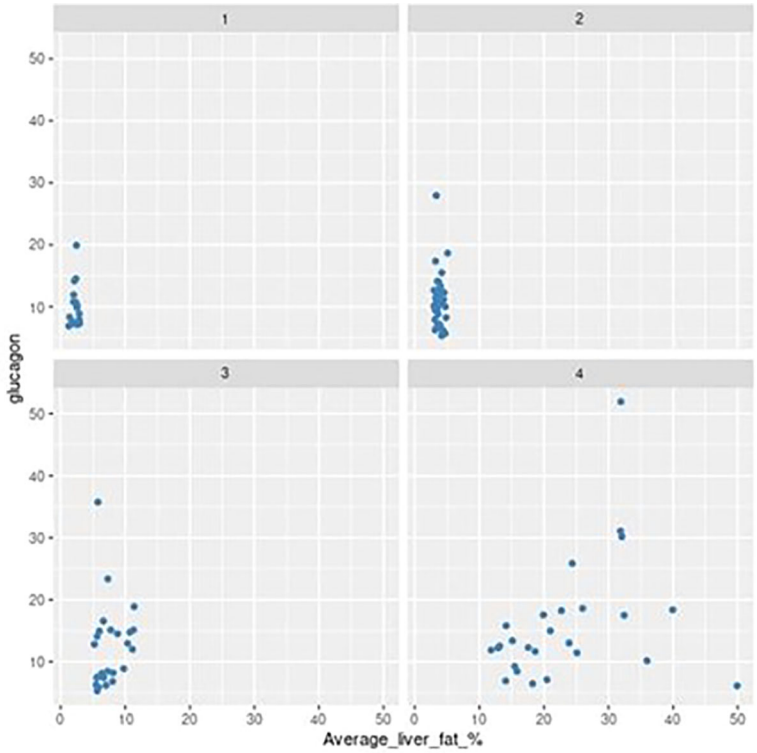


FIGURE 2
Scatter plot showing relationship between average liver fat (%) and fasting glucagon (pmol/L) grouped by MRI quartile (1-4).

TABLE 3 Multivariate regression analysis with standardized coefficients: predictor of glucagon in a pediatric cohort with overweight/obesity (n = 73) matched for BMI-SDS (R2 = 0.336), correct by Bonferroni-Holm algorithm.

	Coefficient	p-value
Anthropometric data		
BMI-SDS	-0.10	1.000
MRI VAT volume (cm**3**)	0.45	0.031*
MRI liver fat content (%)	0.47	0.044*
Metabolic data		
OGTT 120 min. glucose (mmol/L)	-0.04	1.000
Fasting insulin (µIU/mL)	0.40	0.016*
SPISE	0.04	1.000
ALT (µkat/L)	-0.46	0.030*

*$p < 0.05$, tested for multicollinearity.
BMI-SDS, body mas index standard deviation score; MRI, magnetic resonance imaging; VAT, visceral adipose tissue; ALT, alanine aminotransferase; OGTT, oral glucose tolerance test; SPISE, single point insulin sensitivity estimator.

Discussion

This is the first study to examine predictors of hyperglucagonemia in a pediatric NAFLD population. Our data identify visceral adipose tissue and the HIRI index as surrogate for hepatic insulin resistance as determinants of hyperglucagonemia in pediatric NAFLD.

Obesity significantly increases the risk of NAFLD, effecting the pediatric population with obesity in large numbers (24). VAT and WHR, but not BMI, predicted increased levels of glucagon in our cohort. This implies that the accumulation of visceral fat rather than adiposity associates with deranged glucagon metabolism. This is in accordance with the findings of Manell et al., who concluded that high levels of glucagon are

TABLE 4 Multivariate regression analysi with standardized coefficients: determinant of hyperglucagonemia in NAFLD (n = 66) matched for BMI-SDS (Model R^2 = 0.509).

Model	Coefficient	p-value
Average liver fat, %	0.097620	0.477
VAT, cm^3	0.006884	0.017*
HIRI	0.000187	0.003*

*p < 0.05, tested for multicollinearity.
VAT, visceral adipose tissue; HIRI, Hepatic Insulin Resistance Index.

related to VAT, rather than liver fat content, pancreas fat content and subcutaneous adipose tissue (SAT) (3). A positive relation between fasting hyperglucagonemia and increased WHR could also be observed in an adult cohort where WHR turned out to be the best anthropometric predictor of NAFLD (25, 26). This indicates that VAT and central adiposity are closely related to NAFLD occurrence (27). Analyzing data from a different pediatric cohort, our group previously showed that increased WHR is related to increased VAT and fasting insulin levels in children with obesity and increased hepatic liver fat content (28). This is in keeping with a plethora of studies reporting that WHR and VAT can be identified as indirect parameters of insulin resistance (29, 30).

In our study, univariate analysis not only indicated a significant relationship of fasting insulin, VAT, MRI liver fat content with glucagon in our cohort of children and adolescents with overweight and obesity, but also with the liver enzyme alanine transaminase (ALT). Alanine transaminase levels are accepted as surrogates of NAFLD in clinical practice (30, 31), although liver enzymes are known to be limited in sensitivity and specificity in the diagnosis of pediatric NAFLD (31, 32). It is worth mentioning in this context, that children with overweight/obesity and elevated ALT values had a more than 2-fold increased risk for future dysglycemia independent of age, sex and BMI-SDS in a survival analysis of up to 11 years of follow-up of 510 children with overweight and obesity from the Leipzig Childhood Cohort. Hence, elevated transaminases were suggested as an early predictor for glycemic deterioration (31).

The multiple regression model identified VAT and the HIRI index as the best predictive variables for hyperglucagonemia in our pediatric NAFLD cohort. The HIRI index is a dynamic surrogate index derived from the OGTT. Recent studies have evaluated the predictive accuracy of surrogate indices for hepatic insulin resistance derived from dynamic tests, such as the HIRI, suggesting that these are suitable alternatives to describe ß-cell function (20, 22, 33). Similar to our pediatric cohort, D'Adamo and Deivanayagam concluded that an increase in intrahepatic fat is associated with an increase in the HIRI index (34, 35). Additionally, when considering surrogates of insulin sensitivity (HOMA IR, HIRI), adult studies showed that increased circulating levels of fasting glucagon, together with increased insulin levels, are tightly coupled to a reduction of insulin sensitivity in individuals with normal and disturbed glucose metabolism (36–38). In our study cohort a similar relationship could be observed in children with NAFLD. This is an interesting aspect as direct glucagon suppression is caused by insulin stimulation as seen in non-diabetic subjects (39). Recently, evidence of a liver-alpha cell axis in humans was introduced (40, 41). The concept claims that fat accumulation in the liver attenuates the sensitivity of hepatocytes towards glucagon causing impaired hepatic glucagon signaling and consequently results in hyperglucagonemia (41, 42). Our data are in line with previous adult studies (41–43) supporting the existence of such a liver-alpha cell feedback loop as early as during childhood. Faerch et al. showed that glucose regulation during development of insulin resistance was linked not merely with hypersecretion of insulin, but also with a reduced capability to acutely suppress glucagon after glucose intake in adults (37). Hypersecretion of glucagon from pancreatic alpha cells has hence been suggested to be due to an impairment of hepatic glucagon signaling, which then, due to decreased glucagon-induced amino acid turnover, would result in hyperaminoacedemia (14). Lischka et al. recently reported higher levels of plasma branched-chain amino acids in children with NAFLD, suggesting that BCAAs could be an important link between obesity and other metabolic pathways (43). However, the association between amino acid and glucagon metabolism in pediatric NAFLD has yet to be studied.

There are some strengths and limitations that need to be acknowledged. The primary strength of the study was a relatively large study cohort of children with MRI diagnosed NAFLD. Although the gold standard of diagnosis of different stages of NAFLD – steatosis to fibrosis/cirrhosis - would be liver biopsy, MRI scans are well suited to quantify liver fat content which was the aim of this study (16). A limitation of the current study is that the conclusions cannot be translated to other ethnic groups other than Caucasian. Further, a more detailed characterization according to pubertal stages matched by age and BMI-SDS was not feasible due to statistical limitations related to sample size.

In summary, our results identify that average liver fat content is predictive in pediatric overweight and obesity. Visceral adipose tissue (VAT) and the HIRI index were identified as determinants of hyperglucagonemia in pediatric NAFLD, but not average liver fat content.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by ethical committee of city of Salzburg. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

KMa and DW conceived the idea and designed the study. KR designed the tables. SS performed the statistical analysis. HarM, PB, AF, HanM, TP, HA, JK and KMö revised the paper. KMa and DW wrote the manuscript draft. All authors revised and accepted the final version of the manuscript.

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.

References

1. Crespo M, Lappe S, Feldstein AE, Alkhouri N. Similarities and differences between pediatric and adult nonalcoholic fatty liver disease. *Metabolism* (2016) 65:1161–71. doi: 10.1016/j.metabol.2016.01.008

2. Anderson EL, Howe LD, Jones HE, Higgins JPT, Lawlor DA, Fraser A, et al. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. *PloS One* (2015) 10(10):e0140908. doi: 10.1371/journal.pone.0140908

3. Manell H, Kristinsson H, Kullberg J, Ubhayasekera K, Mörwald K, Staaf J, et al. Hyperglucagonemia in youth is associated with high plasma free fatty acids, visceral adiposity and impaired glucose tolerance. *Pediatr Diabetes* (2019) 20 (7):880–91. doi: 10.1111/pedi.12890

4. Lerret SM, Garcia-Rodriguez L, Skelton J, Biank V, Kilway D, Telega G. Predictors of non-alcoholic steatohepatitis in obese children. *Gastroenterol Nurs* (2011) 34:434–7. doi: 10.1097/SGA.0b013e3182371356

5. Newton KP, Hou J, Crimmins NA, Lavine JE, Barlow SE, Xanthakos SA, et al. Prevalence of prediabetes and type 2 diabetes in children with nonalcoholic fatty liver disease. *JAMA Pediatr* (2016) 170(10):e161971. doi: 10.1001/jamapediatrics.2016.1971

6. Utzschneider KM, Largajolli A, Bertoldo A, Marcovina S, Nelson JE, Yeh MM, et al. Serum ferritin is associated with non-alcoholic fatty liver disease and decreased b-cell function in non-diabetic men and women. *J Diabetes Complicat* (2014) 28(2):177–84. doi: 10.1016/j.jdiacomp.2013.11.007

7. Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* (2006) 91:4753–61. doi: 10.1210/jc.2006-0587

8. Chon CW, Kim BS, Cho YK, Sung KC, Bae JC, Kim TW, et al. Effect of nonalcoholic fatty liver disease on the development of type 2 diabetes in nonobese, nondiabetic korean men. *Gut Liver* (2012) 6:368–73. doi: 10.5009/gnl.2012.6.3.368

9. Sung KC, Jeong WS, Wild SH, Byrne CD. Combined influence of insulin resistance, overweight/obesity, and fatty liver as risk factors for type 2 diabetes. *Diabetes Care* (2012) 35:717–22. doi: 10.2337/dc11-1853

10. Albrechtsen Wewer NJ, Kuhre RE, Pedersen J, Knop FK. The biology of glucagon and the consequences of hyperglucagonemia. *Biomark Med Act* (2016) 10 (11):1141–51. doi: 10.2217/bmm-2016-0090

11. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J Clin Invest* (2012) 122(1):4–12. doi: 10.1172/JCI60016

12. Manell H, Staaf J, Manukyan L, Kristinsson H, Cen J, Stenlid R, et al. Altered plasma levels of glucagon, GLP-1 and glicentin during OGTT in adolescents with obesity and type 2 diabetes. *J Clin Endocrinol Metab* (2016) 101(3):1181–9. doi: 10.1210/jc.2015-3885

13. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem* (2004) 279:32345–53. doi: 10.1074/jbc.M313478200

14. Albrechtsen WN, Junker AE, Christensen M, Hædersdal S, Wibrand F, Lund A, et al. Hyperglucagonemia correlates with plasma levels of non-branched-chain amino acids in patients with liver disease independent of type 2 diabetes. *Am J Physiol Gastrointest Liver Physiol* (2018) 314:G91–6. doi: 10.1152/ajpgi.00216.2017

15. Solloway Mark J, Madjidi A, Gu C, Eastham-Andersen J, Clarke HJ, Kljavin N, et al. Glucagon couples hepatic amino acid catabolism to mTOR-dependent regulation of αcell mass. *Cell Rep* (2015) 12:495–510. doi: 10.1016/j.celrep.2015.06.034

16. Mörwald K, Aigner E, Bergsten P, Brunner SM, Forslund A, Kullberg J, et al. Serum ferritin correlates with liver fat in Male adolescents with obesity. *Front Endocrinol (Lausanne).* (2020) 11:340. doi: 10.3389/fendo.2020.00340

17. Disse E, Bastard JP, Bonnet F, Maitrepierre C, Peyrat J, Louche-Pelissier C, et al. A lipid-parameter-based index for estimating insulin sensitivity and identifying insulin resistance in a healthy population. *Diabetes Metab* (2008) 34 (5):457–63. doi: 10.1016/j.diabet.2008.02.009

18. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* (2007) 85(9):660–7. doi: 10.2471/BLT.07.043497

19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* (1985) 28 (7):412–9. doi: 10.1007/BF00280883

20. Yeckel CW, Weiss R, Dziura J, Taksali SE, Dufour S, Burgert TS, et al. Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. *J Clin Endocrinol Metab* (2004) 89(3):1096–101. doi: 10.1210/jc.2003-031503

21. Paulmichl K, Hatunic M, Højlund K, Jotic A, Krebs M, Mitrakou A, et al. Modification and validation of the triglyceride-to-HDL cholesterol ratio as a surrogate of insulin sensitivity in white juveniles and adults without diabetes mellitus: The single point insulin sensitivity estimator (SPISE). *Clin Chem* (2016) 62(9):1211–9. doi: 10.1373/clinchem.2016.257436

22. Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. *Diabetes Care* (2007) 30(1):89–94. doi: 10.2337/dc06-1519

23. Hedderich J, Sachs L. Angewandte statistik. In: *Methodensammlung mit r (16. aufl.)*. Berlin: Springer Spektrum (2018).

24. Kelsey M, Zaepfel A, Bjornstad P, Nadeau K. Age-related consequences of childhood obesity. *Gerontology* (2014) 60:222–8. doi: 10.1159/000356023

25. Czernichowa S, Kengnec AP, Stamatakis E, Hamerd M, Batty D. Body mass index, waist circumference, and waist-hip ratio: which is the better discriminator of cardiovascular disease mortality risk? evidence from an individual-participant metaanalysis of 82,864 participants from nine cohort stud. *Obes Rev* (2011) 12(9):680–7. doi: 10.1111/j.1467-789X.2011.00879.x

26. Moore L, Fals AM, Jennelle PJ, Green JF, Pepe J, Richard T. Analysis of pediatric waist to hip ratio relationship to metabolic syndrome markers. *J Pediatr Health Care* (2015) 29(4):319–24. doi: 10.1016/j.pedhc.2014.12.003

27. Demant M, Bagger JI, Suppli MP, Lund A, Gyldenløve M, Hansen KB, et al. Determinants of fasting hyperglucagonemia in patients with type 2 diabetes and nondiabetic control subjects. *Metab Syndrome Related Disord* (2018) 16(10):530–6. doi: 10.1089/met.2018.0066

28. Weghuber D, Roden M, Franz C, Chmelik M, Torabia S, Nowotny P, et al. Vascular function in obese children with non-alcoholic fatty liver disease. *Int J Pediatr Obes* (2011) 6:120 –127. doi: 10.3109/17477161003792580

29. Vajro P, Lenta S, Socha P, Dhawan A, McKiernan P, Baumann U, et al. Consensus statement, diagnosis of noalcoholic fatty liver disease in children and adolescents: Position paper of the ESPGHAN hepathology committee. *JPGN* (2012) 54:700–13. doi: 10.1097/mpg.0b013e318252a13f

30. Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, et al. Histopathology 7. of pediatric non-alcoholic fatty liver disease. *Hepatology* (2005) 42:641 – 9. doi: 10.1002/hep.20842

31. Koutny F, Weghuber D, Bollow E, Greber-Platzer S, Hartmann K, Körner A, et al. Prevalence of prediabetes and type 2 diabetes in children with obesity and increased transaminases in European German-speaking countries. analysis of the APV initiative. *Pediatr Obes* (2019) 15:1–8. doi: 10.1111/ijpo.12601

32. Ferrannini E, Muscelli E, Natali A, Gabriel R, Mitrakou A, Flyvbjerg A, et al. Association of fasting glucagon and proinsulin concentrations with insulin resistance. *Diabetologia* (2007) 50(11):2342–7. doi: 10.1007/s00125-007-0806-x

33. Muniyappa R, Tella Harsha S, Sortur S, Mszar R, Grewal S, Abel BS, et al. Predictive accuracy of surrogate indices for hepatic and skeletal muscle insulin sensitivity. *J Endocr Soc* (2019) 3(1):108–18. doi: 10.1210/js.2018-00206

34. Deivanayagam S, Mohammed B, Vitola B, Naguib G, Keshen T, Kirk E, et al. Nonalcoholic fatty liver disease is associated with hepatic and skeletal muscle insulin resistance in overweight adolescents. *Am J Clin Nutr* (2008) 88:257–62. doi: 10.1093/ajcn/88.2.257

35. D'Adamo E, Cali AM, Weiss R, Santoro N, Pierpont B, Northrup V, et al. Central role of fatty liver in the pathogenesis of insulin resistance in obese adolescents. *Diabetes Care* (2010) 33:1817–22. doi: 10.2337/dc10-0284

36. Hoffman RP, Vicini P, Cobelli C. Pubertal changes in 32. HOMA and QUICKI: relationship to hepatic and peripheral insulin sensitivity. *Pediatr Diabetes* (2004) 5:122. doi: 10.1111/j.1399-543X.2004.00050.x

37. Færch K, Vistisen D, Pacini G, Torekov SS, Johansen NB, Witte DR, et al. Insulin resistance is accompanied by increased fasting glucagon and delayed glucagon suppression in individuals with normal and impaired glucose regulation. *Diabetes* (2016) 65(11):3473–3481. doi: 10.2337/db16-0240

38. Ahrén B. Glucagon secretion in relation to insulin sensitivity in healthy subjects. *Diabetologia* (2006) 49:117–22. doi: 10.1007/s00125-005-0056-8

39. Junker AE, Gluud L, Holst JJ, Knop FK, Vilsboll T. Diabetic and nondiabetic patients with nonalcoholic fatty liver disease have an impaired incretin effect and fasting hyperglucagonaemia. *J Internal Med* (2016) 279:485–93. doi: 10.1111/joim.12462

40. Holst JJ, Wewer Albrechtsen NJ, Pedersen J, Knop FK. Glucagon and amino acids are linked in a mutual feedback cycle: the liver-α-cell axis. *Diabetes* (2017) 66 (2):235–40. doi: 10.2337/db16-0994

41. Wewer Albrechtsen NJ, Galsgaard KD, Winter-Sørensen M, Suppli MP, Janah L, et al. The liver-α-cell axis and type 2 diabetes. *Endocr Rev* (2019) 40 (5):1353–66. doi: 10.1210/er.2018-00251

42. Gar C, Haschka SJ, Kern-Matschilles S, Rauch B, Sacco V, Prehn C, et al. The liver–alpha cell axis associates with liver fat and insulin resistance: a validation study in women with nonsteatotic liver fat levels. *Diabetologia* (2021) 64(3):512–20. doi: 10.1007/s00125-020-05334-x

43. Lischka J, Schanzer A, Hojreh A, Ssalamah AB, Item Bellarmine C, de Gier C, et al. A branched-chain amino acid-based metabolic score can predict liver fat in children and adolescents with severe obesity. *Pediatr Obes* (2020) 16:e12739. doi: 10.1111/ijpo.12739

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 27 September 2022
DOI 10.3389/fendo.2022.1030809

OPEN ACCESS

EDITED BY
Artur Mazur,
University of Rzeszow, Poland

REVIEWED BY
Daniel Weghuber,
Paracelsus Medical University, Austria
Serhiy Nyankovskyy,
Danylo Halytsky Lviv National Medical University, Ukraine

*CORRESPONDENCE
Mandy Vogel
mandy.vogel@medizin.uni-leipzig.de

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share senior authorship

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 29 August 2022
ACCEPTED 08 September 2022
PUBLISHED 27 September 2022

CITATION
Brunnert L, Puasa ID, Garten A, Penke M, Gaul S, Grafe N, Karlas T, Kiess W, Flemming G and Vogel M (2022) Pediatric percentiles for transient elastography measurements - effects of age, sex, weight status and pubertal stage. *Front. Endocrinol.* 13:1030809. doi: 10.3389/fendo.2022.1030809

Pediatric percentiles for transient elastography measurements - effects of age, sex, weight status and pubertal stage

Lina Brunnert[1†], Ika Damayanti Puasa[1†], Antje Garten[1], Melanie Penke[1], Susanne Gaul[1,2], Nico Grafe[3], Thomas Karlas[4], Wieland Kiess[1,3], Gunter Flemming[1‡] and Mandy Vogel[1,3*‡]

[1]Center for Pediatric Research, University Hospital for Children and Adolescents, Leipzig, Germany, [2]Clinic and Polyclinic for Cardiology, Leipzig University Medical Center, Leipzig, Germany, [3]Leipzig Research Center for Civilization Diseases, LIFE Child, Leipzig, Germany, [4]Department of Medicine II, Division of Gastroenterology, Leipzig University Medical Center, Leipzig, Germany

Background and aims: Transient Elastography is a non-invasive, cost-efficient, non-ionizing, observer-independent and reliable method to detect liver fibrosis using Liver Stiffness Measurement (LSM) and the degree of fat accumulation in the liver using Controlled Attenuation Parameter (CAP). This study aims to derive reference values for both measures from healthy children and adolescents. Further, we aim to assess the potential influence of age, sex, puberty, and BMI-SDS on CAP and LSM.

Methods: Within the LIFE Child study, amongst others, anthropometric data and pubertal status were assessed. Transient Elastography (TE) was performed using the FibroScan® device in a population-based cohort at 982 study visits of 482 healthy children aged between 10 and 18 years. Percentiles for LSM and CAP were estimated, and the effects of age, sex, puberty and weight status were assessed through hierarchical regression models.

Results: There was a strong age dependency for LSM with higher values for older children, most pronounced in the upper percentiles in boys. Contrarily, CAP was relatively stable across the age span without considerable difference between boys and girls. We found a significant positive correlation between BMI-SDS and both CAP and LSM for BMI-SDS >1.28. For BMI-SDS < 1.28, the association was also positive but reached statistical significance only for CAP. Further, the association between BMI-SDS and CAP was significantly stronger in younger than in older children. There was no association between pubertal status and CAP. For LSM, we found that children with a high BMI-SDS but not children with normal weight had significantly higher LSM values in Tanner stage 4.

Conclusions: Age, sex, pubertal status and weight status should be considered when interpreting LSM and CAP in pediatric patients to facilitate and improve

early detection of abnormal liver function, which is associated with common pathologies, such as NAFLD.

KEYWORDS

non-alcoholic fatty liver disease – NAFLD, fibroscan, liver stiffness, reference values, obesity, pediatrics

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in children and adolescents. A recent systematic review and meta-analysis estimated a global NAFLD prevalence of 7.6% in children. Moreover, in studies focusing on children with obesity, the prevalence was as high as 34.2% (1). Due to its association with obesity, NAFLD has already become a health issue of pandemic dimensions (2). Considering the growing number of children and adolescents with obesity worldwide (3), the impact of NAFLD on public health will likely increase even further.

NAFLD can lead to liver fibrosis and cirrhosis and increases the risk of developing hepatocellular carcinoma (HCC) (4, 5). Moreover, NAFLD is associated with an increased risk of cardiovascular disease, type 2 diabetes and increased mortality at adult age (2). Detected at early stages, before the liver is irreversibly damaged, NAFLD is treatable with lifestyle modifications, e. g., improved diet, increased physical activity and weight loss (6). There is a high probability of successful development of future pharmacological treatment options, since several drugs for children with NAFLD have been tested in phase 2 trials recently (2). To facilitate successful treatment, detecting NAFLD in pediatric patients accurately at an early stage is imperative.

Until today, the gold standard for diagnosing NAFLD is the histopathological examination of a liver biopsy. However, liver biopsy in children raises several ethical issues and is therefore reluctantly performed. Children often need general anesthesia, which entails a risk for the patient. Additionally, there is the risk of bleeding or mispuncture. Furthermore, since only a tiny part of the liver is examined, there is a risk of misdiagnosis due to sampling bias (7). Hence, reliable non-invasive diagnostic tools are urgently needed.

Various serum parameters and imaging procedures have been evaluated in several studies over the last years, but mostly with rather disappointing results (2, 6, 8–10). Measurement of alanine transaminase (ALT), for instance, is the most common serum parameter for screening, but physiological levels are no reliable predictor for the absence of NAFLD. Moreover, most imaging procedures bring their own disadvantages. CT detects fibrosis and steatosis reliably; however, it must not be used regularly in pediatric patients because of radiation burden. MRI, which works without radiation and is also very sensitive, is expensive and not widely available, rendering it unsuitable to be the standard procedure for detecting NAFLD. Regular ultrasound, on the other hand, is non-ionizing, inexpensive and widely available, but not reliable in detecting NAFLD (2).

Transient Elastography (TE, by FibroScan ® (Echosens, Paris, France)) has drawn a high amount of academic interest since it is a cost-efficient, observer-independent and non-ionizing method to detect fibrosis and steatosis reliably (11–18). FibroScan ® provides two different methods to examine the liver: liver stiffness measurement (LSM) and controlled attenuation parameter (CAP). While LSM is a parameter to estimate liver fibrosis, CAP quantifies the percentage of liver fat.

However, to use TE in pediatric practice, reliable reference values of healthy children - including the potential influence of age, sex, weight and pubertal status - are needed. By drawing from a large, longitudinal, deeply characterized cohort of healthy children, this study aims to provide percentiles for both LSM and CAP measurement. Moreover, we will examine the potential influence of sex, age, BMI and pubertal status on these two parameters. Hereby, we hope to facilitate a better interpretation of test results and, thus, to make a beneficial contribution to pediatric practice with regard to detecting and, ideally, treating NAFLD.

Materials and methods

This article is structured according to the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) Statement checklist for cohort studies (19).

Study design

The LIFE Child study is a prospective longitudinal population-based cohort study with a life course approach to health and disease (20). As a part of LIFE, a research project conducted at the Leipzig Research Center for Civilization Diseases, LIFE Child aims to monitor healthy child development from birth to adulthood and to understand the

development of non-communicable diseases such as obesity (21). The study was designed in accordance with the Declaration of Helsinki (22). The Ethics Committee of the Medical Faculty of the University of Leipzig approved the study (Reg. No. 26410-19042010), which is registered with ClinicalTrials.gov under the clinical trial number NCT02550236.

Setting

Fully informed and written consent was obtained from all participants (from the age of twelve) and their parents. Each study visit contained age-customized interviews, medical examinations, standardized tests, questionnaires and the collection of biological samples, as well as the implementation of FibroScan® measurements (20, 21).

Participants

Children from Leipzig or neighboring municipalities in Germany were recruited *via* advertisement at different institutions, by media or by word of mouth. The children were primarily healthy, without severe disorders like malignancies, syndromal diseases or diabetes. Accordingly, the acquired test results are qualified for generating reference values. Height was measured using a stadiometer ("Prof. Keller"; Längenmesstechnik GmbH Limbach, Limbach-Oberfrohna, Germany, measurement accuracy 0.10 cm). Participants were weighed with the "Seca701" scale (seca GmbH & Co.KG, Hamburg, Germany, accurate to 50 g). BMI was calculated and transformed into standard deviation scores (SDS) according to the guidelines of the German Obesity Association (23, 24). Overweight and obesity were defined according to the same guidelines (23, 24) as 1.28 < BMI-SDS < 1.88 and BMI-SDS ≥ 1.88, respectively. Pubertal stage was assessed according to Tanner (25, 26) by specially trained and regularly instructed investigators.

Study size

Data from 1491 visits provided by 698 individuals from the LIFE Child cohort with a complete data set (CAP, LSM, sex, age, pubertal stage, and BMI) were available. In N=249 cases, we performed double measurements.

Our exclusion criteria were:

1. Measurements from participants younger than 10 years and older than 18 years of age were excluded (N=71 visits and 41 children), due to the small number of measurements below and above that age.
2. Participants with the intake of at least 1 of 92 potentially hepatotoxic drugs (listed in Supplementary Table 1) at the time of measurement were excluded, N= 62 visits and 10 children.

The remaining visits N=1358 from 647 children were used for the assessment of influence factors (sex, age, BMI-SDS, pubertal status).

For the calculation of LSM and CAP percentiles, we excluded 165 participants (231 visits) with a BMI-SDS < 3rd and >97th percentile (BMI-SDS < -1.88 and BMI-SDS>1.88), resulting in data from 982 visits from 482 individuals.

Glucose and insulin measurements were available from 625 visits from 196 individuals.

Transient elastography measurement

The examination was carried out after an overnight fast by specially trained and regularly re-certified examiners. The participants were asked to lie on the back, the right arm maximally abducted, and to stay immobile during the examination. Those participants who were designated for dual measurements were asked to stay in the same position after the first measurement, and the second measurement was performed by the same examiner immediately afterwards.

LSM and CAP values were measured using the FibroScan® device with the M probe (25 - 65 mm measurement depth) or XL probe (35 - 75 mm measurement depth). The FibroScan® device includes the Automatic Probe Selection (APS) tool, which indicates which of the two probes should be used for measurement. LSM measures the propagation of produced shear waves, and the results are displayed in kilopascals (kPa). CAP measures the attenuation of the above-mentioned shear wave propagation, producing results in decibels per meter (dB/m). The measurement was successful when 10 valid data points could be measured.

Laboratory parameters

Blood samples were taken from the participants after an overnight fast. Serum glucose concentrations were measured by the photometric method (Roche, Basel, Switzerland). Serum insulin concentrations were measured using a quantitative electrochemiluminescence method (Roche) (27). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as described in Matthews et al. (28).

Statistical analyses

Descriptive statistics are given as mean and standard deviations for continuous and counts and percentages for categorical variables.

References for LSM and CAP were estimated as a continuous function of age, stratified by sex using the LMS method as implemented in the package gamlss (29). We corrected for multiple measurements per person by setting weights on the observations accordingly. Subsequently, CAP and LSM measurements were transformed to standard deviation scores applying the new references.

Associations between LSM and CAP as outcome and the assumed predictors (sex, age, BMI-SDS, and pubertal stage) were assessed using hierarchical regression analysis. To assess the effect of puberty, raw measurements of LSM and CAP were used because of the strong dependency between age and puberty; in all other models, the age- and sex-adjusted SDS were used as outcome. All models were adjusted for multiple measurements per subject by adding the subject as random effect. The nature of associations was investigated using non-parametrical generalized additive models. The association between LSM and BMI-SDS required polynomial modeling (3^{rd} degree). Otherwise, linear approximation yielded a sufficient fit. We tested for relevant interactions between predictors. Model terms were only kept if they were necessary. In addition, models were tested for variance inflation. Results were reported as (non-standardized) coefficients and the respective 95%-confidence interval.

To assess intraobserver reliability, we calculated the overall concordance correlation coefficient (OCCC) (30, 31). In addition, we report the components overall precision (OPREC) and overall accuracy (OACCU) and present the respective Bland-Altman plots. The chosen strength-of-agreement categories are orientated to those of the Pearson product-moment correlation: CCC ≥ 0.9 ("excellent"); < 0.9 and ≥ 0.7 ("good"); < 0.7 and ≥ 0.5 ("moderate"); and < 0.5 ("low").

The mediating effect of hepatic insulin resistance was assessed by mediation analyses using HOMA-IR implemented *via* a structural equation model.

Analyses and visualization were performed using the packages gamlss (29), lme4 (32) (version 1.1.30) and ggplot2 (3.3.6) in R (version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria) (33).

Results

Participants

We used the data of 482 (252 male, 231 female) healthy individuals, aged between 10 and 18 years with a BMI-SDS between 3^{rd} and 97^{th} percentile, who were examined between December 2013 and June 2022 in the context of the Leipzig Research Centre for Civilization Diseases (LIFE). Since LIFE Child is a longitudinal study, some participants were measured more than once over the period of 8 years, resulting in a total of 982 (624 male, 587 female) visits for the calculation of the percentiles. Dual measurements for the evaluation of FibroScan® validity were performed in 249 individuals. The population characteristics for the entire study population (N=1358) are listed in Table 1.

Reproducibility/FibroScan ® validity

For both LSM and CAP, we could show an "excellent" OACCU. OCCC and OPREC were "good" for LSM and "moderate" for CAP. The results are shown in Table 2 and Figures 1A, B.

Percentiles for LSM and CAP are influenced by sex and age

The 3^{rd}, 10^{th}, 50^{th}, 90^{th} and 97^{th} percentile curves for LSM and CAP are shown for boys and girls in Figures 2A, B. The respective parameter values are shown in Supplementary Tables 2 (A)-(D).

LSM percentiles show increasing values for both sexes with, in general, higher values for boys, which becomes more pronounced in the upper percentiles (e.g., 16.5 years p50: girls=4.6kPa boys=5.1kPa; p97: girls=7.2kPa boys=8.5kPa). Also, the curve shapes differ from each other with regard to sex: The curves for girls ascend for the first 1.5 years, then slightly flatten for about 1.5 years, after which they ascend again until they reach their peak at about 16.5 years (P50 5.95kPa) which is followed by another slight drop in the 3^{rd}, 10^{th} and 50^{th} percentile. The curves P50, P90 and P97 for boys, on the other hand, show continuous slopes until reaching their peaks, followed by a slight flattening. The age at which boys reach the highest values is comparable with that of girls (about 16.5 years) in P50, 90 and 97. In the lower percentiles, however, the highest values were measured at 18 years.

CAP percentiles show similarly shaped curves for boys and girls. The reference values are comparable as well. Comparing the reference values at the age of ten and 18 years, the lower percentiles show a tendency to descend slightly while the higher percentiles tend to ascend slightly, reaching their peaks at about 14 years. P50 depicts rather stable values during the eight years (boys: 200dB/m at age 11 and 15 years and 198db/m at age 18 years; girls: 188dB/m at age 11 years and 197dB/m from age 14.5 – 18 years).

The parameter tables are provided as part of the R package childs (version 0.8.0). The package also contains functions to transform measurement values into SDS and to create percentile curves. It is available from CRAN (https://cran.r-project.org/package=childsds).

10.3389/fendo.2022.1030809

TABLE 1 Baseline characteristics of the study population.

	[ALL] N = 1358	male N = 692	female N = 666	p.overall
Sex:				
male	692 (51.0%)			
female	666 (49.0%)			
Age (years)	14.0 (2.81)	13.9 (2.87)	14.1 (2.74)	0.432
Pubertal Stage:				<0.001
1	154 (15.3%)	91 (20.5%)	63 (11.2%)	
2	146 (14.5%)	77 (17.3%)	69 (12.2%)	
3	111 (11.0%)	42 (9.46%)	69 (12.2%)	
4	170 (16.8%)	73 (16.4%)	97 (17.2%)	
5	428 (42.4%)	161 (36.3%)	267 (47.3%)	
Weight status:				0.421
underweight/normal weight	885 (65.3%)	455 (65.8%)	430 (64.8%)	
overweight	129 (9.51%)	71 (10.3%)	58 (8.73%)	
obese	342 (25.2%)	166 (24.0%)	176 (26.5%)	
BMI-SDS	0.71 (1.39)	0.64 (1.33)	0.78 (1.45)	0.055

Values are given as mean and standard deviations for continuous and counts and percentages for categorical variables.

TABLE 2 Results of the calculation of the OCCC, the OPREC and the OACCU for LSM and CAP of N=249 dual measurements.

	OCCC	OPREC	OACCU
LSM	0.74	0.76	0.97
CAP	0.66	0.66	1.0

OCCC, overall concordance correlation coefficient; OPREC, overall precision; OACCU, overall accuracy; LSM, Liver Stiffness Measurement; CAP, Controlled Attenuation Parameter. Results were classified as ≥ 0.9 "excellent"; < 0.9 and ≥ 0.7 "good"; < 0.7 and ≥ 0.5 "moderate"; and < 0.5 "low".

Influence of BMI-SDS on LSM and CAP

After establishing percentiles, we assessed the association between weight status and both LSM and CAP values. In children with a BMI-SDS <1.28, there was a slightly positive association between LSM-SDS and BMI-SDS. However, it did not reach statistical significance (beta=0.07, p=0.48). In children with overweight and obesity, the respective effect size was three times as high, and the association became significantly positive (beta=0.26, p=0.025) (see Figure 3A). The two slopes were not significantly different from each other ($beta_{Interaction}$=0.19, p=0.289). The effects of weight status on LSM were not different, regardless of age and sex.

In children with a BMI-SDS <1.28, we found a significant positive association between CAP-SDS and BMI-SDS (beta=0.15, p<0.001). In children with a BMI-SDS >1.28, the effect size was six times as high (beta=0.95, p<0.001) (see Figure 3B). The two slopes were significantly different from each other ($beta_{interaction}$=0.85, p<0.001). In addition, the effect varied significantly with age, having the strongest effect for younger children ($beta_{10years}$=1.6, p<0.001) and the weakest effect for older adolescents ($beta_{18years}$=0.6, p<0.001). The effect of weight status on CAP did not differ between sexes.

Influence of pubertal status on LSM

LSM increased significantly with advancing puberty in boys. The values were significantly higher in Tanner stage (TS) 3 (beta=1.1, p=0.029), TS 4 (beta=1.2, p=0.004), and TS 5 (beta=1.5, p<0.001) than in TS 1. In girls, there was no such distinct pattern. Considering weight status, there was a significant interaction between Tanner stage and BMI-SDS: While we found no effect of puberty in children with a BMI-SDS around or below 0, we found significantly higher LSM values for children with BMI-SDS of 1.88 or higher in TS 4 and 5. The effects were remarkably stronger in TS 4 ($beta_{3BMI-SDS}$=4.3, p<0.001; $beta_{2.6BMI-SDS}$=2.6, p<0.001; $beta_{2BMI-SDS}$=1.5, p=0.005) than in TS 5 ($beta_{3BMI-SDS}$=1.3, p=0.14; $beta_{2.5BMI-SDS}$=1.5, p=0.004; $beta_{2BMI-SDS}$=1.5, p=0.001) (see Figure 4). The association did not differ between sexes. The association of LSM with Tanner stage 4-5 was partly (approximately 1/3, p = 0.047)

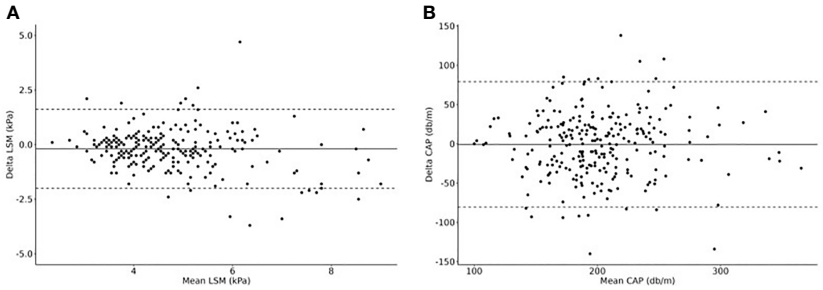


FIGURE 1
Dual measurements of **(A)** LSM and **(B)** CAP to calculate the overall concordance correlation coefficient (OCCC), the overall precision (OPREC) and the overall accuracy (OACCU). Delta values are plotted in relationship to mean values (Bland-Altman Plots). N=249 cases.

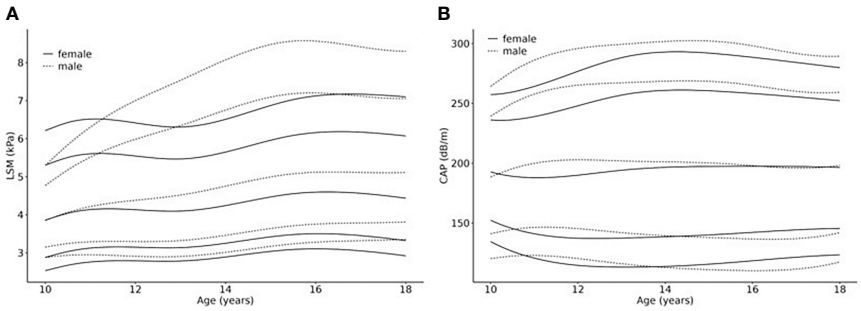


FIGURE 2
Age- and sex-related percentiles of **(A)** LSM (kPa) and **(B)** CAP (dB/m) values. Smoothed percentile curves are shown separately for females and males in relationship to age (10 – 18 years), based on a normal weight reference population from a LIFE Child study sample (N =982 cases (624 male, 587 female) of 482 (252 male, 231 female) healthy individuals). The 3rd (P3), 10th (P10), 50th (P50, median), 90th (P90) and 97th (P97) percentile are shown.

explained by hepatic insulin resistance, which we measured as Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

Influence of pubertal status on CAP

There was no significant association between CAP results and puberty. Moreover, the association between BMI-SDS and CAP did not differ between Tanner stages. Values were, on average, 10 kPa higher for males than for females (p=0.012). There was no interaction between sex and Tanner stage or BMI-SDS.

Discussion

Considering the rising prevalence of obesity and concomitant liver diseases, especially NAFLD, in children and adolescents, non-invasive diagnostic tools to accurately detect liver pathologies in pediatric patients are urgently needed. Transient Elastography has been used extensively to aid the diagnosis of fatty liver disease and fibrosis in the adult population for which TE reference values are available (34–36). Several studies have already postulated the need for reliable TE reference values for children, respectively the necessity of further detailed research on TE measurement in the pediatric context (6, 37).

With the aim to close this knowledge gap, this study provides pediatric reference values and presents the respective percentiles for the Transient Elastography measurements LSM and CAP, based on our investigation of a large and well-characterized cohort of healthy children and adolescents. We decided to include children with overweight when estimating percentiles because our analyses revealed that the influence of BMI-SDS was similar as in children with normal weight. In contrast, increasing BMI-SDS had considerably stronger effects on LSM and CAP for children with obesity (Figure 3).

Furthermore, we analyzed the influence of age, sex, weight and pubertal status on LSM and CAP. Thereby, we enable

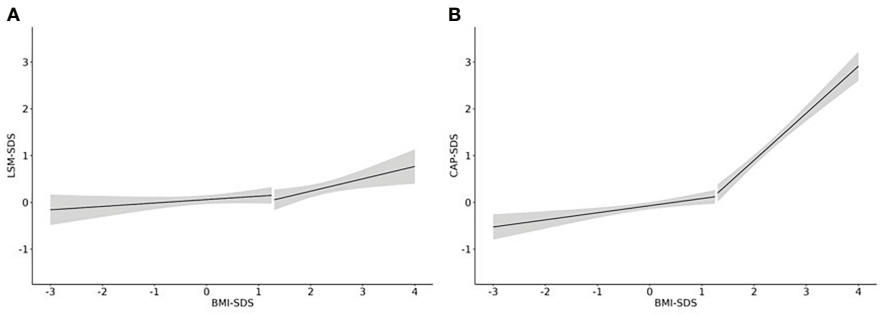


FIGURE 3
Effect of weight status on LSM and CAP. Linear regression curves including a 95%-confidence band are shown for the association of **(A)** LSM-SDS and BMI-SDS and **(B)** CAP-SDS and BMI-SDS. N = 1358 from 647 children.

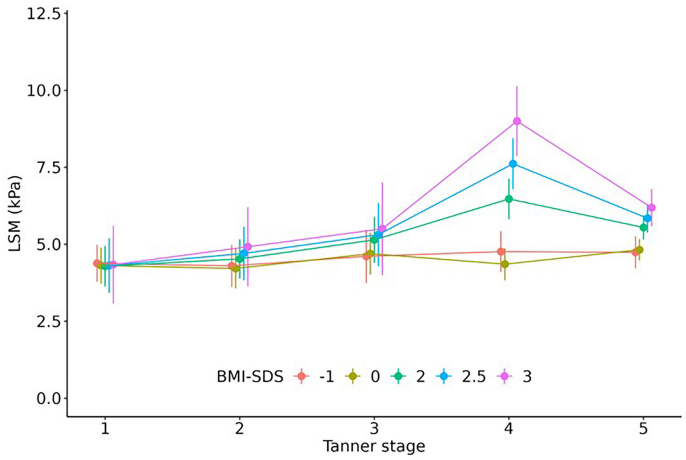


FIGURE 4
Effect of pubertal status on LSM. Children with overweight/obesity (BMI-SDS ≥ 2) but not with normal weight had significantly higher LSM values in Tanner stage 4 and 5. Regression estimates including 95% confidence interval are shown.

examiners and practitioners to interpret LSM and CAP test results in children more accurately, having appropriate reference values at hand.

Our research has shown that LSM is age-dependent, and LSM test results tend to increase with age in the pediatric context. Our reference values for LSM are generally in line with findings of other recent studies examining healthy children (38–43). Likewise, the increase over age was also observed in other studies (38–42). Contrary to this, Ramirez et al., who investigated a cohort of 462 healthy children and provided reference values as well, found no effect of age on LSM (43). Potential reasons for this inconsistency include a different age range (12 – 20 years) as well as a different ethnic and geographic background of the cohort. A recent meta-analysis with 1702 participants, on the other hand, also found that values increase with age (40). Zeng et al. (44) provided reference values for five-year-olds based on a very large cohort. The reference values they established were remarkably lower than ours. Since we included participants starting at age 10 who, from the start, showed higher values compared to those of five-year-olds (Zeng 2019: LSM_{5years} median 3.2 kPa *vs*. Brunnert 2022: $LSM_{10years}$ median 3.9 kPa), we regard their study results, taken together with ours, as strongly supporting the validity of the assumption that pediatric LSM values increase with age. However, Mjelle et al. (39) state that there is about the same number of studies indicating an age-dependency of LSM values as there is for age-independency. This clearly highlights the need to further investigate the age-dependency of LSM values in the pediatric context. In our study, LSM values peak at 14.5 years and stay more or less stable afterwards. This leads to the assumption that after age 18 no further increase in LSM values will occur. This assumption is in line with the so far published studies of the

adult population stating that LSM results show no age-dependency (45, 46).

Moreover, our research has shown that LSM test results are higher for boys. This is also confirmed by results from other relevant recent studies (38, 39, 42). Tokuhara et al., on the other hand, could not find any influence of sex on LSM results (41). Likewise, the above-mentioned study by Ramirez et al. (43) did not find sex-dependent alterations of LSM. Since our study clearly shows the sex-dependency of LSM test results, we expect that future research will further validate this outcome.

We could not identify any correlation between age, sex and CAP. With regard to pediatric CAP measurements, there are only a few published studies providing reference values. Ramirez et al. (43) presented stable, age- and sex-independent CAP values from the ages 12 to 20. Their findings are in line with our observation that CAP values are neither age- nor sex-dependent. This was also shown by a recent study by Ferraioli et al. (47). However, Zeng et al. (44) identified a median CAP value of 171db/m for five-year-olds, so there might be a tendency for lower CAP values at younger ages, if we take into account that our values for older children and adolescents are remarkably higher (median LSM at age 15: 197dB/m for girls, 200dB/m for boys). Since we only analyzed results of children aged 10 years and older, our study could not add further insights on the question of whether CAP values increase below age 10.

We found a positive correlation between weight status and LSM as well as CAP test results, also found by Zeng et al. (44). In addition, Ferraioli et al. (47) examined CAP values of children categorized as 'normal weight', 'overweight' and 'obese'. They, too, found a significant positive association between CAP and weight status (30). Lee et al. evaluated LSM in children with obesity. Values were remarkably higher (16) than in our reference population, which further supports our finding of a considerable impact of weight status on LSM values.

Furthermore, we found that LSM but not CAP values differ across puberty. To our knowledge, we present the first examination of the impact of pubertal status on TE measurements. Partly, the effect might be explained by the increasing hepatic insulin resistance during puberty (48) as our results suggest. Another reason for increased hepatic insulin resistance is obesity (49). Accordingly, we found that adolescents with obesity had significantly higher LSM, especially in Tanner stage 4 and 5. The underlying mechanisms of this phenomenon are unclear and should be subject to future research.

Evaluating dual measurements, we could show that TE is a method with medium reproducibility. Our findings are in line with results of other studies investigating the reproducibility of TE measurements: Ferraioli et al. reported a concordance correlation coefficient (CCC) for CAP of 0.82 for children with normal weight and 0.6 for children with obesity (17). Rowland et al. reported a CCC for LSM of 0.85 (50). We would, therefore, suggest the implementation of a second measurement in case of borderline TE results, to improve the reliability of the results.

There are some limitations to our study. We only used data from a single study center with limited access to subjects with diverse ethnic background. Thus, our results are not necessarily representative of pediatric patients worldwide. Furthermore, families participating in the LIFE Child research project generally have a socio-economic status above average (51) which could also render our findings less representative with regard to both the global pediatric population as well as the general pediatric population of a particular state or region. In addition, the HOMA-IR was only available for a subpopulation (n = 196) which led to less power in the related analyses. Moreover, for evident ethical reasons, we did not perform liver biopsies to validate our test results.

Nevertheless, our study has several strengths. To our knowledge, this paper is the first to provide reference values for both LSM and CAP based on a large pediatric cohort from 10 to 18 years. Additionally, we established that age, sex, BMI-SDS and pubertal status have an impact on TE test results and, thus, should be considered when evaluating LSM and CAP values. Accordingly, we suggest our sex- and age-adapted reference values to interpret TE results in pediatric practice. There are numerous studies evaluating the usefulness and feasibility of TE for pediatric subjects, but most of them only examine patients with NAFLD or obesity. However, in pediatric practice, we need reference values guiding us in our endeavor to identify potential risks or existing diseases in patients. Thus, the reference values and percentiles we present in this paper can help us to red-flag conspicuous test results.

Given the already high and, most likely, further increasing prevalence of liver diseases such as NAFLD, it is paramount to detect potential diseases at an early stage. Our paper attempts to make a valuable contribution to this endeavor in terms of research as well as practice.

Data availability statement

The datasets presented in this article are not readily available because data cannot be shared publicly because there exist ethical restrictions. The LIFE Child study is a study collecting potentially sensitive information. Publishing data sets is not covered by the informed consent provided by the study participants. Furthermore, the data protection concept of LIFE requests that all (external as well as internal) researchers interested in accessing data sign a project agreement. Researchers that are interested in accessing and analyzing data collected in the LIFE Child study may contact the data use and access committee (dm@life.uni-leipzig.de). Requests to access the datasets should be directed to dm@life.uni-leipzig.de.

Ethics statement

This study was reviewed and approved by The Ethics Committee of the Medical Faculty of the University of Leipzig. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Conceptualization, SG, MP, WK, AG and GF. Methodology, TK, NG, GF and MV. Software MV. Validation, IP, NG and MV. Formal analysis IP, LB and MV. Investigation, IP, NG, GF, MV. Resources, TK, GF, WK and MV. Data curation, IP, LB and MV. Writing—original draft preparation LB, IP and MV. Writing—review and editing, LB, AG, GF and MV. Visualization, IP, LB and MV. Supervision, GF, MV, AG and WK. Project administration, AG. Funding acquisition, SG, MP and WK. All authors contributed to the article and approved the submitted version.

Funding

This publication is supported by LIFE – Leipzig Research Center for Civilization Diseases, University of Leipzig. LIFE is funded by means of the European Union, by means of the European Social Fund (ESF, https://ec.europa.eu/regional_policy/en/funding/social-fund/), by the European Regional Development Fund (ERDF, https://ec.europa.eu/regional_policy/en/funding/erdf/), and by means of the Free State of Saxony. This publication was funded by the Open Access Publishing Fund of Leipzig University supported by the German Research Foundation within the program Open Access Publication Funding. Furthermore, we acknowledge support from the Roland-Ernst-Stiftung. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We are deeply grateful to all the families who have taken part in this study, and the whole LIFE Child team.

Conflict of interest

TK received unrestricted research grants from Echosens SA, France, not related to this project. TK took part in a clinical advisory board meeting of Echosens SA.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1030809/full#supplementary-material

References

1. Anderson EL, Howe LD, Jones HE, Higgins JPT, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. *PloS One* (2015) 10(10):e0140908. doi: 10.1371/journal.pone.0140908

2. Draijer L, Benninga M, Koot B. Pediatric NAFLD: an overview and recent developments in diagnostics and treatment. *Expert Rev Gastroenterol Hepatol* (2019) 13(5):447–61. doi: 10.1080/17474124.2019.1595589

3. Abarca-Gómez L, Abdeen ZA, Hamid ZA, Abu-Rmeileh NM, Acosta-Cazares B, Acuin C. 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(10113):2627–42. doi: 10.1016/S0140-6736(17)32129-3

4. Mann J, Valenti L, Scorletti E, Byrne C, Nobili V. Nonalcoholic fatty liver disease in children. *Semin Liver Dis* (2018) 38(01):001–13. doi: 10.1055/s-0038-1627456

5. Khan HH, Klingert CE, Kumar S, Lyons H. Cirrhosis in a young child due to fatty liver; importance of early screening: A case report and review of the literature. *Am J Case Rep* (2020) 21:e923250. doi: 10.12659/AJCR.923250

6. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: Recommendations from the expert committee on NAFLD (ECON) and the north American society of pediatric gastroenterology, hepatology and nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr* (2017) 64(2):319–34. doi: 10.1097/MPG.0000000000001482

7. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* (2005) 128(7):1898–906. doi: 10.1053/j.gastro.2005.03.084

8. Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: A multicenter validation study. *Hepatology* (2009) 50(4):1072–8. doi: 10.1002/hep.23050

9. Draijer LG, Oosterhout JPM, Vali Y, Zwetsloot S, Lee JH, Etten-Jamaludin FS. Diagnostic accuracy of fibrosis tests in children with non-alcoholic fatty liver disease: A systematic review. *Liver Int* (2021) 41(9):2087–100. doi: 10.1111/liv.14908

10. Kwon Y, Kim ES, Choe YH, Kim MJ. Stratification by non-invasive biomarkers of non-alcoholic fatty liver disease in children. *Front Pediatr* (2022) 10:846273. doi: 10.3389/fped.2022.846273

11. Fitzpatrick E, Quaglia A, Vimalesvaran S, Basso MS, Dhawan A. Transient elastography is a useful noninvasive tool for the evaluation of fibrosis in paediatric chronic liver disease. *J Pediatr Gastroenterol Nutr* (2013) 56(1):72–6. doi: 10.1097/MPG.0b013e31826f2760

12. Nobili V, Vizzutti F, Arena U, Abraldes JG, Marra F, Pietrobattista A. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. *Hepatology* (2008) 48(2):442–8. doi: 10.1002/hep.22376

13. Friedrich-Rust M, Romen D, Vermehren J, Kriener S, Sadet D, Herrmann E. Acoustic radiation force impulse-imaging and transient elastography for non-invasive assessment of liver fibrosis and steatosis in NAFLD. *Eur J Radiol* (2012) 81(3):e325–31. doi: 10.1016/j.ejrad.2011.10.029

14. Sasso M, Miette V, Sandrin L, Beaugrand M. The controlled attenuation parameter (CAP): A novel tool for the non-invasive evaluation of steatosis using fibroscan®. *Clin Res Hepatol Gastroenterol* (2012) 36(1):13–20. doi: 10.1016/j.clinre.2011.08.001

15. Nobili V, Monti L, Alisi A, Zupone CL, Pietrobattista A, Tomà P. Transient elastography for assessment of fibrosis in paediatric liver disease. *Pediatr Radiol* (2011) 41(10):1232–8. doi: 10.1007/s00247-011-2143-y

16. Lee JE, Ko KO, Lim JW, Cheon EJ, Song YH, Yoon JM. Correlation between transient elastography (Fibroscan ®) and ultrasonographic and computed tomographic grading in pediatric nonalcoholic steatohepatitis. *Pediatr Gastroenterol Hepatol Nutr* (2022) 25(3):240. doi: 10.5223/pghn.2022.25.3.240

17. Ferraioli G. Quantitative assessment of liver steatosis using ultrasound controlled attenuation parameter (Echosens). *J Med Ultrason* (2021) 48(4):489–95. doi: 10.1007/s10396-021-01106-1

18. Abenavoli L, Beaugrand M. Transient elastography in non-alcoholic fatty liver disease. *Ann Hepatol* (2012) 11(2):172–8. doi: 10.1016/S1665-2681(19)31021-X

19. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Int J Surg* (2014) 12(12):1495–9. doi: 10.1016/j.ijsu.2014.07.013

20. Quante M, Hesse M, Döhnert M, Fuchs M, Hirsch C, Sergeyev E. The LIFE child study: a life course approach to disease and health. *BMC Public Health* (2012) 12(1):1021. doi: 10.1186/1471-2458-12-1021

21. Poulain T, Baber R, Vogel M, Pietzner D, Kirsten T. The LIFE child study: a population-based perinatal and pediatric cohort in Germany. *Eur J Epidemiol* (2017) 32(2):145–58. doi: 10.1007/s10654-016-0216-9

22. World medical association declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA* (2013) 310(20):2191–4. doi: 10.1001/jama.2013.281053

23. Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiss HC, Hesse V. Percentiles of body mass index in children and adolescents evaluated from different regional German studies. *Monatsschr Kinderheilkd* (2001) 149(8):807–18. doi: 10.1007/s001120170107

24. Wabitsch M, Moss A. [Evidence-based (S3) guideline of the working group on childhood and adolescent obesity (AGA) of the German obesity society (DAG) and the German society of pediatrics and adolescent medicine (DGKJ)]. *Arbeitsgemeinschaft Adipositas im Kindes und Jugendalter (AGA)* (2019).

25. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* (1970) 45(239):13–23. doi: 10.1136/adc.45.239.13

26. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child* (1969) 44:291–303. doi: 10.1136/adc.44.235.291

27. Stanik J, Kratzsch J, Landgraf K, Vogel M, Thiery J, Kiess W. The bone markers sclerostin, osteoprotegerin, and bone-specific alkaline phosphatase are related to insulin resistance in children and adolescents, independent of their association with growth and obesity. *Horm Res Paediatr* (2019) 91(1):1–8. doi: 10.1159/000497113

28. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* (1985) 28(7):412–9. doi: 10.1007/BF00280883

29. Rigby RA, Stasinopoulos DM. Generalized additive models for location, scale and shape. *J R Stat Soc Ser C Appl Stat* (2005) 54(3):507–54. doi: 10.1111/j.1467-9876.2005.00510.x

30. Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* (1989) 45(1):255. doi: 10.2307/2532051

31. Barnhart HX, Haber M, Song J. Overall concordance correlation coefficient for evaluating agreement among multiple observers. *Biometrics* (2002) 58(4):1020–7. doi: 10.1111/j.0006-341X.2002.01020.x

32. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw* (2015) 67(1):1–48. doi: 10.18637/JSS.V067.I01

33. R Core Team. *R: A language and environment for statistical computing. r foundation for statistical computing.* (2022).

34. Tovo CV, Villela-Nogueira CA, Leite NC, Panke CL, Port GZ, Fernandes S. Transient hepatic elastography has the best performance to evaluate liver fibrosis in non-alcoholic fatty liver disease (NAFLD). *Ann Hepatol* (2019) 18(3):445–9. doi: 10.1016/j.aohep.2018.09.003

35. Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* (2019) 156(6):1717–30. doi: 10.1053/j.gastro.2019.01.042

36. Noureddin M, Ntanios F, Malhotra D, Hoover K, Emir B, McLeod E. Predicting NAFLD prevalence in the united states using national health and nutrition examination survey 2017–2018 transient elastography data and application of machine learning. *Hepatol Commun* (2022) 6(7):1537–48. doi: 10.1002/hep4.1935

37. Tran LC, Ley D, Bourdon G, Coopman S, Lerisson H, Tillaux C. Noninvasive pediatric liver fibrosis measurement: Two-dimensional shear wave elastography compared with transient elastography. *Front Pediatr* (2022) 10:849815. doi: 10.3389/fped.2022.849815

38. Mărginean CO, Meliț LE, Ghiga DV, Săsăran MO. Reference values of normal liver stiffness in healthy children by two methods: 2D shear wave and transient elastography. *Sci Rep* (2020) 10(1):7213. doi: 10.1038/s41598-020-64320-w

39. Mjelle AB, Mulabecirovic A, Havre RF, Rosendahl K, Juliusson PB, Olafsdottir E. Normal liver stiffness values in children: A comparison of three different elastography methods. *J Pediatr Gastroenterol Nutr* (2019) 68(5):706–12. doi: 10.1097/MPG.0000000000002320

40. Li DK, Khan MR, Wang Z, Chongsrisawat V, Swangsak P, Teufel-Schäfer U. Normal liver stiffness and influencing factors in healthy children: An individual participant data meta-analysis. *Liver Int* (2020) 40(11):2602–11. doi: 10.1111/liv.14658

41. Tokuhara D, Cho Y, Shintaku H. Transient elastography-based liver stiffness age-dependently increases in children. *PloS One* (2016) 11(11):e0166683. doi: 10.1371/journal.pone.0166683

42. Engelmann G, Gebhardt C, Wenning D, Wühl E, Hoffmann GF, Selmi B. Feasibility study and control values of transient elastography in healthy children. *Eur J Pediatr* (2012) 171:353–60. doi: 10.1007/s00431-011-1558-7

43. Ramírez-Vélez R, García-Hermoso A, Correa-Rodríguez M, Izquierdo M. Defining values for controlled attenuation parameter and liver stiffness in youth without liver disease. *Pediatr Res* (2022) 91(4):912–20. doi: 10.1038/s41390-021-01441-6

44. Zeng J, Zhang X, Sun C, Pan Q, Lu WY, Chen Q. Feasibility study and reference values of FibroScan 502 with m probe in healthy preschool children aged 5 years. *BMC Pediatr* (2019) 19(1):129. doi: 10.1186/s12887-019-1487-6

45. Alsebaey A. Normal liver stiffness: A study in living donors with normal liver histology. *World J Hepatol* (2015) 7(8):1149. doi: 10.4254/wjh.v7.i8.1149

46. Suh CH, Kim SY, Kim KW, Lim YS, Lee SJ, Lee MG. Determination of normal hepatic elasticity by using real-time shear-wave elastography. *Radiology* (2014) 271(3):895–900. doi: 10.1148/radiol.14131251

47. Ferraioli G, Calcaterra V, Lissandrin R, Guazzotti M, Maiocchi L, Tinelli C. Noninvasive assessment of liver steatosis in children: the clinical value of controlled attenuation parameter. *BMC Gastroenterol* (2017) 17(1):61. doi: 10.1186/s12876-017-0617-6

48. Suzuki A, Abdelmalek MF, Schwimmer JB, Lavine JE, Scheimann AO, Unalp–Arida A. Association between puberty and features of nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* (2012) 10(7):786–94. doi: 10.1016/j.cgh.2012.01.020

49. Maffeis C, Morandi A. Body composition and insulin resistance in children. *Eur J Clin Nutr* (2018) 72(9):1239–45. doi: 10.1038/s41430-018-0239-2

50. The Cystic Fibrosis Registry of Ireland, The Cystic Fibrosis Liver Disease Research Group, Rowland M, McGee A, Broderick A, Drumm B. Repeatability of transient elastography in children. *Pediatr Res* (2020) 88(4):587–92. doi: 10.1038/s41390-020-0916-4

51. Dathan-Stumpf A, Vogel M, Hiemisch A, Thiery J, Burkhardt R, Kratzsch J. Pediatric reference data of serum lipids and prevalence of dyslipidemia: Results from a population-based cohort in Germany. *Clin Biochem* (2016) 49(10–11):740–9. doi: 10.1016/j.clinbiochem.2016.02.010

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 11 October 2022
DOI 10.3389/fendo.2022.1036808

OPEN ACCESS

EDITED BY
Artur Mazur,
University of Rzeszow, Poland

REVIEWED BY
Barbara Glowinska-Olszewska,
Medical University of Bialystok, Poland
Ewa Otto-Buczkowska,
Medical Center, Poland

*CORRESPONDENCE
Agata Chobot
agata.chobot@uni.opole.pl

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 05 September 2022
ACCEPTED 21 September 2022
PUBLISHED 11 October 2022

CITATION
Seget S, Jarosz-Chobot P, Ochab A, Polanska J, Rusak E, Witoszek P and Chobot A (2022) Body mass index, basal insulin and glycemic control in children with type 1 diabetes treated with the advanced hybrid closed loop system remain stable - 1-year prospective, observational, two-center study. *Front. Endocrinol.* 13:1036808. doi: 10.3389/fendo.2022.1036808

Body mass index, basal insulin and glycemic control in children with type 1 diabetes treated with the advanced hybrid closed loop system remain stable - 1-year prospective, observational, two-center study

Sebastian Seget[1], Przemysława Jarosz-Chobot[1], Agnieszka Ochab[2], Joanna Polanska[3], Ewa Rusak[1], Paulina Witoszek[4] and Agata Chobot[2*]

[1]Department of Children's Diabetology, Medical University of Silesia, Katowice, Poland, [2]Department of Pediatrics, Institute of Medical Sciences, University of Opole, Opole, Poland, [3]Department of Data Science and Engineering, Silesian University of Technology, Gliwice, Poland, [4]Department of Children's Diabetology and Pediatrics, John Paul II Upper Silesian Child Health Centre, Katowice, Poland

Background: Information on the influence of insulin treatment using advanced hybrid closed loop systems (AHCL) on body weight of young patients with type 1 diabetes (T1D) is scarce. The aim of this study was to observe whether there were any changes in body mass index (BMI) of children and adolescents with T1D treated using the Medtronic Minimed 780G AHCL after 1 year of follow up and to analyze potential associations between these changes and the insulin doses.

Materials and methods: For 50 children and adolescents (age 5.4-16.8 years, 24 (48%) boys, T1D for 3.9 ± 2.56 years) using an AHCL system anthropometric and AHCL data were collected prospectively. BMI Z-scores and two-week AHCL records obtained after AHCL enrollment were compared with data after 6 months and also 1 year after starting AHCL.

Results: The BMI Z-score of the patients at 1 year follow-up did not change from time of AHCL initiation (0.51 ± 2.79 vs 0.57 ± 2.85, p>0.05). There was a slight increase in total daily insulin per kg of body weight (0.67 ± 0.21 U/kg vs 0.80 ± 0.21 U/kg, p <0.001), but the percent of basal insulin was unchanged (34.88 ± 6.91% vs 35.08 ± 6.30%, p>0.05). We observed also no change (AHCL start vs after 1 year) in glycemic control parameters: average sensor glucose (131.36± 11.04 mg/dL vs 132.45 ± 13.42 mg/dL, p>0.05), coefficient of variation (34.99± 5.17% vs 34.06 ± 5.38%, p>0.05), glucose management indicator (6.45

± 0.26% vs 6.48 ± 0.32%, p>0.05), and time spent in the range of 70–180 mg/dL (79.28 ± 8.12% vs 80.40 ± 8.25%, p>0.05).

Conclusion: During the 1 year of follow-up the BMI of children and adolescents with T1D treated with an AHCL system remained stable. Although there was a slight increase in the total daily insulin dose, the percent of basal insulin was unchanged. The patients maintained recommended glycemic control.

KEYWORDS

advanced hybrid closed-loop system, type 1 diabetes, children, body mass index, BMI, basal insulin, total daily insulin dose, time in range

Introduction

The prevalence of overweight and obesity among youth with type 1 diabetes (T1D) is steadily increasing and reached even 35% in recent reports (1–4). Excessive body weight complicates attainment of recommended glucose control targets and is often tied with use of higher daily and basal insulin doses (5, 6). Obesity is also an independent, additional risk factor of macro- and microvascular complications, non-alcoholic fatty liver disease and polycystic ovary syndrome in individuals with T1D (7–13). Moreover youth with T1D and obesity are at higher risk of developing peripheral and cardiac autonomic neuropathy (14–16).

Evidence from the Diabetes Control and Complications Trial suggested that using high doses of insulin was related to weight gain in patients with diabetes (17). Although a later 10-year observation did not reach an identical conclusion (18) it seems that the relation between insulin treatment and excessive body weight or weight gain has not been fully explained (4, 8, 19–22).

Worth emphasizing is that children with T1D and obesity face many difficulties when attempting to treat both conditions. For example dieting and exercise, well known methods to decrease body weight, require additional education, selfcare and awareness due to the risk of hypoglycaemia (8, 23–25).

New technologies in the treatment of T1D, including the advanced hybrid closed loop (AHCL) system may perhaps be a good tool to make weight maintenance easier for children and adolescents with T1D (26). On the other hand these new technologies bring more flexibility in daily consumption. Knowing the action of insulin and that the mainstay of treatment for obesity are diet and exercise, one of the necessary approaches is developing treatment strategies with lowest possible daily insulin dose that would at the same not impair the glycemic control (8, 24, 25, 27).

Because of the increasing problem of overweight and obesity among children and adolescents with T1D it seems vital to further investigate this topic and increase the knowledge on how to prevent or treat excessive body weight in these patients. A recent meta-analysis showed no differences in weight gain in children treated with either insulin pumps or multiple daily injections (28, 29). However another study, that analyzed weight in children who switched from MDI to insulin pump, demonstrated different trends in weight gain. The results indicated a positive association between the basal insulin dose and rate of weight gain, while there was no association with the total daily insulin dose (30). Insulin pump treatment using recommended settings might help in reducing the basal insulin dose (31).

Considering the above aspects, our study aimed to observe if there were changes in weight of children and adolescents with T1D using an AHCL system after 1 year of follow up and to analyze potential associations with the daily and basal insulin doses.

Materials and methods

Patients

We enrolled for the study and followed prospectively 50 children and adolescents with T1D, treated with the AHCL system MiniMed 780G in automatic mode, at two regional pediatric diabetes centers (Department of Children's Diabetology, University Clinical Hospital of the Medical University of Silesia in Katowice and Department of Pediatrics, University Clinical Hospital of the University of Opole, Poland), both Centers of Reference of the SWEET (Better control in Pediatric and Adolescent diabeteS: Working to crEate CEnTers of Reference) network. Diabetes care for children in Poland is centralized and carried out in regional centers belonging mostly to academic institutions. Inclusion criteria for the study were: age ≤ 18 years, as well as more

than 70% of the sensor usage time, and more than 70% spent in automatic mode - to obtain reliable continuous glucose monitoring (CGM) and AHCL data.

Methods

The study group was characterized by biometric parameters - age, sex, duration of T1D. Data from the AHCL system was automatically sent to the CareLink server and retrieved using CareLink Professional software (Medtronic MiniMed, USA). Two-week AHCL records as well as anthropometric parameters - body mass and height - were collected prospectively: right after AHCL enrollment, 6 months, and 1 year after starting AHCL. For each time point the body mass index (BMI) z-score was calculated using the individual's weight and height and the World Health Organization (WHO) reference values (32). CGM readings were analyzed using GlyCulator 3.0 software (Medical University of Łódz, Poland) (33).

Statistical analysis

The statistical analyses were performed using the Statistica 13.3 software (StatSoft, Inc., Tulsa, OK, USA). Descriptive statistics (mean, standard deviation, median, interquartile range, minimum and maximum values, coefficient of variation, and their 95% confidence intervals were calculated for each parameter. Data distribution was tested using the Shapiro–Wilk test. The differences between baseline and at 6 months follow-up as well as between baseline and at 1 year follow-up, were established, using Student's t-test for dependent samples or the Wilcoxon signed rank test, whichever was appropriate according to the data distribution. Results were considered significant at p value lower than 0.05.

The study protocol was approved by the Local Bioethics Committee of the Medical University of Silesia in Katowice (Decision no. PCN/0022/KBI/83/2 of March 30, 2021).

Results

The study included 50 children and adolescents with an average age of 9.9 ± 2.4 years (median: 9.7, range: 5.4-16.8), 24 (48%) of them were male. The average onset of T1D was 6.0 ± 2.9 (median: 5.7, range: 0.8-13.0) years and mean T1D duration was 3.9 ± 2.6 years (median: 3.63, range: 0.3-10.7).

BMI z-scores of the studied children and adolescents did not change significantly neither after 6 nor after 12 months of follow-up (Table 1 and Figure 1). There was a slight increase in total daily insulin (TDI) dose from baseline (by 0.1 U/kg at 6 months and by 0.13 U/kg after 1 year of follow-up, p<0.001), however the percent of basal and bolus insulin remained stable. The amount of insulin in auto-corrective boluses increased significantly at 6 and 12 months (respectively by 0.82 U and 1.24 U, p<0.05) (Table 1 and Figure 1).

In general we did not observe changes after 6 or after 12 months of follow-up in glycemic control parameters: average

TABLE 1 Body mass index, daily insulin doses and sensor data of children and adolescents with type 1 diabetes using the advanced hybrid closed-loop system at initiation of the system, at 6 months and 1 year of follow-up.

	AHCL First two weeks	AHCL Two weeks after six months	AHCL Two weeks after 1 year
BMI z-score	0.57 ± 2.85	0.55 ± 2.90	0.51 ± 2.79
Total daily insulin [U/kg]	0.67 ± 0.21	0.77 ± 0.21 *	0,80 ± 0.21 **
Basal insulin [%]	34.88 ± 6.91	33.16 ± 5.09	35.08 ± 6.36
Bolus insulin [%]	65.02 ± 6.9	66.84 ± 5.09	64.92 ± 6.36
Autocorrection [U]	2.85 ± 2.42	3.67 ± 2.64 *	4.09 ± 2.60 **
Sensor use [%]	95.1 ± 3.94	94.04 ± 4.69	93.8 ± 4.52
Smartguard [%]	92.32 ± 14.72	96.62 ± 5.70	97.56 ± 2.79 **
Average sensor glucose [mg/dl]	131.36± 11.04	132.46 ± 11.73	132.45 ± 13.42
CV [%]	34.99± 5.17	33.75 ± 5.02	34.06 ± 5.38
GMI [%]	6.45 ± 0.26	6.48 ± 0.28	6.48 ± 0.32
		Percent of sensor glucose values in range	
>250 mg/dl [%]	2.33 ± 2.52	2.34 ± 2.31	2.68 ± 3.48
180 - 250 mg/dl [%]	13.13 ± 5.74	12.83 ± 5.88	12.59 ± 5.78
70 - 180 mg/dl [%]	79.28 ± 8.12	81.16 ± 7.83	80.40 ± 8.25
54 - 70 mg/dl [%]	4.15 ± 2.70	2.95 ± 1.75 *	3.4 ± 2.34
<54 mg/dl [%]	1.11 ± 1,07	0.73 ± 0.77 *	0.93 ± 0.92

AHCL, Advanced Hybrid Closed-Loop System; U, unit; CV, Coefficient of variation; GMI, Glucose Management Indicator; *, significant difference (p<0.05) between baseline 2 weeks using AHCL and the 2 weeks after 6 months, ** significant difference (p<0.05) between baseline 2 weeks using AHCL and the 2 weeks after 1 year follow-up.

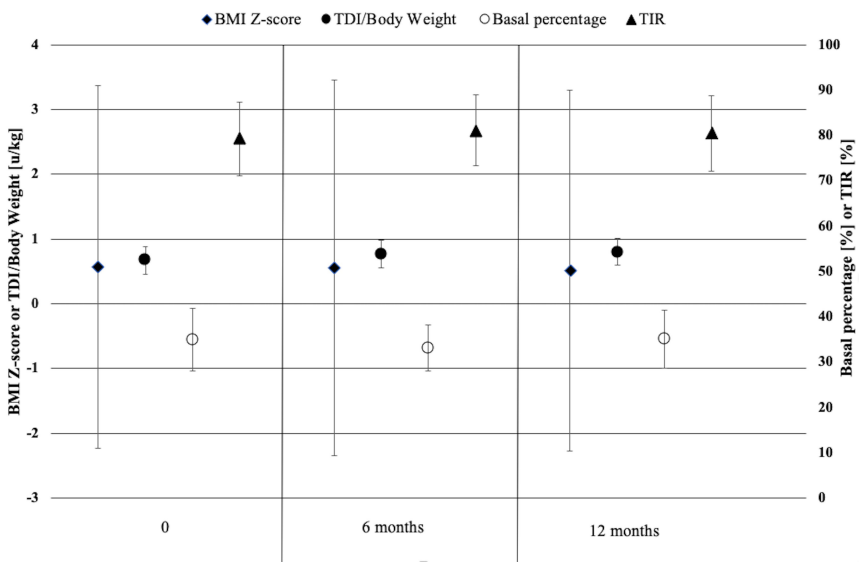


FIGURE 1
Body mass index, daily insulin doses, basal percentage and TIR of children and adolescents with type 1 diabetes using the advanced hybrid closed-loop system at initiation of the system, at 6 months and 1 year of follow-up.

sensor glucose, coefficient of variation, glucose management indicator, and percent of sensor glucose values in different ranges. Only the percent of time spent in range 54-70 mg/dl and <54 mg/dl was significantly reduced after 6 months ($p<0.05$), but did not differ from baseline after one year of treatment (Table 1). After one year, the use of the AHCL auto mode (% of time using Smartguard) increased significantly ($p<0.05$), while maintaining similar time of sensor use (Table 1).

Discussion

We describe stable BMI z-scores of children and adolescents with T1D using the AHCL system during a 1-year follow up which is the first such long observation (34–45). Similar results, also for a pediatric cohort of individuals with T1D, were obtained by Tornese et al., who reported no change in BMI z-score after 6 months of insulin treatment using either the hybrid closed-loop (Minimed 670G) or AHCL (Minimed 780G) systems. Noteworthy, our patients were characterized by more optimal glycemic control (lower GMI 6.48% vs 7.1% and higher TIR 81.16% vs 72%) after 6 months of AHCL use with a similar time spent in auto-mode (96.62% vs 96) and percent of sensor use (94.04% vs 92%) (36). Among the growing number of studies evaluating the AHCL Minimed 780G system these are to our knowledge the only two investigating the associations with BMI z-score (34–45). Former studies that observed BMI in children with T1D using insulin pumps other than AHCL showed not unequivocal results although the latest metaanalysis suggested no change in body mass (28, 29).

Weight gain was linked to the basal insulin dose and seemed to be independent from the TDI dose (30). Our observations from this study stay in line with the previous findings - no change in BMI z-score was accompanied by a slight increase in TDI. The percentage of basal insulin was 34.88% and did not change after 6 or 12 months of AHCL use. Also the largest pediatric AHCL study during which 790 patients 15 years of age were followed for 6 months (time in auto mode at 6 months 94.9%) revealed an increase in TDI (44). In two other investigations the transition from sensor augmented pump with low glucose suspend system (SAP-LGS) or predictive low glucose suspend system (SAP-PLGS) to AHCL - contrary to our findings - was associated with a decrease in basal insulin with a simultaneous increase in bolus insulin, which is most likely due to self-correction (39, 45). This small but significant increase of TDI in our cohort may be partially explained by the fact that the studied population was younger and some of these children might have entered puberty during the observation time.

Another interesting observation from this study is the increased use of the AHCL auto mode and no change of time of sensor use after one year of observation. The high and unchanged sensor use that was found also in another study may result from the necessity to use it to operate the Minimed system in automatic mode (36). The increasing time in auto-mode is optimistic and would suggest that the patients are not only keen to use it as a novelty but with time learn to rely on it and use it more.

The studied cohort showed a high TIR and other sensor parameters very well fitting the recommendations. This optimal control was maintained during the 1 year of follow-up. Authors of shorter, up to 6 months, observations described significant improvements in glycemic control parameters after switching to AHCL from SAP-LGS/PLGS. However the cohort presented in this study as well as the subgroup of patients from Poland that were included in the publication by Arrieta et al. had the best baseline glycemic control (44).

The above discussed aspects suggest that the AHCL may help to sustain good glycemic control without a risk of increasing body weight. The unchanged percent of time <70 and <54 mg/dl can be also interpreted as no increase in severe hypoglycaemia risk. If AHCL could be a beneficial tool for overweight or obese children with T1D in terms facilitaing weight reduction without impairment in glycemic control requires further studies.

The novelty of this study is the longest, 1 year follow-up of the AHCL use in auto-mode in the pediatric population combined with the assessment of BMI z-score changes. Another strength of this investigation is the youngest observed until now (mean age: 9.88 ± 2.44) (34–45) cohort characterized by good glycemic control parameters. Nevertheless we acknowledge the limitations, which include the lack of the assessment of the amount of carbohydrates consumed as well as no detailed dietary evaluation at baseline and follow up that would allow us to note any changes in eating behaviors.

Conclusion

During the 1 year of follow-up the BMI z-score of children and adolescents with T1D using an AHCL system remained stable. Although there was a slight increase in the total daily insulin dose, the percent of basal insulin was unchanged. The patients maintained recommended glycemic control.

Data availability statement

The datasets generated and analyzed for this study are available from the corresponding or first author upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by Local Bioethics Committee of the Medical University of Silesia in Katowice (Decision no. PCN/0022/KBI/83/2 of March 30, 2021). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

SS researched data, performed statistical analysis, participated in data interpretation and drafting the manuscript. PJ-C designed the study and reviewed and edited the manuscript. AO, ER, and PW participated in researching data and contributed to the drafting of the manuscript. JP supervised the statistical analysis and participated in data interpretation. AC reviewed the study design, participated in data interpretation, reviewed and edited the manuscript. All authors approved the final version of the manuscript. AC and SS are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

JP was financed by SUT grant no 02/070/BK_22/0033 project.

Conflict of interest

P-JC has received speaker honoraria from Medtronic, DexCom, Abbott, Ypsomed, and Roche, was a member of the advisory boards for Medtronic and Abbott and received research support from Medtronic. SS has received speaker honoraria from Medtronic and Ypsomed.

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.

Publisher's note

References

1. Ciężki S, Kurpiewska E, Bossowski A, Głowińska-Olszewska B. Multi-faceted influence of obesity on type 1 diabetes in children - from disease pathogenesis to complications. *Front Endocrinol (Lausanne)* (2022) 16:890833(13). doi: 10.3389/fendo.2022.890833

2. Liu LL, Lawrence JM, Davis C, Liese AD, Pettitt DJ, Pihoker C, et al. SEARCH for diabetes in youth study group. Prevalence of overweight and obesity in youth with diabetes in USA: The SEARCH for diabetes in youth study. *Pediatr Diabetes* (2010) 11(1):4–11. doi: 10.1111/j.1399-5448.2009.00519.x

3. Maffeis C, Birkebaek NH, Konstantinova M, Schwandt A, Vazeou A, Casteels K, et al. SWEET study group. Prevalence of underweight, overweight, and obesity in children and adolescents with type 1 diabetes: Data from the international SWEET registry. *Pediatr Diabetes* (2018) 19(7):1211–20. doi: 10.1111/pedi.12730

4. Baskaran C, Volkening LK, Diaz M, Laffel LM. A decade of temporal trends in overweight/obesity in youth with type 1 diabetes after the diabetes control and complications trial. *Pediatr Diabetes* (2015) 16(4):263–70. doi: 10.1111/pedi.12166

5. DuBose SN, Hermann JM, Tamborlane WV, Beck RW, Dost A, DiMeglio LA, et al. Type 1 diabetes exchange clinic network and diabetes prospective follow-up registry. obesity in youth with type 1 diabetes in Germany, Austria, and the united states. *J Pediatr* (2015) 167(3):627–32.e1-4. doi: 10.1016/j.jpeds.2015.05.046

6. Muis MJ, Bots ML, Bilo HJ, Hoogma RP, Hoekstra JB, Grobbee DE, et al. Determinants of daily insulin use in type 1 diabetes. *J Diabetes Complications* (2006) 20(6):356–60. doi: 10.1016/j.jdiacomp.2005.08.006

7. Corbin KD, Driscoll KA, Pratley RE, Smith SR, Maahs DM, Mayer-Davis EJ. Advancing care for type 1 diabetes and obesity network (ACT1ON). obesity in type 1 diabetes: Pathophysiology, clinical impact, and mechanisms. *Endocr Rev* (2018) 1;39(5):629–63. doi: 10.1210/er.2017-00191

8. Van der Schueren B, Ellis D, Faradji RN, Al-Ozairi E, Rosen J, Mathieu C. Obesity in people living with type 1 diabetes. *Lancet Diabetes Endocrinol* (2021) 9 (11):776–85. doi: 10.1016/S2213-8587(21)00246-1

9. Polsky S, Ellis SL. Obesity, insulin resistance, and type 1 diabetes mellitus. *Curr Opin Endocrinol Diabetes Obes* (2015) 22(4):277–82. doi: 10.1097/MED.0000000000000170

10. Purnell JQ, Zinman B, Brunzell JDDCCT/EDIC Research Group. The effect of excess weight gain with intensive diabetes mellitus treatment on cardiovascular disease risk factors and atherosclerosis in type 1 diabetes mellitus: Results from the diabetes control and complications Trial/Epidemiology of diabetes interventions and complications study (DCCT/EDIC) study. *Circulation* (2013) 127(2):180–7. doi: 10.1161/CIRCULATIONAHA.111.077487

11. Pinhas-Hamiel O, Levek-Motola N, Kaidar K, Boyko V, Tisch E, Mazor-Aronovitch K, et al. Prevalence of overweight, obesity and metabolic syndrome components in children, adolescents and young adults with type 1 diabetes mellitus. *Diabetes Metab Res Rev* (2015) 31(1):76–84. doi: 10.1002/dmrr.2565

12. Chillarón JJ, Goday A, Flores-Le-Roux JA, Benaiges D, Carrera MJ, Puig J, et al. Estimated glucose disposal rate in assessment of the metabolic syndrome and microvascular complications in patients with type 1 diabetes. *J Clin Endocrinol Metab* (2009) 94(9):3530–4. doi: 10.1210/jc.2009-0960

13. Gomes MB, Conte D, Drummond KRG, Mallmann F, Pinheiro AA, Leal FSL, et al. Overweight/obesity in adolescents with type 1 diabetes belonging to an admixed population. a Brazilian multicenter study. *Diabetol Metab Syndr* (2022) 14 (1):1. doi: 10.1186/s13098-021-00759-9

14. Jaiswal M, Divers J, Dabelea D, Isom S, Bell RA, Martin CL, et al. Prevalence of and risk factors for diabetic peripheral neuropathy in youth with type 1 and type 2 diabetes: SEARCH for diabetes in youth study. *Diabetes Care* (2017) 40(9):1226–32. doi: 10.2337/dc17-0179

15. Franceschi R, Mozzillo E, Di Candia F, Rosanio FM, Leonardi L, Liguori A, et al. A systematic review of the prevalence, risk factors and screening tools for autonomic and diabetic peripheral neuropathy in children, adolescents and young adults with type 1 diabetes. *Acta Diabetol* (2022) 59(3):293–308. doi: 10.1007/s00592-022-01850-x

16. Kazamel M, Stino AM, Smith AG. Metabolic syndrome and peripheral neuropathy. *Muscle Nerve* (2021) 63(3):285–93. doi: 10.1002/mus.27086

17. The DCCT Research Group. Weight gain associated with intensive therapy in the diabetes control and complications trial. the DCCT research group. *Diabetes Care* (1988) 11(7):567–73. doi: 10.2337/diacare.11.7.567

18. Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular outcomes in type 1 diabetes: The DCCT/EDIC study 30-year follow-up. *Diabetes Care* (2016) 39(5):686–93. doi: 10.2337/dc15-1990

19. Casu A, Bilal A, Pratley RE. Advancing care for type 1 diabetes, obesity network (ACT1ON). pharmacological therapies to address obesity in type 1 diabetes. *Curr Opin Endocrinol Diabetes Obes* (2020) 27(4):194–206. doi: 10.1097/MED.0000000000000555

20. Driscoll KA, Corbin KD, Maahs DM, Pratley R, Bishop FK, Kahkoska A, et al. Advancing care for type 1 diabetes and obesity network (ACT1ON). biopsychosocial aspects of weight management in type 1 diabetes: A review and next steps. *Curr Diabetes Rep* (2017) 17(8):58. doi: 10.1007/s11892-017-0892-1

21. Myśliwiec A, Lejk A, Skalska M, Jastrzębska J, Sztangierska B, Jastrzębski Z. Assessment of the diet of male adolescents suffering from type 1 diabetes. *Pediatr Endocrinol Diabetes Metab* (2021) 27(1):7–11. doi: 10.5114/pedm.2020.101805

22. Minges KE, Whittemore R, Grey M. Overweight and obesity in youth with type 1 diabetes. *Annu Rev Nurs Res* (2013) 31:47–69. doi: 10.1891/0739-6686.31.47

23. Zaharieva DP, Addala A, Simmons KM, Maahs DM. Weight management in youth with type 1 diabetes and obesity: Challenges and possible solutions. *Curr Obes Rep* (2020) 9(4):412–23. doi: 10.1007/s13679-020-00411-z

24. Mottalib A, Kasetty M, Mar JY, Elseaidy T, Ashrafzadeh S, Hamdy O. Weight management in patients with type 1 diabetes and obesity. *Curr Diabetes Rep* (2017) 17(10):92. doi: 10.1007/s11892-017-0918-8

25. Chetty T, Shetty V, Fournier PA, Adolfsson P, Jones TW, Davis EA. Exercise management for young people with type 1 diabetes: A structured approach to the exercise consultation. *Front Endocrinol (Lausanne)* (2019) 10:326. doi: 10.3389/fendo.2019.00326

26. Biester T, Tauschmann M, Chobot A, Kordonouri O, Danne T, Kapellen T, et al. The automated pancreas: A review of technologies and clinical practice. *Diabetes Obes Metab* (2022) 24 Suppl 1:43–57. doi: 10.1111/dom.14576

27. Russell-Jones D, Khan R. Insulin-associated weight gain in diabetes–causes, effects and coping strategies. *Diabetes Obes Metab* (2007) 9(6):799–812. doi: 10.1111/j.1463-1326.2006.00686.x

28. Yeh HC, Brown TT, Maruthur N, Ranasinghe P, Berger Z, Suh YD, et al. Comparative effectiveness and safety of methods of insulin delivery and glucose monitoring for diabetes mellitus: a systematic review and meta-analysis. *Ann Intern Med* (2012) 157(5):336–47. doi: 10.7326/0003-4819-157-5-201209040-00508

29. Study Group REPOSE. Relative effectiveness of insulin pump treatment over multiple daily injections and structured education during flexible intensive insulin treatment for type 1 diabetes: cluster randomised trial (REPOSE). *BMJ* (2017) 356: j1285. doi: 10.1136/bmj.j1285

30. Boucher-Berry C, Parton EA, Alemzadeh R. Excess weight gain during insulin pump therapy is associated with higher basal insulin doses. *J Diabetes Metab Disord* (2016) 15:47. doi: 10.1186/s40200-016-0271-5

31. Oldham V, Mumford B, Lee D, Jones J, Das G. Impact of insulin pump therapy on key parameters of diabetes management and diabetes related emotional distress in the first 12 months. *Diabetes Res Clin Pract* (2020) 166:108281. doi: 10.1016/j.diabres.2020.108281

32. World Health Organization. *BMI* . Available at: https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/bmi-for-age (Accessed 25 August 2022).

33. *GlyCulator 3.0 software*. Available at: https://glyculator.btm.umed.pl/ (Accessed 25 August 2022).

34. Elbarbary NS A, Ismail EAR. Glycemic control during Ramadan fasting in adolescents and young adults with type 1 diabetes on MiniMed™ 780G advanced hybrid closed–loop system: A randomized controlled trial. *Diabetes Res Clin Pract* (2022) 191:110045. doi: 10.1016/j.diabres.2022.110045

35. Karakuş KE, Yeşiltepe Mutlu G, Gökçe T, Eviz E, Can E, Muradoğlu S, et al. Insulin requirements for basal and auto-correction insulin delivery in advanced hybrid closed-loop system: 4193 days' real-world data of children in two different age groups. *J Diabetes Sci Technol* (2022) 30. doi: 10.1177/19322968221106194

36. Tornese G, Buzzurro F, Carletti C, Faleschini E, Barbi E. Six-month effectiveness of advanced *vs.* standard hybrid closed-loop system in children and adolescents with type 1 diabetes mellitus. *Front Endocrinol (Lausanne)* (2021) 12:766314. doi: 10.3389/fendo.2021.766314

37. Petrovski G, Al Khalaf F, Campbell J, Day E, Almajaly D, Hussain K, et al. Successful transitioning children and adolescents with type 1 diabetes from multiple daily injections to advanced hybrid closed-loop system in 10 days: a prospective intervention study on MiniMed 780G system. *Acta Diabetol* (2022) 59 (5):743–6. doi: 10.1007/s00592-022-01851-w

38. Bergenstal RM, Nimri R, Beck RW, Criego A, Laffel L, Schatz D, et al. FLAIR study group. a comparison of two hybrid closed-loop systems in adolescents and young adults with type 1 diabetes (FLAIR): A multicentre, randomised, crossover trial. *Lancet* (2021) 397(10270):208–19. doi: 10.1016/S0140-6736(20)32514-9

39. Beato-Víbora PI, Gallego-Gamero F, Ambrojo-López A, Gil-Poch E, Martín-Romo I, Arroyo-Díez FJ. Rapid improvement in time in range after the implementation of an advanced hybrid closed-loop system in adolescents and

adults with type 1 diabetes. *Diabetes Technol Ther* (2021) 23(9):609–15. doi: 10.1089/dia.2021.0037

40. Carlson AL, Sherr JL, Shulman DI, Garg SK, Pop-Busui R, Bode BW, et al. Safety and glycemic outcomes during the MiniMed™ advanced hybrid closed-loop system pivotal trial in adolescents and adults with type 1 diabetes. *Diabetes Technol Ther* (2022) 24(3):178–89. doi: 10.1089/dia.2021.0319

41. Collyns OJ, Meier RA, Betts ZL, Chan DSH, Frampton C, Frewen CM, et al. Improved glycemic outcomes with medtronic MiniMed advanced hybrid closed-loop delivery: Results from a randomized crossover trial comparing automated insulin delivery with predictive low glucose suspend in people with type 1 diabetes. *Diabetes Care* (2021) 44(4):969–75. doi: 10.2337/dc20-2250

42. Matejko B, Juza A, Kieć-Wilk B, Cyranka K, Krzyżowska S, Chen X, et al. Transitioning of people with T1D from multiple daily injections and self-monitoring of blood glucose directly to MiniMed 780G advanced hybrid closed loop system: A two-center, randomized, controlled study. *Diabetes Care* (2022) 15:dc220470. doi: 10.2337/dc22-0470

43. Piccini B, Pessina B, Barni F, Benucci F, Casalini E, Farina S, et al. Metabolic control in type 1 diabetes patients switching to advanced hybrid closed loop. *Pediatr Diabetes* (2021) 22(Suppl. S30):65. doi: 10.1111/pedi.13269

44. Arrieta A, Battelino T, Scaramuzza AE, Da Silva J, Castañeda J, Cordero TL, et al. Comparison of MiniMed 780G system performance in users aged younger and older than 15 years: Evidence from 12 870 real-world users. *Diabetes Obes Metab* (2022) 24(7):1370–9. doi: 10.1111/dom.14714

45. Seget S, Rusak E, Polanska J, Jarosz-Chobot P. Prospective open-label, single-arm, single-center follow-up study of the application of the advanced hybrid closed loop system in well-controlled children and adolescents with type 1 diabetes. *Diabetes Technol Ther* (2022) 4. doi: 10.1089/dia.2022.0148

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 18 October 2022
DOI 10.3389/fendo.2022.1032491

OPEN ACCESS

EDITED BY
Artur Mazur,
University of Rzeszow, Poland

REVIEWED BY
Sabina Galiniak,
University of Rzeszow, Poland
Łukasz Dembiński,
Medical University of Warsaw, Poland

*CORRESPONDENCE
Vidhu V. Thaker
vvt2114@cumc.columbia.edu

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 30 August 2022
ACCEPTED 29 September 2022
PUBLISHED 18 October 2022

CITATION
Rajamoorthi A, LeDuc CA and Thaker VV (2022) The metabolic conditioning of obesity: A review of the pathogenesis of obesity and the epigenetic pathways that "program" obesity from conception. *Front. Endocrinol.* 13:1032491. doi: 10.3389/fendo.2022.1032491

The metabolic conditioning of obesity: A review of the pathogenesis of obesity and the epigenetic pathways that "program" obesity from conception

Ananthi Rajamoorthi[1], Charles A. LeDuc[1,2] and Vidhu V. Thaker[1,2,3]*

[1]Department of Pediatrics, Columbia University Medical Center, New York, NY, United States, [2]The Naomi Berrie Diabetes Center, Columbia University IRVING Medical Center, New York, NY, United States, [3]Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, United States

Understanding the developmental origins of health and disease is integral to overcome the global tide of obesity and its metabolic consequences, including atherosclerotic cardiovascular disease, type 2 diabetes, hyperlipidemia, and nonalcoholic fatty liver disease. The rising prevalence of obesity has been attributed, in part, to environmental factors including the globalization of the western diet and unhealthy lifestyle choices. In this review we argue that *how* and *when* such exposures come into play from conception significantly impact overall risk of obesity and later health outcomes. While the laws of thermodynamics dictate that obesity is caused by an imbalance between caloric intake and energy expenditure, the drivers of each of these may be laid down before the manifestation of the phenotype. We present evidence over the last half-century that suggests that the temporospatial evolution of obesity from intrauterine life and beyond is, in part, due to the conditioning of physiological processes at critical developmental periods that results in maladaptive responses to obesogenic exposures later in life. We begin the review by introducing studies that describe an association between perinatal factors and later risk of obesity. After a brief discussion of the pathogenesis of obesity, including the systemic regulation of appetite, adiposity, and basal metabolic rate, we delve into the mechanics of how intrauterine, postnatal and early childhood metabolic environments may contribute to adult obesity risk through the process of metabolic conditioning. Finally, we detail the specific epigenetic pathways identified both in preclinical and clinical studies that synergistically "program" obesity.

KEYWORDS

childhood obesity, metabolic programing, epigenetics, *in utero* environment, cardiometabolic risk, postnatal conditions

Key Messages

1. Obesity is the result of a dynamic interplay between genetics, physiology, behavior, and environment, that accumulate over time from conception and can predispose to energy homeostasis perturbations throughout the lifespan.
2. Developmental plasticity allows for the conditioning or "programming" of obesity in response to the metabolic environments of intrauterine, postnatal, and early childhood, that may be responsible for resistance to weight loss efforts in later life.
3. The metabolic environments in the prenatal, postnatal, and early childhood periods, reflective of maternal nutrition, postnatal feeding, and early childhood adiposity, respectively, significantly contribute to adult health outcomes.
4. Emerging evidence suggests that *in utero*, postnatal and early childhood metabolic environments affect adult basal metabolic rate and, in turn, obesity risk through metabolic programming, that needs further exploration.
5. Altered maternal levels of metabolic substrates and hormones in obesity contribute to maternal insulin resistance, oxidative stress and inflammation, resulting in perturbations in glucose and lipid homeostasis and feeding circuitry in the perinatal period, contributing to long-term obesity risk.
6. The *in utero* environment of maternal obesity, and the concomitant short-term aberrations in energy homeostasis during the perinatal period, translate to long-term obesity risk through both central and peripheral epigenetic modifications that regulate energy homeostatic set points.
7. Epigenetic changes are not exclusive to the perinatal period; these changes may occur during childhood and beyond in response to behavioral and environmental factors.
8. Epigenetic pathways that contribute to the conditioning of obesity are highly dynamic and reversible. Currently known pathways include variations in methylation status of obesity-related genes, which occur in response to maternal, postnatal, and early childhood nutrition, and have been shown to be associated with differences in adiposity and body composition in infancy and beyond.

Introduction

Early life exposures "program" long-term obesity risk

The global impact of childhood obesity

Childhood obesity has become one of the most pervasive global health crises of this century. In 2020, 39 million children worldwide under the age of 5 years had overweight or obesity. The prevalence of overweight and obesity in children aged 5-19 years across genders quadrupled from 4% in 1975 to over 18% in 2016. Further, what was once considered to be an epidemic of the Western world, childhood obesity has infiltrated both low- and middle-income countries. Since 2000, there has been a 24% increase in the prevalence of overweight/obesity in children under 5 years in Africa, while 50% of all children under 5 years with overweight and obesity lived in Asia in 2019 (1).

Obesity impacts growth and development from infancy, and is associated with increased risk of body dissatisfaction, depressive symptoms and low self-esteem during childhood and adolescence (2, 3). Childhood obesity persists into adulthood and is associated with higher prevalence of cardiometabolic risks including atherosclerotic cardiovascular disease (ASCVD), type 2 diabetes (T2D), hyperlipidemia and nonalcoholic fatty liver disease (NAFLD) (4).

Systemic regulation of adiposity and metabolic rate

Many genes associated with body mass index (BMI) are expressed in the hypothalamus, which receives sensory inputs from peripheral organs including the gastrointestinal (GI) tract, liver, adipose tissue, pancreas, and skeletal muscle regarding the overall metabolic status of the organism, to regulate food intake, appetite, and body weight (5). The "gut-brain-axis" is critical for some of these inputs due to the presence of mechanosensors and chemoreceptors in the intestinal epithelial wall that sense both volume and nutrient content of ingested food. This information is communicated centrally *via* two mechanisms: vagal innervation and endocrine hormones. GI-derived hormones, such as glucagon like peptide-1 (GLP-1) and ghrelin, not only behave in a paracrine manner to affect the local absorption and metabolism of nutrients, but can also act centrally through signaling pathways to affect feeding behavior and whole-body energy homeostasis (6). Hormones produced by adipose tissue, pancreas, skeletal muscle, and liver also serve as sensory inputs for the hypothalamus, including leptin, cholecystokinin, peptide YY, insulin, fibroblast growth factor 21 (FGF21) and other

"adipokines" and "myokines," which regulate systemic lipid and carbohydrate homeostasis (7). The level of leptin, produced by adipose tissue, is directly proportional to its mass. Its functional roles during early stages of development are diverse (8). In adults, however, reduction and/or absence of leptin promotes food intake and decreases energy expenditure (9). Together with the corticolimbic system and hindbrain, the hypothalamus integrates peripherally derived inputs to regulate appetite and food intake.

In addition to food intake, energy expenditure (EE) is an important component of metabolic homeostasis and is largely a combination of basal metabolism, thermogenesis, and physical activity. While central neuronal networks play a role in the regulation of EE, the critical component of EE, basal metabolic rate (BMR), is primarily determined by fat-free mass. Fat-free or "lean" mass is composed of skeletal muscle, which together with other metabolically active organs including the liver, heart, brain and kidneys, contribute to BMR (10). Skeletal muscle is a major player for insulin mediated glucose disposal; and dysregulation of skeletal muscle metabolism can strongly influence glucose homeostasis and insulin sensitivity predisposing to obesity related metabolic diseases (11).

Based on the laws of thermodynamics, obesity is a consequence of an imbalance of food intake and energy expenditure. But *how* and *when* such perturbations take place, and in what circumstances and metabolic environments they are more likely to occur, plays a vital role in determining the overall risk of obesity which will be discussed in the following sections.

The role of "programming" in the pathogenesis and treatment of obesity

Obesity is the result of a dynamic interplay between genetics, physiology, behavior, and environment, that accumulate over time since conception and can predispose to perturbations in energy homeostasis throughout life. The most widely studied and accepted interventions for obesity are lifestyle modifications, including exercise and dietary changes. However, both the efficacy and sustainability of such interventions are questionable, as the majority of individuals with obesity are unable to maintain reduced weight despite effective weight loss (12).

> *KEY MESSAGE 1*
>
> Obesity is the result of a dynamic interplay between genetics, physiology, behavior, and environment, that accumulate over time since conception and can predispose to energy homeostasis perturbations through lifespan.

Socioeconomic factors, including accessibility to resources, are important limitations to the overall success of lifestyle interventions. As obesity often affects populations of limited socioeconomic resources, lifestyle modifications are challenging to initiate and sustain in the populations that would most benefit from such interventions, often due to a lack of resources and low health literacy (13, 14).

Further, if obesity was simply driven by an imbalance between food intake and expenditure at a single time point, lifestyle interventions would result in more positive and consistent outcomes. However, despite many public health measures promoting healthy lifestyle habits and health care spending on the screening and prevention of obesity and metabolic syndrome, obesity related ASCVD remains the leading cause of morbidity and mortality in the US and worldwide (15, 16).

There are several physiological mechanisms that explain why sustained weight loss through lifestyle modifications remains difficult (7, 17). Caloric restriction, both in short- and long-term, reduces 24-hour EE (18–20). In addition, several neuroendocrine hormones fluctuate in response to either caloric restriction or acute weight loss, including reductions in leptin and cholecystokinin and an increase in ghrelin, which ultimately function to increase appetite and promote weight regain. Sumithran et al. found that these hormonal fluctuations and increased appetite following 10% weight loss over 8 weeks (and subsequent weight stabilization), persist at 1 year (7). What causes these acute and chronic fluctuations in neuroendocrine signaling that ultimately thwart intervention and promote obesity? While genetic predisposition, socioeconomic factors, lifestyle habits and environmental exposures all contribute to the risk of obesity, the metabolic environment during early development lays the critical foundation for the conditioning of obesity and long-term ASCVD risk (Figure 1).

Studies discussed in the following sections show a clear association between perinatal and childhood metabolic exposures including maternal under- and over-nutrition, maternal diabetes, breastfeeding, early infancy weight gain, and later health outcomes, including obesity and T2D. We hypothesize that developmental plasticity allows for the conditioning or "programming" of obesity in response to the metabolic environments of intrauterine, postnatal, and early childhood life, that play an important role in the resistance to weight loss efforts in later life. Further, if obesity is, in part, the result of conditioning or "programming" of certain physiologic processes established during critical developmental periods of early life, then lifestyle interventions initiated during adulthood

> *KEY MESSAGE 2*
>
> Developmental plasticity allows for the conditioning or "programming" of obesity in response to the metabolic environments of intrauterine, postnatal, and early childhood, that may be responsible for resistance to weight loss efforts in later life.

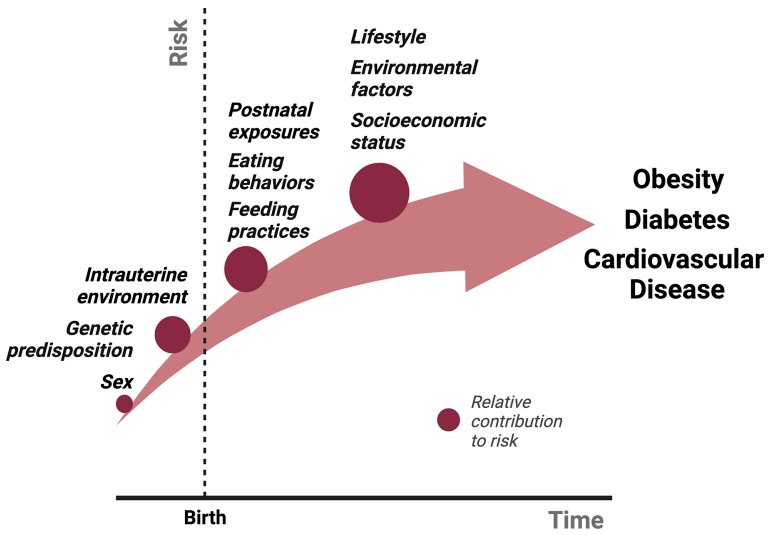


FIGURE 1
The relative contributions of various exposures to the cumulative risk for obesity over time from conception. Several factors contribute to the pathogenesis of obesity. We propose that intrauterine and postnatal exposures make relatively large contributions to the cumulative risk for obesity, in addition to the genetic predisposition. Studies described in this review have shown that postnatal exposures could potentially reverse the pathological events of intrauterine life in the setting of either maternal undernutrition or obesity, suggesting a more significant role for the postnatal environment in the "programming" obesity. We hypothesize that obesity risk acquired beyond early life, primarily in adolescence and adulthood through poor eating and lifestyle habits potentiated by socioeconomic status and other environmental factors, make an even greater contribution to obesity risk due to the sheer amount of time and opportunities available to accrue risk, although this is largely dependent on the individual.

will be insufficient to revert the initial "code" (21, 22). Addressing the risk for obesity in mothers prior to conception or during these critical developmental periods will likely produce favorable long-term outcomes.

The "developmental origins of health and disease" hypothesis and defining the phenomenon of metabolic programming

Metabolic programming (also called developmental programming) refers to the contribution of environmental exposures and nutrient imbalance from conception to early postnatal life – the critical times of organ development and maturation – to the risk of obesity and metabolic syndrome in adulthood, even after the absence or reversal of such exposures (17, 21–23). This is a more focused topic within a larger field known as the developmental origins of health and disease, or simply called "DOHaD." Barker and other experts coined this concept as the "fetal origins of disease" in the 1990s (17, 23–26). Both terms refer to a similar phenomenon in which exposures during critical developmental periods affect long-term health outcomes.

Many experts argue that the term "programming" is not accurate or reflective of the reversible and dynamic nature of these physiologic yet nonadaptive responses and prefer the term "conditioning" instead (17). Throughout this review, accepting both the limitations of the word "programming" as well as its prevalent use in scientific literature, we will interchangeably use these terms referring to the same phenomenon.

Epidemiological studies supporting the role of metabolic programming of obesity

Risk factors for obesity acquired during different periods of development

Prenatal period

The metabolic environments in prenatal, postnatal, and early childhood, which are, in turn, reflective of maternal nutrition, postnatal feeding and early childhood adiposity, respectively, contribute to adult health outcomes. One of the earliest insights into the effects of the *in utero* environment on risk of obesity is from the evaluation of the Dutch "Hunger Winter:" the tragic food restriction imposed upon much of the Northern Holland population by Germany during World War II (27). Over a 6-month period (October 1944 to April 1945), caloric intake was markedly decreased from 1500 to 500 kcal, including that for pregnant women. Exposure to maternal undernutrition during the first or second trimesters increased the prevalence of obesity and cardiometabolic risk in the

KEY MESSAGE 3

The metabolic environments in the prenatal, postnatal, and early childhood periods, reflective of maternal nutrition, postnatal feeding and early childhood adiposity, respectively, significantly contribute to adult health outcomes.

offspring at 18 years of age, when compared to those exposed in the third trimester.

There is significant controversy on the association of birth weight and later obesity risk. In a series of studies conducted since 1989, Barker et al. showed that individuals from the UK with low birth weight had higher risk of coronary heart disease and other risk factors associated with metabolic syndrome including T2D and elevated blood pressure in adulthood (23–26). Based on these studies, Barker proposed that chronic, degenerative conditions of adulthood, including heart disease and T2D, may be triggered by malnutrition during the *in utero* period (fetal "programming"), remain latent for many years, and manifest later in life. Deng et al. found that children from Guangzhong, China with high birth weight had higher odds of overweight/obesity compared to infants with normal birth weight (Odds ratio [OR] 2.42, 95% confidence interval (CI): 1.56; 3.76) (28). Large scale studies have confirmed a U-shaped relationship between birth weight and long-term outcomes, with both higher and lower levels associated with increased risk of different magnitude (29, 30). Further, there is an additive interaction of high birth weight and insufficient physical activity, such that children with high birth weight and lack of physical activity were 3.75 times more likely of being overweight or obese at age 7-9 years compared to those with normal birth weight and sufficient activity (OR 3.75, 95% CI: 2.06; 6.83) (28).

But why such discrepancies? Birth weight, although can be considered a marker of fetal health, is not a holistic reflection of the *dynamics* of intrauterine metabolic environment over time. As shown in the Dutch famine study, the gestational age during which maternal caloric restriction occurs may affect later obesity risk differentially, suggesting the presence of critical developmental windows. Further, there are several paths that can lead to a low birth weight independent of maternal body composition, food/caloric intake, or nutritional status. Mild prematurity, as well as intrauterine growth restriction secondary to uteroplacental insufficiency in the setting of chronic or gestational hypertension, smoking exposure, et cetera may also contribute to low birth weight, although studies in the UK, USA and Australia have shown that the associations between low birth weight and cardiovascular disease risk are not the result of such confounding variables (31–34).

More importantly though, while Barker's hypothesis on the effects of intrauterine life on later health outcomes has been confirmed, the "fetal origins of disease" hypothesis does not consider the metabolic environment in the postnatal period, that is also known to affect future obesity risk. Indeed, considering the exponential rise in the prevalence of ASCVD since Barker's studies, individuals with birth weight across the spectrum are likely to be impacted.

Other prenatal factors such as maternal diabetes and obesity, have been shown to contribute to obesity risk in children both during infancy and later in life. A large population-based comprehensive cohort study evaluated the association between diabetes in the mother (diabetes with insulin treatment, non-insulin treated diabetes and gestational diabetes), stratified by maternal prepregnancy BMI, and large for gestational age (LGA) at birth (35). Pregnant mothers with increasing BMI in the absence of diabetes had increasing odds of having an LGA offspring (36, 37). The adjusted OR for mothers with prepregnancy BMI of 25-29, 30-34 and ≥ 35 having LGA infants were 1.91, 2.45 and 3.38, respectively (95% CI: 1.83-2.00; 2.29-2.62; 3.08-3.71). Risk for LGA in offspring of mothers with obesity doubled in the presence of gestational diabetes and roughly tripled with T2D. Mothers who had insulin-treated diabetes had the highest risk of having LGA offspring regardless of BMI (35).

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study, designed to assess the impact of hyperglycemia on short- and long-term outcomes on the offspring, showed higher frequency of birth weight over 90th percentile, primary Caesarean section, and cord-blood serum C-peptide over 90th percentile (a marker of fetal hyperinsulinemia) with increasing hyperglycemia while adjusting for maternal prepregnancy BMI (38, 39). Further, children of mothers with hyperglycemia showed higher levels of obesity, glucose intolerance and insulin resistance at 10-14 years of age (40, 41). These data suggest that maternal hyperglycemia, even if not clinically considered gestational diabetes, is an important risk factor for perturbations in energy homeostasis and obesity in children, independent from maternal hyperinsulinemia, insulin resistance or obesity, perhaps driven by a hyperglycemic *in utero* environment.

The 1996 National Longitudinal Survey of Youth, Child and Young Adult in the US showed that children at 2-14 years of age born to mothers with obesity prior to pregnancy were approximately four times more likely of being obese (95% CI: 2.6; 6.4, $P < 0.001$) (42). Additionally, a meta-analysis of data acquired from birth cohort studies from Europe, North America and Australia showed that higher maternal prepregnancy BMI and gestational weight gain were associated with higher risk of

childhood overweight/obesity, most significantly in late childhood (10-18 years) (43).

Postnatal period

In the postnatal period, breastfeeding is found to have a protective effect against obesity (44). In a systematic review, Horta et al. showed that children (age 1-9 years), adolescents (age 10-19 years) and adults (≥ 20 years) who were breastfed as infants, have a reduction in the prevalence of overweight or obesity by 26% (95% CI: 21%; 32%), 37% (95% CI: 27%; 46%) and 12% (95% CI: 6%; 18%) respectively (45). The protective effect was more substantial in youth compared to adults, likely due to the accumulation of other environmental factors that may dilute the impact of breastfeeding over time. Further, while most studies were from high-income settings, the effect was consistent across income classifications. Breastfeeding during infancy is also associated with reduced odds of T2D in subjects aged 10-19 years [pooled OR: 0.46 (95% CI: 0.33; 0.66)]. However, this effect was not statistically significant in subjects older than 20 years of age, re-emphasizing the impact of other contributors in adulthood (45).

Early childhood

Weight gain during the first two years of life, is often used as a surrogate marker for overall metabolic status and has been shown to affect persistent risk for obesity (46, 47). Rapid weight gain during early infancy, especially during the first 4-6 months of life, is associated with higher risk of obesity both in childhood (at 6-8 years of age) and in adulthood (at 20 years). Eid et al. showed that the prevalence of obesity was greater in infants with rapid weight gain (9.4%) in the first 6 months of life compared to those with slower weight gain (1.9%) as early as 1970 (48). In 2002, in a large-scale prospective cohort study, Stettler et al. demonstrated a 38% (95% CI: 32%-44%) increased risk of overweight status in children aged 7 years for each 100 gram per month increase in weight gain during the first 4 months of life, independent of birth weight (49). Over two dozen studies have since shown a similar association between rapid early childhood weight gain and later obesity risk (50–54).

Accelerated weight gain during infancy also increases the long-term risk of insulin resistance (55). Singhal et al. measured 32-33 split proinsulin, a serum marker of insulin resistance, in adolescent populations (age 13-16 years) with a history of prematurity who received either nutrient-enriched or lower-nutrient diet during infancy. Premature infants fed nutrient-enriched formula had greater levels of fasting 32-33 split proinsulin (7.2 pmol/L, 95% CI 6.4–8.1) in adolescence compared to those fed lower-nutrient diet (5.9 pmol/L, 95% CI 5.2–6.4), with a mean difference of 20.6% (p = 0.01). Furthermore, fasting 32-33 split proinsulin concentration was associated with greater weight gain in the first two weeks of life (13.2% [5.4–20.9] change per 100 g weight increase; p = 0.001). These data indicate that relative undernutrition during the postnatal period in premature infants may be protective against development of insulin resistance later in life.

The UK-based Avon longitudinal study of parents and children (ALSPAC), found a significant association between weight gain in infancy and seven other independently associated factors with the risk of obesity in 7-year-old children: birth weight, parental obesity, sleep duration, television viewing, size in early life, catch-up growth and early adiposity or BMI rebound (before 43 months) (56, 57). Children in the highest quartile for weight at age 8 months and 18 months were more likely to have obesity at age 7 years with an OR of 3.13 (95% CI: 1.43; 6.85, p = 0.004) and 2.65 (95% CI: 1.25; 5.59, p = 0.011), respectively. Children with a history of rapid catch-up growth between 0-2 years were approximately 2.6 times more likely to be obese at 7 years of age (OR 2.60, 95% CI: 1.09;6.16, p = 0.002). Adiposity rebound, which corresponds to the second rise in BMI that typically occurs between 5-7 years of age, was associated with later obesity risk. Specifically, early adiposity rebound (by 61 months) and very early adiposity rebound (by 43 months) were both associated with risk of obesity at 7 years, with ORs of 2.01 (95% CI: 0.81; 5.20, p < 0.001) and 15.00 (95% CI: 5.32; 42.30, p < 0.001) respectively.

Collectively, these studies describe associations between *in utero*, postnatal, and early infancy factors that affect one's metabolic environment, predisposing not only to childhood obesity but also metabolic syndrome. Before taking a deeper dive into why such associations may exist, we will first define obesity and briefly summarize the factors that affect whole-body adiposity and metabolic rate, both of which contribute to the pathogenesis of obesity.

A word on BMI and other measures of obesity

In studies discussed throughout this review, various markers of obesity were used as proxy measures of fat accumulation. BMI (weight [in kg]/height^2 [in m]) is often used as a marker of excessive fat accumulation and is the most common measure of obesity in epidemiological studies over the age of 2 years. The World Health Organization (WHO) defines obesity in adults as BMI ≥ 30 kg/m^2. In children under 5 years, BMI-for-age, and weight-for-height measurements according to specific growth standards are frequently used (47). The WHO defines obesity as weight-for-height ≥ 3 standard deviations above the median WHO Child Growth standards, while the Centers for Disease Control (CDC) defines childhood obesity as BMI ≥ 95th percentile for sex and age as per the CDC 2000 growth charts.

There are several limitations to the use of BMI as a marker of fat accumulation. The calculation of BMI includes both body

weight and height. However, body weight reflects both fat mass and fat-free mass. Thus, BMI is not an accurate depiction of one's fat mass. Furthermore, several factors have been shown to affect BMI including age, race, physical training, et cetera. For example, BMI may not be an accurate measure of fat mass in various South Asian populations where the "thin yet fat" phenotype is prevalent (58), or in athletes where BMI overestimates adiposity (59).

Body mass and body composition are more accurate measures of adiposity. However, these are not easily accessible and expensive for large-scale studies. Nonetheless, various instrumentations have been used to measure lean and fat mass in humans and animals including dual energy X-ray absorptiometry (DEXA), bioelectrical impedance analysis (BIA), nuclear magnetic resonance (NMR), air displacement plethysmography, and doubly labeled isotope water, many of which are becoming more easily accessible in academic centers (47). Other methods to study adiposity include measuring waist and/or hip circumference, as markers of central obesity, and skinfold thickness, as a marker of subcutaneous or visceral adiposity (triceps, subscapular biceps, abdomen). However, the lack of accuracy and reproducibility of such measurements limits their use (47, 58). More accurate, cost-effective, and easily attainable and reproducible measures of adiposity should continue to be developed to improve the validity of these studies and should be considered an important limitation of studies of obesity in children and adults conducted thus far.

While the WHO and CDC have a clear definition of obesity from a macroscopic standpoint, on a cellular and physiologic level, obesity is an interplay of complex factors involving several organ systems. Importantly, although fat accumulation is reflective of the obese phenotype, a relative decrease in lean mass, which contributes to BMR, plays an important contribution to the development of obesity.

KEY MESSAGE 4

Emerging evidence suggests that in utero, postnatal, and early childhood metabolic environments affect adult basal metabolic rate and, in turn, later obesity risk through the process of metabolic programming, that needs further exploration.

The role of fat free mass in the conditioning of obesity

While measures of body weight and adiposity in childhood and adulthood are associated with one's perinatal metabolic environment, the role of fat-free mass and BMR in the conditioning and pathogenesis of obesity remains unclear.

It was initially hypothesized that birth weight, which contributes to obesity in adulthood, would be negatively associated with fat-free mass and adult BMR. However, several studies have shown contrary results (60–62). Weyer et al. showed that while birth weight is positively associated with both fat-free mass as well as 24-hour adult EE, it is negatively associated with sleeping metabolic rate (62). While the clinical significance of this finding with regards to the pathogenesis of obesity is unclear, more thorough investigation may be needed to assess the effects of intrauterine metabolic milieu on adult fat-free mass and BMR. Further, the use of birth weight itself as a proxy measure for intrauterine nutritional status, is highly contentious. Perhaps more appropriate, future studies will determine the association between postnatal factors such as breastfeeding and early infancy weight gain, and adult fat-free mass to gain a better understanding of the role of BMR in the pathogenesis of obesity.

Thus far we have (1) discussed the global impact of childhood obesity, (2) defined obesity and factors which contribute to its pathogenesis including central and peripheral regulation of appetite, adiposity and BMR, (3) introduced the concept of metabolic conditioning and its role in the pathogenesis of obesity, and (4) presented data that supports the phenomenon of programming, which show an association between *in utero*, postnatal and early infancy metabolic factors and long-term obesity risk. In the next section, we will discuss *how* early developmental metabolic conditioning contributes to adult obesity risk.

Mechanisms of metabolic conditioning of obesity and cardiovascular disease risk

The conditioning of obesity in response to maternal undernutrition vs maternal obesity

The concept of metabolic programming for obesity was first demonstrated preclinically in the 1960s, although the term "metabolic programming" would be coined a few decades later (46). Robert A. McCance, an English scientist, found that overfeeding rats during the early postnatal period increased their body size in adult life. A similar outcome was demonstrated in male infant baboons by Lewis et al, where feeding a nutrient-enriched formula, with 30% excess calories, resulted in increased mesenteric and omental fat depots at 5 years (46, 63).

The Dutch famine study linked the metabolic environment of the fetus and later obesity risk with the specific gestational period of maternal undernutrition. While human fetal adipose depots develop between 14-24 weeks of gestation, they undergo

significant expansion only in the third trimester, which is dependent on fat cell replication (64, 65). On the other hand, hypothalamic neurons develop by 15-18 weeks' gestation and hormone activity in the hypothalamic-pituitary-adrenal axis can be seen as early as 8-12 weeks' gestation and continues to mature in subsequent weeks (66). In the cohort exposed to poor nutrition in the third trimester, it is possible that the lack of maternal caloric intake restricted expansion of fetal adipose tissue, resulting in the programming of adipocytes with decreased propensity for replication, even in the presence of sufficient caloric intake. On the other hand, children exposed to nutrient deprivation in the first two trimesters, were programmed for increased responsiveness to caloric cues, cravings, or appetite due to insufficient energy substrate availability during the critical development period of the hypothalamus.

Malnutrition during the first two trimesters results in a "thrifty" phenotype, commonly known as the "predictive adaptive response". Such conditioning becomes maladaptive in the presence of sufficient or excess nutrition later in childhood, adolescence, or adulthood. It is this mismatch between developmental conditioning and paradoxical exposures later that ultimately lead to adverse health outcomes and the risk for non-communicable disease. When the environment changes, the conditioning becomes nonadaptive. We hypothesize that a more thorough understanding of the temporo-spatial progression of obesity from early development will aide in the discovery of therapeutic interventions to prevent or reverse these maladaptive processes.

The concept of mismatch has been studied more comprehensively in preclinical models (21, 67–69). Using a rat model, Vickers et al. showed that maternal undernutrition during pregnancy promotes obesity and metabolic syndrome in male and female offspring, that is worsened by high-fat diet (HFD) feeding in the postnatal period (68). Administering leptin in the early neonatal period of 3-13 days, a critical period characterized by a high degree of developmental plasticity, reversed this phenotype (68). Gluckman et al. showed that leptin treatment in this critical postnatal period modified the transcription and methylation of genes expressed in the liver that regulate whole-body energy homeostasis including peroxisomal proliferator-activated receptor alpha (*PPARα*); however, the metabolic "program" and direction of these changes depended on the initial perinatal environment (21). Using the same rat model of maternal undernutrition with postnatal hypercaloric nutrition, Vickers et al. also showed that IGF-1 treatment alleviated hyperphagia, obesity, hyperinsulinemia and hyperleptinemia in offspring from malnourished dams (69).

This concept of developmental mismatch, however, does not apply when the exposure remains consistent. While maternal *undernutrition* may condition a "thrifty phenotype" resulting in offspring who develop obesity with exposure to high caloric diet later in life, how does maternal *obesity* result in obese offspring? One could hypothesize that like the evolutionary anticipation of possible future undernutrition in offspring born to malnourished mothers, offspring born to obese mothers should also develop a "predictive adaptive response" for future *overnutrition* and thus have *reduced* propensity for adipocyte replication and *increased* levels of satiety with feeding. However, several epidemiological studies, discussed previously, have shown the contrary: maternal obesity *increases* adiposity in offspring and programs later obesity possibly through obesogenic modifications in appetite regulation and energy metabolism (35–37, 42, 43). Preclinical studies have demonstrated the effects of maternal obesity on offspring adiposity phenotype (70–73). For example, Chen et al. noted that HFD feeding in female rats resulted in increased body weight and adiposity, hyperlipidemia and glucose intolerance in male pups at postnatal day 20 (71). White et al. showed that rat offspring at 18 weeks of age from dams fed a HFD prior to pregnancy, weighed significantly more than offspring from dams fed a normal diet (74).

Hanson and Gluckman in their comprehensive review of the DOHaD hypothesis, argue that maternal undernutrition (an integral thread throughout human history) has been recognized, evolutionarily, as harmful for normal development thus requiring a predictive adaptive response in order to produce fertile, viable offspring (17). Maternal obesity, on the other hand, is a relatively newer "stress" which is not yet recognized in our collective epigenetic or genetic code as maladaptive to development, since it was vanishingly rare only 10 generations ago, and the majority of offspring from mothers with obesity are viable into adulthood and capable of reproduction (17). In contrast to maternal undernutrition, maternal obesity likely plays a *pathophysiological* role in the development of obesity in offspring by altering the metabolic milieu of intrauterine life (Figure 2). We submit that altered maternal levels of metabolic substrates and hormones in the setting of obesity, which contribute to maternal insulin resistance, oxidative stress and inflammation, cause perturbations in glucose and lipid homeostasis and feeding circuitry in the perinatal period, ultimately contributing to long-term obesity risk. These pathophysiological factors will be discussed in detail in the next section.

KEY MESSAGE 5

Altered maternal levels of metabolic substrates and hormones in obesity, which contribute to maternal insulin resistance, oxidative stress, and inflammation, resulting in perturbations in glucose and lipid homeostasis and feeding circuitry in the perinatal period, contributing to long-term obesity risk.

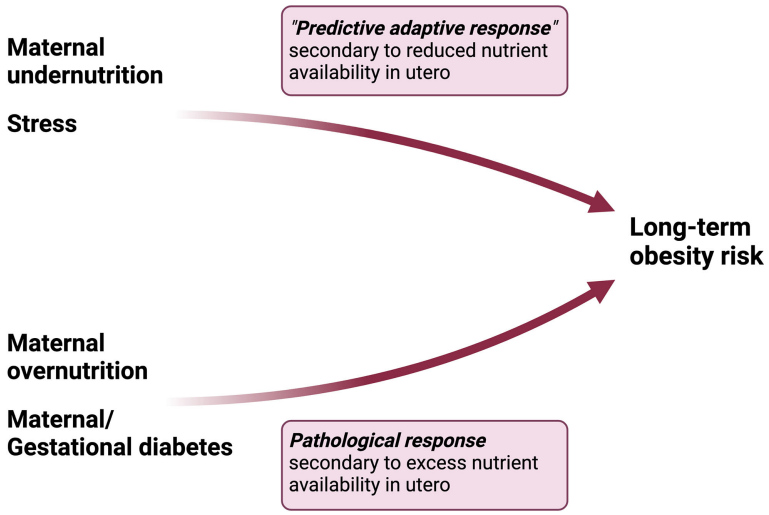


FIGURE 2
Differential pathways to obesity in response to maternal undernutrition vs overnutrition. Maternal undernutrition is hypothesized to condition obesity through a "predictive adaptive response," in which the fetus programs its homeostatic set points in anticipation of persistent caloric deficit. However, the adaptive response becomes maladaptive in the setting of a mismatch between the anticipated environment and paradoxical exposures later in life. On the other hand, maternal overnutrition activates a pathological response in the fetus characterized by dysregulation in hormonal pathways involved in energy homeostasis.

Maternal obesogenic factors that mediate the conditioning of obesity

Circulating metabolic substrates

Maternal obesity is associated with elevated levels of metabolic substrates including free fatty acids (FFAs), glucose and altered lipoprotein profile. FFAs and glucose can independently contribute to fetal overgrowth due to increased availability of substrates (75). Development of maternal insulin resistance and a proinflammatory state in the setting of hyperglycemia and hyperlipidemia may also contribute to an abnormal metabolic transition in early postnatal life (75). Further, exposure to elevated metabolic substrates may cause direct glucotoxic and lipotoxic effects on placental proteins, which in turn have been shown to contribute to oxidative stress, endoplasmic reticulum (ER) stress and proinflammatory pathways affecting overall placental function (76). In response to increased placental transport of glucose to the fetus, fetal hyperinsulinemia develops which is hypothesized to contribute to obesity conditioning in the offspring (77). Intrahypothalamic administration of insulin to rat pups during early development of central feeding circuitry increases body weight, impairs glucose tolerance, and promotes development of obesity and diabetes later in life (78), emphasizing its role in body weight regulation in addition to being a potent growth factor (79).

Circulating hormones

Insulin and leptin

Maternal obesity is associated with hyperinsulinemia, however maternal insulin does not cross the placenta (75, 80). While the pregnant state is naturally associated with peripheral insulin resistance to maximize glucose availability for the developing fetus, pregnant women with obesity have 50-60% higher postprandial insulin levels and are more glucose intolerant than those without obesity. Regardless, the placenta remains normosensitive to insulin in women with obesity, thus, increased placental insulin activation, along with leptin, has been shown to promote mTOR signaling leading to increased glucose and amino acid transport across the placental barrier and fetal overgrowth (81).

It is unclear which components of abnormal glucose homeostasis associated with maternal obesity – maternal hyperglycemia, hyperinsulinemia, or insulin resistance – contribute to the dysregulation of metabolic homeostasis in offspring and future obesity risk. As discussed before, the HAPO study showed that maternal hyperglycemia, in the absence of overt diabetes, increases frequency of LGA and fetal hyperinsulinemia (38), aligning with the concept that increased glucose availability and placental uptake contributes to fetal overgrowth (75). Although insulin does not cross the placental barrier, maternal hyperinsulinemia may exert effects on the

placenta allowing for increased fetal nutrient uptake. While difficult to isolate the effects of maternal insulin resistance without overt diabetes on outcomes in humans, using a heterozygous knockout mouse model for insulin receptor, Carmody et al. studied the effects of maternal insulin resistance with or without maternal HFD on progeny. Maternal insulin resistance alone did not affect body weight, body composition, glucose homeostasis or expression of hypothalamic neuropeptides that regulate feeding behavior, suggesting that the intersection of maternal insulin resistance with HFD feeding is required for the metabolic programming of obesity (80). Future clinical and preclinical studies are required to isolate the individual contributions of maternal hyperglycemia, hyperinsulinemia, and insulin resistance on perturbations in energy homeostasis and offspring obesity risk.

Leptin is produced by adipose tissue, and circulating levels in weight stable adults correspond to the degree of adiposity (82). At the placental level, leptin has been shown to increase system A amino acid transport activity and stimulates the release of proinflammatory cytokine IL-6, possibly contributing to abnormal placental function (75). In the hypothalamus, insulin and leptin modify expression of neuropeptides that regulate appetite and feeding behavior. Specifically, they increase expression of anorexigenic (appetite-suppressing) precursor of alpha melanocyte stimulating hormone (α–MSH) pro-opio melanocortin (POMC) and decrease expression of orexigenic (appetite-promoting) neuropeptide Y (NPY), overall functioning to suppress appetite (82). Thus, with worsening adiposity and hyperglycemia, increasing circulating levels of leptin and insulin aid to maintain the homeostatic set point by suppressing appetite. Ensuing central and peripheral resistance to these anorexigenic hormones both characterizes and contributes to obesity and metabolic syndrome. Although not extensively studied in models of metabolic programming, insulin and leptin resistance in offspring exposed to maternal HFD *in utero* may also contribute to deregulation in energy homeostasis (73, 83–85). In a rat model, Gupta et al. showed that maternal HFD feeding *in utero* increased expression of leptin long receptor (*ObRb*) and insulin receptor b-subunit (*Ir-β*) in the offspring. The downstream leptin and insulin signaling components including signal transducers and activators of transcription-3 (STAT3) and insulin receptor substrate-2 (*Irs2*), respectively, were decreased (73). Morris et al. found that maternal overnutrition during gestation in Sprague-Dawley rats significantly decreased expression of ObRb, without significant alteration in STAT3 expression (85). Isolated elevated developmental leptin exposure can alter adult weight homeostasis in mice. Using an inducible leptin mouse model, Skowronski et al. expressed leptin untethered to obesity and demonstrated that leptin overexpression during the weaning period (corresponding to the third trimester in human brain development) caused the mice to be overly suspectable to obesity when subsequently presented a highly palatable diet (86).

Preclinical studies have shown that HFD feeding in rats and mice promotes maternal obesity as well as maternal hyperinsulinemia and hyperleptinemia. Offspring of HFD-fed rats have elevated levels of insulin and leptin that alters expression of neuropeptide hormones Npy and Pomc and may exert long-term impact on the structure and function of hypothalamic feeding circuits (71–74, 85). Chen et al, for example, found that offspring of HFD-fed have increased mRNA expression of appetite-promoting *Npy* but lower expression of appetite-suppressing *Pomc* at 20 days of life, which may represent an adaptive response to obesity (71).

The contrasting role of leptin in maternal models of undernutrition vs HFD feeding may be perplexing at first glance. We previously discussed that leptin administration in pups exposed to undernutrition *in utero* reversed the obese phenotype. However, in offspring exposed to a HFD *in utero*, both leptin and insulin are initially elevated in early life. The leptin surge in the perinatal period is critical for the normal development of hypothalamic circuitry regulating energy homeostasis, appetite and feeding behaviors (87). Both the *timing* and *magnitude* of that surge appears to be critical and if either absent (as in states of maternal undernutrition) or inappropriately elevated over a prolonged period of time (as in states of maternal overnutrition), an obese, hyperphagic phenotype ensues (77, 88).

These studies suggest that insulin and leptin play critical roles in maintaining whole body energy homeostasis and perturbations in their expression during critical time periods of development may impair long-term regulation of appetite and feeding behaviors, ultimately contributing to an obese phenotype later in life.

Insulin-like growth factor

IGF-1 and IGF-2, which are predominantly synthesized in the liver, regulate fetal growth and development, as well as carbohydrate metabolism (75). Both IGF-1 and IGF-2 deficiency in mice demonstrate prenatal and postnatal growth failure (89, 90). In a human choriocarcinoma cell line, IGF-1 was shown to promote trophoblast proliferation and stimulate glucose and amino acid transport, and IGF-2 deletion reduced mouse placental growth and passive nutrient delivery, suggesting key roles for IGFs in promoting fetal overgrowth (75, 90, 91).

Adiponectin

Adiponectin inhibits insulin signaling in trophoblasts. Maternal obesity is associated with *low* levels of adiponectin, which is thought to promote placental insulin signaling, fetal nutrient transport and in turn fetal overgrowth (75). Adiponectin supplementation in pregnant mice normalizes maternal insulin sensitivity, placental insulin/mTORC1 signaling, nutrient transport and fetal growth, despite minimal changes in maternal visceral adiposity (92). A long-term study in the same mouse model showed *in utero* adiponectin treatment ameliorated the

obesogenic phenotype in 14-week-old adult male offspring, with significant reductions in body weight, fat mass and normalization of insulin sensitivity and hepatic steatosis (93).

Placental signaling contributes to the effects of maternal obesity on offspring

Maternal circulating levels of metabolic substrates and hormones, as discussed above, significantly impact placental function, likely mediated by mTOR signaling on the placenta by activating amino acid and glucose transport as well as mitochondrial biogenesis and protein synthesis, ultimately contributing to excess fetal nutrient delivery with increased risk for fetal overgrowth in obese women (75, 76).

Maternal obesogenic factors could be conveyed into long-term obesity risk in offspring *via* epigenetic modifications

Several maternal obesogenic factors contribute to obesity in offspring, including glucose, FFAs, insulin, leptin, IGF-1 and adiponectin. Together, they promote maternal insulin resistance, hyperglycemia, hyperleptinemia, oxidative stress and inflammation *in utero* which perturb the development of central feeding circuitry and energy homeostasis during the perinatal transition. The ensuing fetal hyperleptinemia, insulinemia and transient neonatal hypoglycemia increase the risk for fetal overgrowth and LGA (Figure 3). But how does this short-term dysregulation in energy homeostasis during the perinatal period affect long-term risk for obesity? We posit that the *in utero* environment caused by maternal obesity, and the concomitant short-term perinatal aberrations in energy

KEY MESSAGE 6

The *in utero* environment of maternal obesity, and the concomitant short-term aberrations in energy homeostasis during the perinatal period, translate to long-term obesity risk through both central and peripheral epigenetic modifications that regulate energy homeostatic set points.

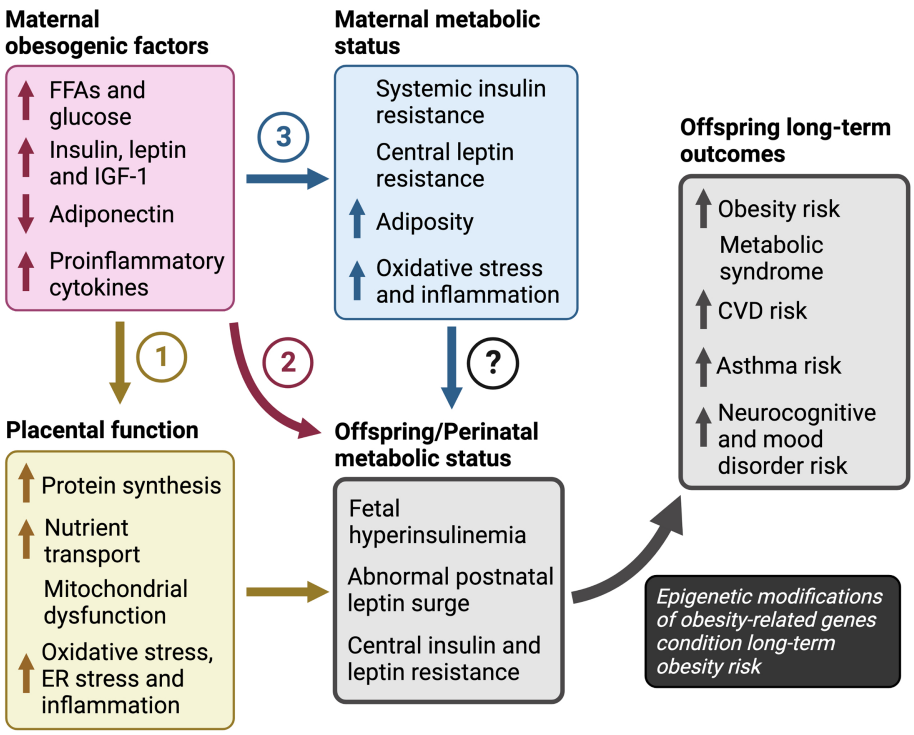


FIGURE 3
Pathways to obesity in children in response to maternal overnutrition The pathways of maternal obesogenic factors towards long-term obesity risk include: (1) dysregulation of placental function in which excess nutrients promote placental protein synthesis, mitochondrial dysfunction and increased nutrient transport, (2) increased nutrient availability, that may have a direct effect on fetal growth and development independent of changes in placental function, and (3) altered maternal sensitivity to hormones leptin and insulin. However, the roles of maternal insulin and leptin resistance, independent of maternal hyper-insulinemia or -leptinemia, are unclear and further investigation is required to determine their impact on the perinatal metabolic transition to life and outcomes in offspring. Finally, we propose that increased risk for obesity, metabolic syndrome, and atherosclerotic cardiovascular disease, depends on an abnormal metabolic status during the perinatal period in response to maternal factors. CVD; cardiovascular disease, ER; endoplasmic reticulum, FFAs; free fatty acids, IGF-1; insulin-like growth factor-1.

homeostasis, translate to long-term obesity risk through epigenetic modifications in both central and peripheral markers that regulate energy homeostatic set points.

Epigenetics is the study of heritable changes in gene expression secondary to chromatin modifications such as DNA methylation, as well as histone and miRNA modifications, without an actual change in DNA sequence (94). DNA methylation contributes to gene expression and is considered one of the primary mechanisms of cellular memory. Specifically, methylation of the 5' position of cytosine by DNA methyltransferases to form 5-methylcytosine (5mC) impedes binding of transcription factors, which ultimately represses transcription of the gene. On the other hand, hypermethylation of CpG islands can promote transcriptional activation (95). Such epigenetic changes to gene expression are critical for normal cell differentiation and development and maturation of organ systems early in life (96, 97). In response to an abnormal metabolic environment *in utero* and in the perinatal period, alterations in epigenetic modifications of obesity-related genes can have lasting effects on gene expression, ultimately contributing to obesity risk (21, 98–100).

Many epigenetic modifications have been identified to play a role in the pathogenesis of obesity. For example, altered methylation patterns have been seen in genes that regulate systemic energy homeostasis such as *HIF3A* (hypoxia-inducible factor 3A), *LEP* (leptin) and *ADP* (adiponectin), as well as *POMC*, *PPARα*, *PGC1α*, *IGF-2*, *IRS-1*, *IL-6* (other genes involved in whole-body lipid and carbohydrate metabolism, insulin signaling and inflammation). Hypermethylation patterns have also been identified in genes related to the circadian rhythm –*CLOCK*, *BMAL1* (in leukocytes and adipocytes). Histone modifications of genes related to adipogenesis and adipocyte differentiation include *PREF-1*, *C/EBPα/β*, *PPARγ* and *aP2*. Finally, expression of certain miRNAs are associated with increased fat storage in adipocytes and may upregulate adipogenesis in obese states. For example, miR-26b promotes proliferation of preadipocytes and expression of *PPARγ* (94, 95). Epigenetic changes can be stable in an organism from one cell to its progeny through cellular division and mitosis, but also across generations and, are thus, considered inheritable. For example, the DNA methylation status of the Agouti focus, responsible in determining fur coloration, is passed on transgenerationally through maternal inheritance (94, 95).

An elegant study performed by Masuyama et al. showed that exposure to a HFD *in utero* results in an obese phenotype in offspring over three generations through the maternal line along with elevated plasma and tissue level expression of leptin and reduction in adiponectin (101). The adiponectin level was driven by increased acetylation and decreased methylation of H3K9 at the promoter region of adiponectin gene in offspring of HFD-fed mice. In contrast, monomethyl H4K20 levels were increased in the *Lep* promoter region of these same offspring. Reversal of the phenotype and epigenome was only possible by chow fed diet feeding in three subsequent generations (101).

Obesity conditioning occurs primarily during critical developmental periods

The critical periods of metabolic programming extend from fetal, perinatal, as well as early postnatal stages (17, 102). During the fetal stage and metabolic transition to postnatal life, there is a high degree of plasticity and epigenetic modifications have been demonstrated in key regulatory genes which affect the development of the hypothalamus and its peripheral connections (66, 94, 95). Further in mammals, significant structural and functional development of adipose tissue, liver and gut occurs after birth. Thus, the postnatal period serves as an important continuation of the fetal phase (102).

For example, in rats and mice, the hypothalamic-pituitary axis is immature at the time of birth and undergoes maturation in the first 2 weeks of life (66, 87, 94, 103). Variations in DNA methylation between hypothalamic and non-neuronal cells are established in the postnatal period (104). In humans, DNA methylation of prefrontal cortex involved in appetite control, satiation, and food craving, increases steadily overtime, exhibiting prolonged postnatal maturation (94, 102–104).

In rodents, white adipose tissue (WAT) is minimal at the time of birth and undergoes postnatal maturation (102). Epididymal WAT is composed of progenitor cells that lack the capacity to differentiate from postnatal days 1 to 4, following which dividing cells are observed. In humans, although WAT is present from the second trimester of gestation, significant growth occurs from birth to 6 weeks of age, during which time body fat doubles from approximately 10% to 20%. After birth, both adipocyte size and number increases accounting for the large adipose tissue expansion (64, 65, 94, 102).

Finally, the liver acquires its metabolic functions and undergoes most of its methylation postnatally (102). Indeed, regulatory regions, including promoters and enhancers of genes involved in lipid and glucose metabolism in the liver, undergo programmed active DNA demethylation in a time-dependent manner after birth. This process has been shown to be partly

KEY MESSAGE 7

Epigenetic changes are not exclusive to the perinatal period; these changes may occur during childhood and beyond in response to various behavioral and environmental factors.

dependent on the activity of ten-eleven translocation dioxygenases, otherwise known as "TET" enzymes (*Tet2* and *Tet3*), which promote DNA demethylation (105).

Despite the high degree of plasticity during the perinatal period, several other factors may result in epigenetic modifications that alter the long-term ability to mobilize fat beyond these critical developmental periods. These include environmental toxins known as "obesogens," changes in gut microbiota and excess dietary intake during early childhood (94, 102). We argue that epigenetic changes are not exclusive to the perinatal period and that these changes may occur along any time in response to various behavioral and environmental factors.

Epigenetic modifications, in response to early environmental exposures, are known to persist into adulthood. For example, the cohort of children from the Dutch Hunger Winter exposed to maternal caloric restriction in the first trimester of gestation had less DNA methylation of the IGF2 gene compared with their unexposed siblings six decades later (94, 106). However, epigenetic changes are not confined by longevity or permanence. Indeed, contrary to the initial understanding of epigenetic mechanisms in the programming of obesity, we now know that these changes are highly dynamic and reversible.

If one's metabolic milieu is altered in favor of weight loss, the epigenetic alterations in DNA methylation may be reversible (94). As discussed previously, the methylation patterns of the promoter regions of leptin and adiponectin genes in mice exposed to a HFD *in utero*, were reversed in three generations upon transitioning to a normal chow diet (101). Further, dietary changes, exercise, and surgical interventions may reverse epigenetic changes. For example, maternal bariatric surgery and associated weight loss improved the metabolic profile of children during their adolescent period compared to their siblings born prior to weight loss surgery, with decreased birth weight, obesity incidence, blood pressure and adiposity, as well as improved insulin sensitivity and lipid profile (107, 108). Siblings born after weight loss surgery exhibited differences in epigenetic modifications in genes related to glucose homeostasis, insulin resistance, inflammation, and vascular disease (99).

The discovery of ten-eleven translocation dioxygenases enzymes, which reverse DNA methylation, has made the mechanistic underpinnings of methylation reversal a possibility (102, 105). "Ten-Eleven Translocation dioxygenases" or TETs convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), that can then undergo serial oxidation resulting in active DNA demethylation *via* removal and substitution with unmethylated cytosine *via* base excision repair. Indeed, 5hmC is detected in several different cell types near regulatory regions (promoters/enhancers) and positively correlates with gene expression (105).

KEY MESSAGE 8

The epigenetic pathways that contribute to the conditioning of obesity are highly dynamic and reversible. Currently known pathways include variations in methylation status of obesity-related genes, which occur in response to maternal, postnatal, and early childhood nutrition, and have been shown to be associated with differences in adiposity and body composition in infancy and beyond.

Epigenetic modifications during different time periods of development affect later obesity risk

Prenatal period

The currently known epigenetic pathways that contribute to the conditioning of obesity include variations in methylation status of obesity-related genes associated with differences in adiposity and body composition in infancy and beyond. An elegant longitudinal clinical study measured the methylation status of CpGs in the promoters of candidate genes from umbilical cord tissue of neonates obtained at birth along with clinical measures of adiposity at 9 years of age (98). Higher methylation level of retinoid X receptor-alpha (*RXRα*) at birth was strongly correlated with adiposity in childhood. RXRα is a transcription factor that regulates expression of genes including *PPARα* involved in whole-body lipid and glucose metabolism, insulin sensitivity and adipogenesis, suggesting an association between early life epigenetic modifications and later obesity risk.

Maternal weight loss surgery is known to reduce the risk of offspring obesity without large maternal weight loss (100, 108). Children born to mothers who underwent biliopancreatic diversion prior to pregnancy had significantly reduced odds of developing obesity from 2-18 years of age, even if the mothers remained overweight after surgery. Altered methylation patterns and expression of genes involved in glucose metabolism, insulin sensitivity, immune and inflammatory-related functions was noted in the cord blood after the surgery (99). We hypothesize that overall improved nutritional status during gestation with concomitant decreased maternal insulin resistance, hyperglycemia, hyperlipidemia, and hyperinsulinemia contribute to a healthy *in utero* environment translating to better offspring outcomes.

Preclinical studies have also shown that variations in the methylation status of obesity-related genes, in response to abnormal maternal diet *in utero*, are associated with weight gain and obesity in the offspring (109–112). Jousse et al. found that maternal undernutrition in mice during gestation and

lactation produces hyperphagic offspring with higher food intake throughout life (112). Mice exposed to maternal undernutrition showed persistent changes in serum levels, mRNA expression and methylation patterns of leptin. Fetal malnutrition results in lower leptin levels with corresponding decreased adipose-specific mRNA expression, as well as long-term changes in methylation of the leptin promoter (112).

Lillycrop et al. found that maternal protein restriction in female rats during pregnancy decreased methylation of hepatic glucocorticoid receptor (*Gr*) and *Pparα*, and increased expression of mRNA transcripts in pups at 6 days of life (109). These epigenetic changes persisted after weaning (while pups were no longer on decreased protein intake). A follow-up study showed persistence of changes in methylation of *Pparα* at 34 and 80 days, corresponding to the adolescent and adult periods respectively (113). Further, folic acid supplementation either during gestation or in adolescence prevented or reversed these epigenetic modifications respectively (109, 114). As DNA methylation requires S-adenosylmethionine as a methyl donor, which is dependent upon serine, glycine, folate, and vitamin B12, epigenetic modifications may be altered by availability of these amino acids and micronutrients *in utero*. These data suggest that maternal nutritional status alters the fetal epigenome and the expression of energy homeostasis genes throughout lifespan (109, 110, 113).

Gluckman et al. showed that maternal protein restriction and post-weaning hypercaloric nutrition followed by leptin treatment from days 3 to 10 of life, reversed the obese phenotype in offspring described previously, and resulted in stable and long-term changes in the methylation patterns and expression of various markers later in life (day 170) (21). Methylation of the *Pparα* promoter was either (1) decreased by leptin treatment in offspring exposed to maternal undernutrition *in utero*, corresponding to enhanced transcript expression, or (2) increased by leptin treatment in offspring without exposure, corresponding to decreased transcript expression. Offspring transitioned to a HFD post-weaning obscured the effects of leptin on *Pparα* expression. Methylation of the *Gr* promoter, on the other hand, was elevated by leptin in maternally well-nourished offspring but unaffected in offspring exposed to maternal protein restriction *in utero*. Finally, leptin also had bidirectional effects on the expression of hepatic 11β-hydroxysteroid dehydrogenase type 2 (*11β-Hsd2*), which is an enzyme that inactivates glucocorticoids. While neonatal leptin treatment decreased 11β-Hsd2 in offspring from undernourished mothers, it increased 11β-Hsd2 in offspring from well-nourished mothers (21). Using a rat model of overfeeding in which mothers developed obesity preconception, Borengasser et al. showed altered methylation patterns and corresponding expression of adipogenic genes in white adipose tissue of offspring (115). Offspring of obese dams showed decreased methylation of *C/ebp-β*, *Zfp324* and *Pparγ*, which are involved in the development of adipose tissue, as well as corresponding increased expression of their downstream targets, ultimately resulting in enhanced adipogenic differentiation and greater adiposity in offspring. Fernandez-Twinn et al. showed that HFD-feeding during gestation in a mouse model produced offspring with unchanged body composition, energy expenditure, activity levels or glucose tolerance at 8 weeks of age; however, fasting insulin levels were significantly higher compared to mice from chow-fed mothers (116). Interestingly, protein expression of Irβ was downregulated in epididymal fat in offspring from HFD-fed mothers, in addition to further downstream markers of insulin signaling including Irs-1, Pi3k, Akt1 and Akt2. Further miR-126 was found to regulate the expression of Irs-1, with a potential role in the epigenetic programming of insulin resistance.

In a genetic mouse model of maternal obesity and diabetes, offspring at 12 weeks of age displayed elevated liver lipid contents, as well as elevated serum leptin (117). Offspring exposed to maternal obesity and diabetes and subsequently challenged with a western-style diet gained significantly more weight and demonstrated glucose intolerance and insulin resistance, compared to offspring from control mice. These phenotypic changes corresponded to variations in hepatic gene expression, specifically those regulating mitochondrial activity such as *Atpase6* and *Cytb*. The epigenetic alterations were not restricted to genes involved in energy homeostasis but were widespread relating to embryonic and tissue/organ development.

In another mouse model of diet-induced maternal obesity, significant alterations in histone acetylation and methylation of hepatic transcripts involved in lipid metabolism were observed in offspring exposed to maternal overnutrition during gestation, which persisted into adulthood (5 weeks of age) (118). Specifically, *Sirt1*, which encodes a histone deacetylase with functions in lipid metabolism and obesity (serving as a marker for cellular energy levels), was significantly reduced in offspring exposed to maternal obesity and persisted into adulthood. Further, differential histone modifications were also seen in *Pparα*, *Pparγ*, *Rora* and *Rxrα* in the offspring from obese mothers that were not sustained at 5 weeks.

Postnatal period

Several human studies show a differential methylation pattern of *LEP* promoter in breast fed infants (102, 119–122). A cross-sectional study in 120 Dutch children found that longer breastfeeding duration reduced the average CpG methylation of *LEP* promoter in peripheral blood and was negatively associated with plasma leptin and infant BMI at an average age of 1.4 years (120). In a prospective cohort study, Pauwels et al. noted that every extra month of breastfeeding was associated with a 0.217% increase in *RXRα* CpG2 methylation (95% CI: 0.103, 0.330; $p < 0.001$). Similarly, increased levels of CpG3 methylation of *LEP* promoter was seen with 7-9 months of breastfeeding (6.1%) compared to 1-3 months (4.3%; $p = 0.007$) that did not persist at 10-12 months. However, infant weight and BMI-for-age at 1

year was significantly lower in children who were breastfed for 10-12 months, suggesting that breastfeeding-mediated epigenetic modifications in *RXRα* and *LEP* may have a role in childhood obesity. In a longer prospective cohort study, Sherwood et al. demonstrated that *LEP* methylation at four CpG sites at 10 years of age was associated with exclusive breastfeeding (121).

Rodent models also show an association between postnatal feeding and differential methylation patterns of obesity-related genes. Rats overnourished during the breastfeeding period by reduction in litter size have increased growth rate, hyperinsulinemia and hyperleptinemia, and overweight throughout life with central leptin and insulin resistance in adulthood (123–125). In another rat model of postnatal overnutrition, Mahmood et al. demonstrated hyperinsulinemia persistent in the post-weaning period in newborn rats fed a high-carbohydrate milk formula (126–128). These animals developed hyperphagic obesity in adult life on a standard chow diet along with associated epigenetic alterations in *Npy* and *Pomc* (128). While *Npy* mRNA expression was increased in the hypothalami of both 16- and 100-day old high-carb fed rats, with increased methylation of specific CpG positions and histone acetylation, *Pomc* expression was reduced, accompanied by a decrease in histone acetylation.

Postnatal exposures can reverse the gestational programming of obesity

Several studies suggest that postnatal exposures may have a greater impact compared to *in utero* exposures (100, 129). Using a rat model of diet-induced obesity in which Sprague-Dawley rats were selectively bred for diet-induced obesity and resistance, Gorski et al, found that obesity-prone pups fostered to lean dams at birth remained obese in spite of reduction in food intake and gradual improvement in insulin sensitivity compared to obesity-prone pups fostered to obese dams (129). They noted an upregulation in hypothalamic expression of leptin receptor (*Lepr-b*) and insulin receptor (*Ir*), suggesting improved leptin sensitivity. On the other hand, lean pups fostered to obese dams during lactation develop obesity and insulin resistance when fed high-caloric diet, with a decrease in expression of *Lepr-b* and *Insr*.

Obesity programming may also have a role in the development of the neuronal projections relevant to energy homeostasis. In a study of offspring born to dams fed a HFD during lactation, Vogt et al. observed no differences in the expression of *Pomc*, *Npy* and agouti-related peptide (*AgRP*). However, they noted impaired *Pomc*- and *Agrp*- neuronal projections to target sites within the hypothalamus (130).

Finally, as discussed previously, rat models of maternal food restriction during pregnancy cause intrauterine growth restriction in offspring who later show rapid catch-up growth with adult obesity and metabolic syndrome (21, 109, 111–113). This phenotype can be prevented through continued maternal undernutrition during the lactation period, where availability of nutrients is also limited to the newborn (131, 132). Tosh et al. showed that maternal food restriction during both pregnancy and lactation decreased hepatic mRNA and protein expression of *Igf1*, that persisted at 9 months of age (132). Transitioning the dams to a regular diet during the lactation period rescued the expression of *Igf1* in the adult offspring, both in transcript expression and serum levels.

Discussion

Future directions to prevent the evolution of obesity from conception

Summary and additional remarks

Early exposure to maternal obesogenic factors from conception "programs" long-term obesity risk through epigenetic modifications in genes involved in energy homeostasis. While there are several risk factors for childhood obesity, maternal overnutrition during pregnancy has consistently been shown to alter the fetal epigenome. The effects of epigenetic alterations often persist into adulthood, contributing to the long-term obesity risk in offspring. Observational studies suggest that maternal diet, rather than maternal weight in isolation, may be the driver of the epigenetic modifications during the perinatal period. Previously described study of siblings born before and after maternal bariatric surgery found that significant weight reduction or normalization of BMI was not necessary to reduce the risk of obesity in offspring, although the systemic effects of bariatric surgery on programming of feeding behavior and whole-body metabolism in offspring remain unclear (108). Further, the Dutch famine study and studies on rodent models have shown that maternal undernutrition also predisposes to longer-term obesity in offspring (21, 82, 106, 109, 111–113). Thus, we hypothesize that the *in utero* metabolic environment, which is significantly influenced by maternal diet, determines the predisposition to obesity in offspring.

In this review, we have described several markers that have been shown to be susceptible to epigenetic modifications during critical developmental periods to promote an obesogenic phenotype during lifespan. These obesity-related genes are primarily expressed in the hypothalamus, liver, adipose tissue, and pancreas and serve as potential therapeutic targets for both the treatment and prevention of obesity, summarized in Table 1.

A preclinical study on the long-term effects of epigenetic changes in offspring in response to maternal undernutrition showed that some epigenetic modifications do not persist into adulthood (121). While it is unclear why some genes are more susceptible to reversal of epigenetic modifications compared to

TABLE 1 Tissue-specific pathways and genes involved in the metabolic programming of obesity.

Tissue	Obesogenic pathway	Target gene(s)	References (preclinical)	References (clinical)
Hypothalamus	Central feeding dysregulation	*NPY*	(71, 73, 84, 124, 127)	
		POMC	(71, 73, 84, 127, 129)*	
		AgRP	(73, 129)*	
		MC4R	(84)	
	Central leptin resistance	ObRb, Stat-3	(73, 84), 122)**	
	Central insulin resistance	*IR-β, STAT-3 IRS-2*	(73, 123)**	
Adipose Tissue	Adipogenesis	*C/EBP-β*	(114)	
		PPARγ	(114)	
	Peripheral insulin resistance	*IR-β, IRS-1, PI3K, AKT1/2*	(115)	
		ADP	(100)	
	Abnormal postnatal leptin surge	*LEP*	(21, 67, 68)	
	Persistent hyperleptinemia	*LEP*	(100)	(119, 120, 121)
Pancreas	Fetal hyperinsulinemia	*INS*	(72, 78)	
Liver	Lipid and glucose metabolic dysregulation	*PPARα*	(21, 108, 110, 112, 113, 117)	
		RXRα	(117)	(97, 121)
		SIRT1	(117)	
		GR	(21, 108, 110, 113)	
	Abnormal growth and development	*IGF1*	(69)	
		IGF2	(105)	

Several markers that regulate energy homeostasis have been found to contribute to the epigenetic programming of obesity. Here, we have included data primarily from animal models, which have identified changes in tissue-specific (1) epigenetic regulation and/or (2) mRNA/protein expression of these genes in response to either maternal undernutrition or overnutrition during the prenatal and/or postnatal periods. We have further stratified these markers into tissue-specific obesogenic pathways. The clinical data referenced, notably, did not investigate tissue-level changes as the human samples obtained were of blood, buccal swab or placental tissue. Nonetheless, they too were stratified into tissue-specific obesogenic pathways based on the known functional role of the marker and interpreting the results of the clinical study within the context of preclinical data referenced here investigating the same marker.
*This study did not show changes in expression or methylation patterns of POMC or AgRP but rather observed altered neuronal projections to target hypothalamic sites in response to postnatal maternal HFD feeding.
**Central leptin or insulin resistance was evaluated in these studies by measuring the firing rate of arcuate neurons in response to exogenous leptin or insulin, respectively, in rats postnatally overnourished, not by measuring the expression or methylation patterns of downstream signaling molecules.

others, we argue that the *effects* of those initial epigenetic alterations likely contribute to the cumulative risk for obesity throughout life. Epigenetic alterations sustained during early critical developmental time periods can be considered as the "first-hit" (of many) in the pathogenesis of obesity. While, in the setting of maternal overnutrition, hypothalamic circuitry has been re-programmed towards lower levels of satiety and increased appetite, peripheral metabolism has been conditioned towards increased lipid storage and insulin resistance. Thus, we argue that the homeostatic set points which are established in early life initiate the temporospatial evolution of obesity and contribute to cumulative risk of metabolic syndrome, even if some epigenetic modifications which conditioned those set points may not persist. The mechanisms behind this hypothesis are unclear, and further investigation is required to understand the factors mediating the changes caused by these epigenetic modifications throughout lifespan.

Transgenerational transfer of metabolic risk

We have outlined evidence from human and animal studies on the metabolic risk caused by intrauterine and maternal exposures (F0 generation) to the offspring (F1 generation). The transmission of this risk to the subsequent generations resulting in transgenerational risk of metabolic disease into F2/F3 generation and beyond is possible. Epidemiological evidence suggests that exposure to maternal obesity and T2D results in higher prevalence of obesity, T2D (133, 134) and cardiometabolic risk in adult children (135), that may potentially remain in subsequent generation(s). Animal studies, that allow isolation of the gestational and the postnatal environment have established that the transmission of the risks conferred by maternal/gestational exposures may persist for up to 4 generations through both maternal and paternal germlines (136, 137). Evidence for such transmission is available in many animal species by environmental exposures. As an example, sex-specific inheritance of impact of high fat diet induced changes was demonstrated in females in F3 generation *via* paternal transmission in mice (138) that could be abolished by normal diet for three generations (101). Many similar examples are available in the review by King et al. (136) and Mohajer et al. (137) emphasizing the need and urgency of interrupting the cycle of transgenerational transfer of metabolic risk.

Reversing the epigenetic modifications which "program" obesity

We previously described both clinical and preclinical studies that showed a reversal of epigenetic modifications of obesity-related genes with improvement in metabolic status *in utero* or in later life (94, 99, 101). The discovery of TET enzymes made the mechanistic reversal of DNA methylation a possibility, however, how this process is activated, and under what conditions, remains unknown.

Dietary supplementation with folic acid has been shown to modulate the epigenetic modifications by altering availability of substrates for methylation of DNA, thus potentially reversing the epigenetic alterations of obesity-related genes (109). Natural compounds from dietary sources such as quercetin, curcumin, genistein, resveratrol and lycopene, may act as epigenetic modifiers (139). These compounds have been studied extensively in cancer biology, as they simultaneously possess anti-inflammatory, antioxidant and anticancer properties and may have a potential benefit in targeting the conditioning of obesity (140, 141).

Cancer and immunology research have further provided data for the use of "epigenetic drugs" (141, 142). Certain classes of epigenetic modifiers, such as histone deacetylase inhibitors (HDACi), histone acetyltransferase inhibitors (HATi), DNA methyltransferase inhibitors (DNMTis), histone demtheylating inhibitors (HDMis) and sirtuin-activating compounds (STACs), are currently being used for the treatment of cancer, urea cycle disorders, epilepsy, and hypertension. It is possible that these or other similar drugs may have a potential for the treatment and prevention of metabolic diseases such as obesity and diabetes. Early studies of synthetic and natural compounds including resveratrol (STAC), curcumin (HATi), hydralazine (DNMTi), valproic acid (HDAi) and sodium phenylbutyrate (HDAi) in obesity and diabetes are promising (140).

Limitations of epigenetic studies

Evaluating a cause-and-effect relationship between early life epigenetic modifications in response to maternal exposures and later obesity risk in *humans* is challenging for two primary reasons. First, most clinical studies investigating the epigenetic programming of obesity use blood, easy-to-obtain epithelial (i.e., buccal swabs) or placental samples (i.e., cord blood or tissue) to measure epigenetic modifications during the perinatal period and later in childhood. There is, for obvious reasons, limited access to central and peripheral tissues including the hypothalamus, adipose tissue, liver, and skeletal muscle which would otherwise help to more thoroughly understand the functional roles of obesity-related genes that undergo epigenetic alterations during critical time periods of development and condition obesity. Discovery of circulating epigenetic markers that more accurately reflect tissue-specific changes will be important moving forward in this field.

The second limitation of both preclinical and clinical studies investigating the role of epigenetics in the conditioning of obesity is simply the vast number of epigenetic modifications possible for one gene, which synergistically affect its expression. In other words, while most studies discussed in this review have focused on measuring differential methylation patterns of gene promoters or CpG islands, several other DNA and histone modifications shape the expression patterns of genes including histone acetylation and non-coding RNAs (i.e. microRNAs). While it is time-consuming and challenging to measure multiple epigenetic modifications, it is also unclear whether long-term expression of genes can be extrapolated based on single or few epigenetic changes that can be currently studied.

Lifestyle modifications remain vital in the prevention and treatment of childhood obesity

This and other prior reviews regarding the epigenetic conditioning of obesity discuss data on the contribution of *in utero* exposures on long-term obesity risk due to the abnormal "programming" of energy homeostatic set points likely making it more challenging to initiate and maintain weight loss. We hypothesize that lifestyle modifications may lead to better outcomes when applied earlier in life during periods of developmental plasticity. Preclinical studies suggest that improved nutrition during lactation can reverse and/or prevent the consequences of poor maternal diet *in utero*, although how maternal diet and exposures during postnatal feeding affect breastmilk content requires further investigation. Nevertheless, although lifestyle modifications in adults are not successful in promoting long-term maintenance of weight loss, we hypothesize that maternal lifestyle modifications either before pregnancy, during gestation and/or in the postnatal period, will improve long-term outcomes *in children* and reduce their risk for obesity in later life.

Further, although the perinatal period likely has the highest degree of central and peripheral plasticity; central feeding circuitry, peripheral innervations and metabolically active tissues are still susceptible to epigenetic modifications, and the consequences of *in utero* exposures may likely be reversed when lifestyle modifications are applied during childhood and even the adolescent period.

Proven to be more efficacious and successful than lifestyle modifications, maternal bariatric surgery prior to pregnancy has been shown to reduce long-term obesity risk in children; however, is typically only performed in patients with significant obesity (BMI ≥ 40 or BMI ≥ 35 in patients with significant comorbidities) (108, 143). Although less efficacious than bariatric surgery, several pharmaceutical interventions have been approved by the Food and Drug Administration (FDA) for the treatment of obesity (143–148). Of note, almost all current drug therapies *are more efficacious* in weight loss when combined with lifestyle modifications. Treatments such as GLP-1 receptor agonists, with or without other peptides, significantly improve overall metabolic status by

increasing insulin sensitivity and modifying absorption of carbohydrates (149). As discussed before, improvement in maternal metabolic status appears to be more important than reduction of maternal adiposity alone to reduce long-term risk for obesity in children, although, clinically, adiposity and insulin resistance are often interdependent especially in the setting of maternal overnutrition.

We hypothesize that treatments targeting adiposity and insulin resistance simultaneously, in patients with or without overt diabetes, may be efficacious in both treating obesity and reducing offspring risk for obesity and diabetes. Increasing number of drugs, including GLP-1 receptor analogues, have been approved for treatment of obesity and diabetes in youth 12 years and older. Further, targeted drug therapy for specific genetic variants involved in the leptin melanocortin pathway are approved for children 6 years and older; ongoing clinical trials may expand this armamentarium in the future (150).

Clinical practice recommendations to reduce long-term risk of obesity in children

Based on data presented in this review, we recommend mothers with obesity to incorporate lifestyle modifications including consuming a well-balanced diet and physical activity prior to conception, during gestation (within reasonable limits) as well as during lactation to reduce long-term obesity risk in children.

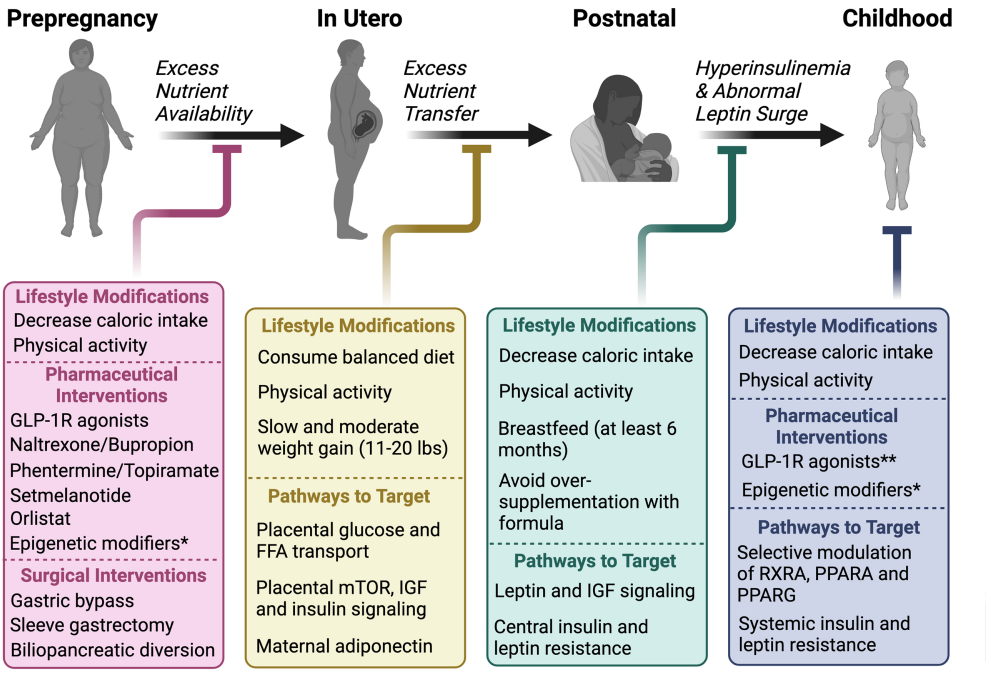


FIGURE 4
Time-dependent interventions to prevent the temporospatial evolution of obesity from prepregnancy to childhood Maternal lifestyle modifications, despite lack of effectiveness in long-term weight loss maintenance in adults, remain critical for the prevention of obesity in children. Excess nutrient availability, which concomitantly leads to an over-nourished *in utero* environment, is the initial insult in maternal obesity that predisposes children to obesity. Lifestyle modifications including a reduction in caloric intake and physical activity, as well as surgical and pharmaceutical interventions are available to ameliorate excess nutrient availability to a potential fetus. Although weight loss is not recommended while pregnant, methods to target excess nutrient transfer to the fetus include consuming a balanced diet and maintaining a moderate level of physical activity with slow weight gain (a maximum of 11-20 lbs is recommended by the NIH for pregnant women with obesity). In addition, research efforts should be focused on targeting pathways that promote nutrient transport to the fetus including energy substrate transporters, as well as mTOR, IGF and insulin signaling. Maternal adiponectin treatment in rodent models has been shown to improve metabolic status in offspring and is another possible therapeutic option in humans during this period. After birth, maternal lifestyle modifications again are critical although how these affect breastmilk content remains unclear. Nonetheless, breastfeeding for at least 6 months has been shown to reduce risk for obesity in children. Further, avoiding overfeeding and rapid weight gain during infancy is critical in reducing long-term risk for metabolic syndrome. Finally, in children with obesity, current interventions remain limited. Based on preclinical and clinical data discussed, we hypothesize that obesity treatments, which may help to reverse the epigenetic programming of obesity, may be more effective during childhood vs adulthood as obesity-related genes and signaling pathways regulating central feeding circuitry and basal metabolism are likely more susceptible to reversal of epigenetic alterations during this time. In addition to epigenetic modifiers, other pathways to target include systemic insulin and leptin, as well as RXRA, PPARA and PPARG signaling which regulate feeding behavior, as well as systemic lipid and glucose homeostasis, respectively.*Epigenetic modifiers (both natural and synthetic compounds) are not currently approved for the treatment or prevention of obesity but have excellent therapeutic potential based on data discussed in this review.**Several drugs are approved for use in youth ≥ 12 years of age and others in clinical trials.

Rapid weight gain during early infancy has been shown to significantly increase the risk for obesity later in life. We propose that breastfeeding should be encouraged in all infants, especially those of mothers with obesity and formula supplementation should be limited in breastfed infants to reduce risk of overfeeding. Further, based on the study by Huh et al. (151), the introduction of solid foods should be withheld prior to 4 months of age to avoid overnutrition in the first few months of life – a critical window of development during maturation of energy homeostatic systems setting the course for future feeding behavior and basal metabolism.

Below is a summary of clinical practices based on both human and animal studies regarding the metabolic programming of obesity, to reduce the long-term risk of obesity in children. Many of these have been recommended by professional organizations including the International Federation of Gynecology and Obstetrics (FIGO), American College of Obstetricians and Gynecologists (ACOG), American Academy of Pediatrics (AAP) and National Institutes of Health (NIH) (152). Figure 4 incorporates these recommendations and reviews current therapeutic strategies to target obesity, as well as epigenetic modifiers and potential pathways to target based on preclinical and clinical studies discussed in this review, both for the prevention and treatment of childhood obesity.

CLINICAL PRACTICE RECOMMENDATIONS FOR MOTHERS WITH OBESITY TO REDUCE LONG-TERM RISK OF METABOLIC DISEASE IN CHILDREN

1. Lifestyle modifications, including lower caloric intake and physical activity, even without significant reduction in fat mass or normalization of BMI prepregnancy, will improve long-term outcomes in children.
2. Although weight loss is not recommended during pregnancy, healthy eating habits, moderate physical activity and slow, moderate weight gain (specifically 11-20 lbs for women with BMI ≥ 30 kg/m^2) will improve maternal metabolic status *in utero*, likely reducing risk for obesity in children.
3. Lower caloric intake in women with BMI ≥ 30 kg/m^2 during the lactation period will likely reduce risk for obesity in children.
4. Breastfeeding for at least 6 months or longer has been shown to reduce risk for obesity in children later in life.
5. Avoiding (1) over-supplementation with formula in breastfeed infants and (2) early introduction to solid foods (before 4 months of age) will reduce long-term risk for obesity in children.

Conclusion

Here, we have summarized the mechanistic underpinnings of the metabolic programming of obesity, as well as the epigenetic pathways that "program" obesity, which should be considered as potential therapeutic targets both for the prevention and treatment of childhood obesity. We hope that this review challenges the commonly held belief that obesity is the result of a lack of motivation or conviction to practice healthy lifestyle choices, both in the individual and as a community. Several factors resist changes in feeding behavior and adiposity, some of which have been described in this review. We hope that further understanding of the metabolic conditioning of obesity both in the clinical and research arenas will help us collectively address the global epidemic of obesity.

Author contributions

AR, CL, and VT conceived the manuscript and undertook research. AR wrote the first draft of the manuscript. VVT is responsible for the integrity of the content. All authors contributed to the article and approved the submitted version.

Funding

Research reported in this publication was supported by National Institute of Diabetes and Digestive and Kidney DIseases of the National Institutes of Health under award number: K23DK110539 and P30DK026687. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgments

The authors would like to thank Drs. Alicja A. Skowronski and Rudy Leibel for the helpful discussions about metabolic programming. The figures were created with BioRender.com.

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

References

1. World Health Organization. *Obesity and overweight: World health organization* (2021). Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

2. Paxton SJ, Damiano SR. The development of body image and weight bias in childhood. *Adv Child Dev Behav* (2017) 52:269–98. doi: 10.1016/bs.acdb.2016.10.006

3. Sagar R, Gupta T. Psychological aspects of obesity in children and adolescents. *Indian J Pediatr* (2018) 85(7):554–9. doi: 10.1007/s12098-017-2539-2

4. Rolland-Cachera MF, Deheeger M, Maillot M, Bellisle F. Early adiposity rebound: Causes and consequences for obesity in children and adults. *Int J Obes (Lond).* (2006) 30 Suppl 4:S11–7. doi: 10.1038/sj.ijo.0803514

5. Berthoud HR, Munzberg H, Morrison CD. Blaming the brain for obesity: Integration of hedonic and homeostatic mechanisms. *Gastroenterology* (2017) 152 (7):1728–38. doi: 10.1053/j.gastro.2016.12.050

6. Decarie-Spain L, Kanoski SE. Ghrelin and glucagon-like peptide-1: A gut-brain axis battle for food reward. *Nutrients* (2021) 3(3):977. doi: 10.3390/nu13030977

7. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Long-term persistence of hormonal adaptations to weight loss. *N. Engl J Med* (2011) 365(17):1597–604. doi: 10.1056/NEJMoa1105816

8. LeDuc CA, Skowronski AA, Rosenbaum M. The role of leptin in the development of energy homeostatic systems and the maintenance of body weight. *Front Physiol* (2021) 12:789519. doi: 10.3389/fphys.2021.789519

9. Rosenbaum M, Leibel RL. 20 years of leptin: Role of leptin in energy homeostasis in humans. *J Endocrinol* (2014) 223(1):T83–96. doi: 10.1530/JOE-14-0358

10. Zurlo F, Larson K, Bogardus C, Ravussin E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J Clin Invest* (1990) 86(5):1423–7. doi: 10.1172/JCI114857

11. Wu H, Ballantyne CM. Skeletal muscle inflammation and insulin resistance in obesity. *J Clin Invest* (2017) 127(1):43–54. doi: 10.1172/JCI88880

12. Wadden TA. Treatment of obesity by moderate and severe caloric restriction. results of clinical research trials. *Ann Intern Med* (1993) 119(7 Pt 2):688–93. doi: 10.7326/0003-4819-119-7_part_2-199310011-00012

13. Trust for America's Health. The state of obesity 2021. Available at: https://www.tfah.org/wp-content/uploads/2021/09/2021ObesityReport_Fnl.pdf.

14. Chrissini MK, Panagiotakos DB. Health literacy as a determinant of childhood and adult obesity: A systematic review. *Int J Adolesc Med Health* (2021) 33(3):9–39. doi: 10.1515/ijamh-2020-0275

15. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics-2019 update: A report from the American heart association. *Circulation* (2019) 139(10):e56–e528. doi: 10.1161/CIR.0000000000000659

16. Mensah GA, Roth GA, Fuster V. The global burden of cardiovascular diseases and risk factors: 2020 and beyond. *J Am Coll Cardiol* (2019) 74(20):2529–32. doi: 10.1016/j.jacc.2019.10.009

17. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: Physiology or pathophysiology? *Physiol Rev* (2014) 94(4):1027–76. doi: 10.1152/physrev.00029.2013

18. Aronne LJ, Hall KD, Jakicic MJ, Leibel RL, Lowe MR, Rosenbaum M, et al. Describing the weight-reduced state: Physiology, behavior, and interventions. *Obes (Silver. Spring).* (2021) 29 Suppl 1:S9–s24. doi: 10.1002/oby.23086

19. Ravussin E, Smith SR, Ferrante AWJr. Physiology of energy expenditure in the weight-reduced state. *Obes (Silver. Spring).* (2021) 29 Suppl 1:S31–s8. doi: 10.1002/oby.23095

20. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. *N. Engl J Med* (1995) 332(10):621–8. doi: 10.1056/NEJM199503093321001

21. Gluckman PD, Lillycrop KA, Vickers MH, Pleasants AB, Phillips ES, Beedle AS, et al. Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. *Proc Natl Acad Sci U.S.A.* (2007) 104 (31):12796–800. doi: 10.1073/pnas.0705667104

22. Gluckman PD, Hanson M, Zimmet P, Forrester T. Losing the war against obesity: The need for a developmental perspective. *Sci Transl Med* (2011) 3 (93):93cm19. doi: 10.1126/scitranslmed.3002554

23. Barker DJ, Eriksson JG, Forsen T, Osmond C. Fetal origins of adult disease: Strength of effects and biological basis. *Int J Epidemiol* (2002) 31(6):1235–9. doi: 10.1093/ije/31.6.1235

24. Barker DJ. Fetal origins of coronary heart disease. *Br Heart J* (1993) 69 (3):195–6. doi: 10.1136/hrt.69.3.195

25. Barker DJ, Fall CH. Fetal and infant origins of cardiovascular disease. *Arch Dis Child* (1993) 68(6):797–9. doi: 10.1136/adc.68.6.797

26. Barker DJP. Fetal origins of cardiovascular disease. *Ann Med* (1999) 31 (sup1):3–6. doi: 10.1080/07853890.1999.11904392

27. Dietz WH. Periods of risk in childhood for the development of adult obesity–what do we need to learn? *J Nutr* (1997) 127(9):1884S–6S. doi: 10.1093/jn/127.9.1884S

28. Deng JR, Tan WQ, Yang SY, Ao LP, Liang JP, Li LX, et al. High birth weight and its interaction with physical activity influence the risk of obesity in early school-aged children. *World J Pediatr* (2020) 16(4):385–92. doi: 10.1007/s12519-020-00336-6

29. Qiao Y, Ma J, Wang Y, Li W, Katzmarzyk PT, Chaput JP, et al. Birth weight and childhood obesity: A 12-country study. *Int J Obes Suppl* (2015) 5(Suppl 2):S74–9. doi: 10.1038/ijosup.2015.23

30. Kapral N, Miller SE, Scharf RJ, Gurka MJ, DeBoer MD. Associations between birthweight and overweight and obesity in school-age children. *Pediatr Obes* (2018) 13(6):333–41. doi: 10.1111/ijpo.12227

31. Liang J, Xu C, Liu Q, Fan X, Xu J, Zhang L, et al. Association between birth weight and risk of cardiovascular disease: Evidence from uk biobank. *Nutr Metab Cardiovasc Dis* (2021) 31(9):2637–43. doi: 10.1016/j.numecd.2021.05.017

32. Arnold L, Hoy W, Wang Z. Low birthweight increases risk for cardiovascular disease hospitalisations in a remote indigenous Australian community–a prospective cohort study. *Aust N. Z. J Public Health* (2016) 40 Suppl 1:S102–6. doi: 10.1111/1753-6405.12426

33. Sjoholm P, Pahkala K, Davison B, Niinikoski H, Raitakari O, Juonala M, et al. Birth weight for gestational age and later cardiovascular health: A comparison between longitudinal Finnish and indigenous Australian cohorts. *Ann Med* (2021) 53(1):2060–71. doi: 10.1080/07853890.2021.1999491

34. Wang YX, Li Y, Rich-Edwards JW, Florio AA, Shan Z, Wang S, et al. Associations of birth weight and later life lifestyle factors with risk of cardiovascular disease in the USA: A prospective cohort study. *EClinicalMedicine* (2022) 51:101570. doi: 10.1016/j.eclinm.2022.101570

35. Kong L, Nilsson IAK, Gissler M, Lavebratt C. Associations of maternal diabetes and body mass index with offspring birth weight and prematurity. *JAMA Pediatr* (2019) 173(4):371–8. doi: 10.1001/jamapediatrics.2018.5541

36. Kim SY, Sharma AJ, Sappenfield W, Wilson HG, Salihu HM. Association of maternal body mass index, excessive weight gain, and gestational diabetes mellitus with Large-for-Gestational-Age births. *Obstet. Gynecol.* (2014) 123(4):737–44. doi: 10.1097/AOG.0000000000000177

37. Bowers K, Laughon SK, Kiely M, Brite J, Chen Z, Zhang C. Gestational diabetes, pre-pregnancy obesity and pregnancy weight gain in relation to excess fetal growth: Variations by Race/Ethnicity. *Diabetologia* (2013) 56(6):1263–71. doi: 10.1007/s00125-013-2881-5

38. Group HSCR, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, et al. Hyperglycemia and adverse pregnancy outcomes. *N. Engl J Med* (2008) 358 (19):1991–2002. doi: 10.1056/NEJMoa0707943

39. Group HSCR. Hyperglycemia and adverse pregnancy outcome (Hapo) study: Associations with neonatal anthropometrics. *Diabetes* (2009) 58(2):453–9. doi: 10.2337/db08-1112

40. Lowe WLJr., Lowe LP, Kuang A, Catalano PM, Nodzenski M, Talbot O, et al. Maternal glucose levels during pregnancy and childhood adiposity in the hyperglycemia and adverse pregnancy outcome follow-up study. *Diabetologia* (2019) 62(4):598–610. doi: 10.1007/s00125-018-4809-6

41. Scholtens DM, Kuang A, Lowe LP, Hamilton J, Lawrence JM, Lebenthal Y, et al. Hyperglycemia and adverse pregnancy outcome follow-up study (Hapo fus): Maternal glycemia and childhood glucose metabolism. *Diabetes Care* (2019) 42 (3):381–92. doi: 10.2337/dc18-2021

42. Yu Z, Han S, Zhu J, Sun X, Ji C, Guo X. Pre-pregnancy body mass index in relation to infant birth weight and offspring Overweight/Obesity: A systematic review and meta-analysis. *PloS One* (2013) 8(4):e61627. doi: 10.1371/journal.pone.0061627

43. Voerman E, Santos S, Patro Golab B, Amiano P, Ballester F, Barros H, et al. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. *PloS Med* (2019) 16(2):e1002744. doi: 10.1371/journal.pmed.1002744

44. Victora CG, Bahl R, Barros AJ, Franca GV, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. *Lancet* (2016) 387(10017):475–90. doi: 10.1016/S0140-6736(15)01024-7

45. Horta BL, Loret de Mola C, Victora CG. Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure and type 2 diabetes:

A systematic review and meta-analysis. *Acta Paediatr* (2015) 104(467):30–7. doi: 10.1111/apa.13133

46. Singhal A. Does weight gain in infancy influence the later risk of obesity? *J Pediatr Gastroenterol Nutr* (2010) 51 Suppl 3:S119–20. doi: 10.1097/MPG.0b013e3181efd077

47. Yang Z, Huffman SL. Nutrition in pregnancy and early childhood and associations with obesity in developing countries. *Matern. Child Nutr* (2013) 9 Suppl 1:105–19. doi: 10.1111/mcn.12010

48. Eid EE. Follow-up study of physical growth of children who had excessive weight gain in first six months of life. *Br Med J* (1970) 2(5701):74–6. doi: 10.1136/bmj.2.5701.74

49. Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. *Pediatrics* (2002) 109 (2):194–9. doi: 10.1542/peds.109.2.194

50. Singh AS, Mulder C, Twisk JW, van Mechelen W, Chinapaw MJ. Tracking of childhood overweight into adulthood: A systematic review of the literature. *Obes Rev* (2008) 9(5):474–88. doi: 10.1111/j.1467-789X.2008.00475.x

51. Aris IM, Rifas-Shiman SL, Li LJ, Kleinman KP, Coull BA, Gold DR, et al. Patterns of body mass index milestones in early life and cardiometabolic risk in early adolescence. *Int J Epidemiol* (2019) 48(1):157–67. doi: 10.1093/ije/dyy286

52. Arisaka O, Ichikawa G, Koyama S, Sairenchi T. Childhood obesity: Rapid weight gain in early childhood and subsequent cardiometabolic risk. *Clin Pediatr Endocrinol* (2020) 29(4):135–42. doi: 10.1297/cpe.29.135

53. Lu Y, Pearce A, Li L. Weight gain in early years and subsequent body mass index trajectories across birth weight groups: A prospective longitudinal study. *Eur J Public Health* (2020) 30(2):316–22. doi: 10.1093/eurpub/ckz232

54. Woo JG. Infant growth and long-term cardiometabolic health: A review of recent findings. *Curr Nutr Rep* (2019) 8(1):29–41. doi: 10.1007/s13668-019-0259-0

55. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet* (2003) 361 (9363):1089–97. doi: 10.1016/S0140-6736(03)12895-4

56. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: Systematic review of size and growth in infancy and later obesity. *BMJ* (2005) 331(7522):929. doi: 10.1136/bmj.38586.411273.E0

57. Reilly JJ, Armstrong J, Dorosty AR, Emmett PM, Ness A, Rogers I, et al. Early life risk factors for obesity in childhood: Cohort study. *BMJ* (2005) 330 (7504):1357. doi: 10.1136/bmj.38470.670903.E0

58. Kuriyan R. Body composition techniques. *Indian J Med Res* (2018) 148 (5):648–58. doi: 10.4103/ijmr.IJMR_1777_18

59. Ode JJ, Pivarnik JM, Reeves MJ, Knous JL. Body mass index as a predictor of percent fat in college athletes and nonathletes. *Med Sci Sports. Exerc.* (2007) 39 (3):403–9. doi: 10.1249/01.mss.0000247008.19127.3e

60. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Size at birth, fat-free mass and resting metabolic rate in adult life. *Horm Metab Res* (2002) 34(2):72–6. doi: 10.1055/s-2002-20518

61. Sandboge S, Moltchanova E, Blomstedt PA, Salonen MK, Kajantie E, Osmond C, et al. Birth-weight and resting metabolic rate in adulthood - sex-specific differences. *Ann Med* (2012) 44(3):296–303. doi: 10.3109/07853890.2010.549147

62. Weyer C, Pratley RE, Lindsay RS, Tataranni PA. Relationship between birth weight and body composition, energy metabolism, and sympathetic nervous system activity later in life. *Obes Res* (2000) 8(8):559–65. doi: 10.1038/oby.2000.72

63. Lewis DS, Bertrand HA, McMahan CA, McGill HCJr., Carey KD, Masoro EJ. Preweaning food intake influences the adiposity of young adult baboons. *J Clin Invest* (1986) 78(4):899–905. doi: 10.1172/JCI112678

64. Poissonnet CM, Burdi AR, Garn SM. The chronology of adipose tissue appearance and distribution in the human fetus. *Early. Hum Dev* (1984) 10(1-2):1–11. doi: 10.1016/0378-3782(84)90106-3

65. Enzi G, Zanardo V, Caretta F, Inelmen EM, Rubaltelli F. Intrauterine growth and adipose tissue development. *Am J Clin Nutr* (1981) 34(9):1785–90. doi: 10.1093/ajcn/34.9.1785

66. Ng PC. The fetal and neonatal hypothalamic-Pituitary-Adrenal axis. *Arch Dis Child Fetal. Neonatal. Ed.* (2000) 82(3):F250–4. doi: 10.1136/fn.82.3.f250

67. Ellis PJ, Morris TJ, Skinner BM, Sargent CA, Vickers MH, Gluckman PD, et al. Thrifty metabolic programming in rats is induced by both maternal undernutrition and postnatal leptin treatment, but masked in the presence of both: Implications for models of developmental programming. *BMC Genomics* (2014) 15:49. doi: 10.1186/1471-2164-15-49

68. Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A, et al. Neonatal leptin treatment reverses developmental programming. *Endocrinology* (2005) 146(10):4211–6. doi: 10.1210/en.2005-0581

69. Vickers MH, Ikenasio BA, Breier BH. Igf-I treatment reduces hyperphagia, obesity, and hypertension in metabolic disorders induced by fetal programming. *Endocrinology* (2001) 142(9):3964–73. doi: 10.1210/endo.142.9.8390

70. Schoonejans JM, Ozanne SE. Developmental programming by maternal obesity: Lessons from animal models. *Diabetes Med* (2021) 38(12):e14694. doi: 10.1111/dme.14694

71. Chen H, Simar D, Lambert K, Mercier J, Morris MJ. Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. *Endocrinology* (2008) 149(11):5348–56. doi: 10.1210/en.2008-0582

72. Srinivasan M, Katewa SD, Palaniyappan A, Pandya JD, Patel MS. Maternal high-fat diet consumption results in fetal malprogramming predisposing to the onset of metabolic syndrome-like phenotype in adulthood. *Am J Physiol Endocrinol Metab* (2006) 291(4):E792–9. doi: 10.1152/ajpendo.00078.2006

73. Gupta A, Srinivasan M, Thamadilok S, Patel MS. Hypothalamic alterations in fetuses of high fat diet-fed obese female rats. *J Endocrinol* (2009) 200(3):293–300. doi: 10.1677/JOE-08-0429

74. White CL, Purpera MN, Morrison CD. Maternal obesity is necessary for programming effect of high-fat diet on offspring. *Am J Physiol Regul Integr Comp Physiol* (2009) 296(5):R1464–72. doi: 10.1152/ajpregu.91015.2008

75. Kelly AC, Powell TL, Jansson T. Placental function in maternal obesity. *Clin Sci (Lond).* (2020) 134(8):961–84. doi: 10.1042/CS20190266

76. Brombach C, Tong W, Giussani DA. Maternal obesity: New placental paradigms unfolded. *Trends Mol Med* (2022) 28(10):823–835. doi: 10.1016/j.molmed.2022.05.013

77. Sullivan EL, Grove KL. Metabolic imprinting in obesity. *Forum Nutr* (2010) 63:186–94. doi: 10.1159/000264406

78. Plagemann A, Heidrich I, Gotz F, Rohde W, Dorner G. Lifelong enhanced diabetes susceptibility and obesity after temporary intrahypothalamic hyperinsulinism during brain organization. *Exp Clin Endocrinol* (1992) 99(2):91–5. doi: 10.1055/s-0029-1211143

79. Hill DJ, Milner RDG. Insulin as a growth factor. *Pediatr Res* (1985) 19 (9):879–86. doi: 10.1203/00006450-198509000-00001

80. Carmody JS, Wan P, Accili D, Zeltser LM, Leibel RL. Respective contributions of maternal insulin resistance and diet to metabolic and hypothalamic phenotypes of progeny. *Obesity* (2011) 19(3):492–9. doi: 10.1038/oby.2010.245

81. Castillo-Castrejon M, Jansson T, Powell TL. No evidence of attenuation of placental insulin-stimulated akt phosphorylation and amino acid transport in maternal obesity and gestational diabetes mellitus. *Am J Physiol Endocrinol Metab* (2019) 317(6):E1037–E49. doi: 10.1152/ajpendo.00196.2019

82. Ahima RS, Saper CB, Flier JS, Elmquist JK. Leptin regulation of neuroendocrine systems. *Front Neuroendocr.* (2000) 21(3):263–307. doi: 10.1006/frne.2000.0197

83. Levin BE, Dunn-Meynell AA. Reduced central leptin sensitivity in rats with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* (2002) 283(4):R941–8. doi: 10.1152/ajpregu.00245.2002

84. Lustig RH, Sen S, Soberman JE, Velasquez-Mieyer PA. Obesity, leptin resistance, and the effects of insulin reduction. *Int J Obes Relat Metab Disord* (2004) 28(10):1344–8. doi: 10.1038/sj.ijo.0802753

85. Morris MJ, Chen H. Established maternal obesity in the rat reprograms hypothalamic appetite regulators and leptin signaling at birth. *Int J Obes* (2009) 33 (1):115–22. doi: 10.1038/ijo.2008.213

86. Skowronski AA, LeDuc CA, Foo KS, Goffer Y, Burnett LC, Egli D, et al. Physiological consequences of transient hyperleptinemia during discrete developmental periods on body weight in mice. *Sci Transl Med* (2020) 12(524). doi: 10.1126/scitranslmed.aax6629

87. Skowronski AA, Shaulson ED, Leibel RL, LeDuc CA. The postnatal leptin surge in mice is variable in both time and intensity and reflects nutritional status. *Int J Obes* (2022) 46(1):39–49. doi: 10.1038/s41366-021-00957-5

88. Amitani M, Asakawa A, Amitani H, Inui A. The role of leptin in the control of insulin-glucose axis. *Front Neurosci* (2013) 7:51. doi: 10.3389/fnins.2013.00051

89. DeChiara TM, Efstratiadis A, Robertson EJ. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor ii gene disrupted by targeting. *Nature* (1990) 345(6270):78–80. doi: 10.1038/345078a0

90. Constancia M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, et al. Placental-specific igf-ii is a major modulator of placental and fetal growth. *Nature* (2002) 417(6892):945–8. doi: 10.1038/nature00819

91. Kniss DA, Shubert PJ, Zimmerman PD, Landon MB, Gabbe SG. Insulinlike growth factors. their regulation of glucose and amino acid transport in placental trophoblasts isolated from first-trimester chorionic villi. *J Reprod Med* (1994) 39 (4):249–56.

92. Aye IL, Rosario FJ, Powell TL, Jansson T. Adiponectin supplementation in pregnant mice prevents the adverse effects of maternal obesity on placental

function and fetal growth. *Proc Natl Acad Sci U.S.A.* (2015) 112(41):12858–63. doi: 10.1073/pnas.1515484112

93. Paulsen ME, Rosario FJ, Wesolowski SR, Powell TL, Jansson T. Normalizing adiponectin levels in obese pregnant mice prevents adverse metabolic outcomes in offspring. *FASEB J* (2019) 33(2):2899–909. doi: 10.1096/fj.201801015R

94. Lopomo A, Burgio E, Migliore L. Epigenetics of obesity. *Prog Mol Biol Transl Sci* (2016) 140:151–84. doi: 10.1016/bs.pmbts.2016.02.002

95. van Dijk SJ, Tellam RL, Morrison JL, Muhlhausler BS, Molloy PL. Recent developments on the role of epigenetics in obesity and metabolic disease. *Clin Epigenet* (2015) 7:66. doi: 10.1186/s13148-015-0101-5

96. Razin A, Shemer R. DNA Methylation in early development. *Hum Mol Genet* (1995) 4:1751–5. doi: 10.1093/hmg/4.suppl_1.1751

97. Ehrlich M. Expression of various genes is controlled by DNA methylation during mammalian development. *J Cell Biochem* (2003) 88(5):899–910. doi: 10.1002/jcb.10464

98. Godfrey KM, Sheppard A, Gluckman PD, Lillycrop KA, Burdge GC, McLean C, et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes* (2011) 60(5):1528–34. doi: 10.2337/db10-0979

99. Guénard F, Deshaies Y, Cianflone K, Kral JG, Marceau P, Vohl MC. Differential methylation in glucoregulatory genes of offspring born before vs. after maternal gastrointestinal bypass surgery. *Proc Natl Acad Sci U.S.A.* (2013) 110(28):11439–44. doi: 10.1073/pnas.1216959110

100. Dearden L, Ozanne SE. Early life origins of metabolic disease: Developmental programming of hypothalamic pathways controlling energy homeostasis. *Front Neuroendocr.* (2015) 39:3–16. doi: 10.1016/j.yfrne.2015.08.001

101. Masuyama H, Mitsui T, Nobumoto E, Hiramatsu Y. The effects of high-fat diet exposure in utero on the obesogenic and diabetogenic traits through epigenetic changes in adiponectin and leptin gene expression for multiple generations in female mice. *Endocrinology* (2015) 156(7):2482–91. doi: 10.1210/en.2014-2020

102. Marousez L, Lesage J, Eberle D. Epigenetics: Linking early postnatal nutrition to obesity programming? *Nutrients* (2019) 11(12):2966. doi: 10.3390/nu11122966

103. Zeltser LM. Feeding circuit development and early-life influences on future feeding behaviour. *Nat Rev Neurosci* (2018) 19(5):302–16. doi: 10.1038/nrn.2018.23

104. Howland MA, Sandman CA, Glynn LM. Developmental origins of the human hypothalamic-Pituitary-Adrenal axis. *Expert Rev Endocrinol Metab* (2017) 12(5):321–39. doi: 10.1080/17446651.2017.1356222

105. Wu X, Zhang Y. Tet-mediated active DNA demethylation: Mechanism, function and beyond. *Nat Rev Genet* (2017) 18(9):517–34. doi: 10.1038/nrg.2017.33

106. Painter RC, Osmond C, Gluckman P, Hanson M, Phillips DI, Roseboom TJ. Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. *BJOG* (2008) 115(10):1243–9. doi: 10.1111/j.1471-0528.2008.01822.x

107. Smith J, Cianflone K, Biron S, Hould FS, Lebel S, Marceau S, et al. Effects of maternal surgical weight loss in mothers on intergenerational transmission of obesity. *J Clin Endocrinol Metab* (2009) 94(11):4275–83. doi: 10.1210/jc.2009-0709

108. Kral JG, Biron S, Simard S, Hould FS, Lebel S, Marceau S, et al. Large Maternal weight loss from obesity surgery prevents transmission of obesity to children who were followed for 2 to 18 years. *Pediatrics* (2006) 118(6):e1644–9. doi: 10.1542/peds.2006-1379

109. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* (2005) 135(6):1382–6. doi: 10.1093/jn/135.6.1382

110. Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE. Maternal nutrition and fetal development. *J Nutr* (2004) 134(9):2169–72. doi: 10.1093/jn/134.9.2169

111. Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *Br J Nutr* (2007) 97 (6):1064–73. doi: 10.1017/S000711450769196X

112. Jousse C, Parry L, Lambert-Langlais S, Maurin AC, Averous J, Bruhat A, et al. Perinatal undernutrition affects the methylation and expression of the leptin gene in adults: Implication for the understanding of metabolic syndrome. *FASEB J* (2011) 25(9):3271–8. doi: 10.1096/fj.11-181792

113. Lillycrop KA, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC. Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic ppar alpha promoter of the offspring. *Br J Nutr* (2008) 100(2):278–82. doi: 10.1017/S0007114507894438

114. Burdge GC, Lillycrop KA, Phillips ES, Slater-Jefferies JL, Jackson AA, Hanson MA. Folic acid supplementation during the juvenile-pubertal period in rats modifies the phenotype and epigenotype induced by prenatal nutrition. *J Nutr* (2009) 139(6):1054–60. doi: 10.3945/jn.109.104653

115. Borengasser SJ, Zhong Y, Kang P, Lindsey F, Ronis MJ, Badger TM, et al. Maternal obesity enhances white adipose tissue differentiation and alters genome-scale DNA methylation in Male rat offspring. *Endocrinology* (2013) 154(11):4113–25. doi: 10.1210/en.2012-2255

116. Fernandez-Twinn DS, Alfaradhi MZ, Martin-Gronert MS, Duque-Guimaraes DE, Piekarz A, Ferland-McCollough D, et al. Downregulation of irs-1 in adipose tissue of offspring of obese mice is programmed cell-autonomously through post-transcriptional mechanisms. *Mol Metab* (2014) 3(3):325–33. doi: 10.1016/j.molmet.2014.01.007

117. Li CC, Young PE, Maloney CA, Eaton SA, Cowley MJ, Buckland ME, et al. Maternal obesity and diabetes induces latent metabolic defects and widespread epigenetic changes in isogenic mice. *Epigenetics* (2013) 8(6):602–11. doi: 10.4161/epi.24656

118. Suter MA, Ma J, Vuguin PM, Hartil K, Fiallo A, Harris RA, et al. *In utero* exposure to a maternal high-fat diet alters the epigenetic histone code in a murine model. *Am J Obstet. Gynecol.* (2014) 210(5):463 e1– e11. doi: 10.1016/j.ajog.2014.01.045

119. Hartwig FP, Loret de Mola C, Davies NM, Victora CG, Relton CL. Breastfeeding effects on DNA methylation in the offspring: A systematic literature review. *PloS One* (2017) 12(3):e0173070. doi: 10.1371/journal.pone.0173070

120. Obermann-Borst SA, Eilers PH, Tobi EW, de Jong FH, Slagboom PE, Heijmans BT, et al. Duration of breastfeeding and gender are associated with methylation of the leptin gene in very young children. *Pediatr Res* (2013) 74 (3):344–9. doi: 10.1038/pr.2013.95

121. Sherwood WB, Bion V, Lockett GA, Ziyab AH, Soto-Ramirez N, Mukherjee N, et al. Duration of breastfeeding is associated with leptin (Lep) DNA methylation profiles and bmi in 10-Year-Old children. *Clin Epigenet* (2019) 11(1):128. doi: 10.1186/s13148-019-0727-9

122. Pauwels S, Symons L, Vanautgaerden EL, Ghosh M, Duca RC, Bekaert B, et al. The influence of the duration of breastfeeding on the infant's metabolic epigenome. *Nutrients* (2019) 11(6):1408. doi: 10.3390/nu11061408

123. Davidowa H, Plagemann A. Decreased inhibition by leptin of hypothalamic arcuate neurons in neonatally overfed young rats. *Neuroreport* (2000) 11(12):2795–8. doi: 10.1097/00001756-200008210-00037

124. Davidowa H, Plagemann A. Insulin resistance of hypothalamic arcuate neurons in neonatally overfed rats. *Neuroreport* (2007) 18(5):521–4. doi: 10.1097/WNR.0b013e32805dfb93

125. Plagemann A, Harder T, Rake A, Waas T, Melchior K, Ziska T, et al. Observations on the orexigenic hypothalamic neuropeptide y-system in neonatally overfed weanling rats. *J Neuroendocr.* (1999) 11(7):541–6. doi: 10.1046/j.1365-2826.1999.00357.x

126. Srinivasan M, Patel MS. Metabolic programming in the immediate postnatal period. *Trends Endocrinol Metab* (2008) 19(4):146–52. doi: 10.1016/j.tem.2007.12.001

127. Patel MS, Srinivasan M. Metabolic programming in the immediate postnatal life. *Ann Nutr Metab* (2011) 58 Suppl 2:18–28. doi: 10.1159/000328040

128. Mahmood S, Smiraglia DJ, Srinivasan M, Patel MS. Epigenetic changes in hypothalamic appetite regulatory genes may underlie the developmental programming for obesity in rat neonates subjected to a high-carbohydrate dietary modification. *J Dev Orig. Health Dis* (2013) 4(6):479–90. doi: 10.1017/S2040174413000238

129. Gorski JN, Dunn-Meynell AA, Hartman TG, Levin BE. Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. *Am J Physiol Regul Integr Comp Physiol* (2006) 291(3):R768–78. doi: 10.1152/ajpregu.00138.2006

130. Vogt MC, Paeger L, Hess S, Steculorum SM, Awazawa M, Hampel B, et al. Neonatal insulin action impairs hypothalamic neurocircuit formation in response to maternal high-fat feeding. *Cell* (2014) 156(3):495–509. doi: 10.1016/j.cell.2014.01.008

131. Desai M, Gayle D, Babu J, Ross MG. Programmed obesity in intrauterine growth-restricted newborns: Modulation by newborn nutrition. *Am J Physiol Regul Integr Comp Physiol* (2005) 288(1):R91–6. doi: 10.1152/ajpregu.00340.2004

132. Tosh DN, Fu Q, Callaway CW, McKnight RA, McMillen IC, Ross MG, et al. Epigenetics of programmed obesity: Alteration in iugr rat hepatic Igf1 mrna expression and histone structure in rapid vs. delayed postnatal catch-up growth. *Am J Physiol Gastroint. Liver. Physiol* (2010) 299(5):G1023–9. doi: 10.1152/ajpgi.00052.2010

133. Dabelea D, Crume T. Maternal environment and the transgenerational cycle of obesity and diabetes. *Diabetes* (2011) 60(7):1849–55. doi: 10.2337/db11-0400

134. Dabelea D, Mayer-Davis EJ, Lamichhane AP, D'Agostino RBJr., Liese AD, Vehik KS, et al. Association of intrauterine exposure to maternal diabetes and obesity with type 2 diabetes in youth: The search case-control study. *Diabetes Care* (2008) 31(7):1422–6. doi: 10.2337/dc07-2417

135. Kankowski L, Ardissino M, McCracken C, Lewandowski AJ, Leeson P, Neubauer S, et al. The impact of maternal obesity on offspring cardiovascular health: A systematic literature review. *Front Endocrinol (Lausanne).* (2022) 13:868441. doi: 10.3389/fendo.2022.868441

136. King SE, Skinner MK. Epigenetic transgenerational inheritance of obesity susceptibility. *Trends Endocrinol Metab* (2020) 31(7):478–94. doi: 10.1016/j.tem.2020.02.009

137. Mohajer N, Joloya EM, Seo J, Shioda T, Blumberg B. Epigenetic transgenerational inheritance of the effects of obesogen exposure. *Front Endocrinol (Lausanne).* (2021) 12:787580. doi: 10.3389/fendo.2021.787580

138. Dunn GA, Bale TL. Maternal high-fat diet effects on third-generation female body size *Via* the paternal lineage. *Endocrinology* (2011) 152(6):2228–36. doi: 10.1210/en.2010-1461

139. Aggarwal R, Jha M, Shrivastava A, Jha AK. Natural compounds: Role in reversal of epigenetic changes. *Biochem (Mosc).* (2015) 80(8):972–89. doi: 10.1134/S0006297915080027

140. Arguelles AO, Meruvu S, Bowman JD, Choudhury M. Are epigenetic drugs for diabetes and obesity at our door step? *Drug Discovery Today* (2016) 21(3):499–509. doi: 10.1016/j.drudis.2015.12.001

141. Ghasemi S. Cancer's epigenetic drugs: Where are they in the cancer medicines? *Pharmacogen. J* (2020) 20(3):367–79. doi: 10.1038/s41397-019-0138-5

142. Abhimanyu, Ontiveros CO, Guerra-Resendez RS, Nishiguchi T, Ladki M, Hilton IB, et al. Reversing post-infectious epigenetic-mediated immune suppression. *Front Immunol* (2021) 12:688132. doi: 10.3389/fimmu.2021.688132

143. Jackson VM, Breen DM, Fortin JP, Liou A, Kuzmiski JB, Loomis AK, et al. Latest approaches for the treatment of obesity. *Expert Opin Drug Discovery* (2015) 10(8):825–39. doi: 10.1517/17460441.2015.1044966

144. Dragano NRV, Ferno J, Dieguez C, Lopez M, Milbank E. Recent updates on obesity treatments: Available drugs and future directions. *Neuroscience* (2020) 437:215–39. doi: 10.1016/j.neuroscience.2020.04.034

145. Knudsen LB, Lau J. The discovery and development of liraglutide and semaglutide. *Front Endocrinol (Lausanne).* (2019) 10:155. doi: 10.3389/fendo.2019.00155

146. Sherman MM, Ungureanu S, Rey JA. Naltrexone/Bupropion er (Contrave): Newly approved treatment option for chronic weight management in obese adults. *P. T.* (2016) 41(3):164–72.

147. Bays H. Phentermine, topiramate and their combination for the treatment of adiposopathy ('Sick fat') and metabolic disease. *Expert Rev Cardiovasc Ther* (2010) 8(12):1777–801. doi: 10.1586/erc.10.125

148. Sharma S, Garfield AS, Shah B, Kleyn P, Ichetovkin I, Moeller IH, et al. Current mechanistic and pharmacodynamic understanding of melanocortin-4 receptor activation. *Molecules* (2019) 24(10):1892. doi: 10.3390/molecules24101892

149. Ryan PM, Seltzer S, Hayward NE, Rodriguez DA, Sless RT, Hawkes CP. Safety and efficacy of glucagon-like peptide-1 receptor agonists in children and adolescents with obesity: A meta-analysis. *J Pediatr* (2021) 236:137–47 e13. doi: 10.1016/j.jpeds.2021.05.009

150. Rosenbaum M, Thaker V. Special considerations relevant to pediatric obesity. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al, editors. *Endotext*. South Dartmouth (MA: MDText.com, Inc (2022).

151. Huh SY, Rifas-Shiman SL, Taveras EM, Oken E, Gillman MW. Timing of solid food introduction and risk of obesity in preschool-aged children. *Pediatrics* (2011) 127(3):e544–51. doi: 10.1542/peds.2010-0740

152. McAuliffe FM, Killeen SL, Jacob CM, Hanson MA, Hadar E, McIntyre HD, et al. Management of prepregnancy, pregnancy, and postpartum obesity from the figo pregnancy and non-communicable diseases committee: A figo (International federation of gynecology and obstetrics) guideline. *Int J Gynaecol. Obstet.* (2020) 151 Suppl 1:16–36. doi: 10.1002/ijgo.13334

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 11 November 2022
DOI 10.3389/fendo.2022.1041718

OPEN ACCESS

EDITED BY
Malgorzata Wojcik,
Jagiellonian University Medical College, Poland

REVIEWED BY
Julio Álvarez-Pitti,
Hospital General Universitario De Valencia, Spain
Rosita Gabbianelli,
University of Camerino, Italy

*CORRESPONDENCE
Christina Kanaka-Gantenbein
chriskan@med.uoa.gr
c.kanaka-gantenbein@med.uoa.gr

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 11 September 2022
ACCEPTED 25 October 2022
PUBLISHED 11 November 2022

CITATION
Gantenbein KV and Kanaka-Gantenbein C (2022) Highlighting the trajectory from intrauterine growth restriction to future obesity.
Front. Endocrinol. 13:1041718.
doi: 10.3389/fendo.2022.1041718

Highlighting the trajectory from intrauterine growth restriction to future obesity

Katherina Vicky Gantenbein[1] and Christina Kanaka-Gantenbein[2]*

[1]Swiss Children's Rehab, University Children's Hospital Zurich, Zurich, Switzerland, [2]Division of Endocrinology, Diabetes and Metabolism, First Department of Pediatrics Medical School, National and Kapodistrian University of Athens, Aghia Sophia Children's Hospital, Athens, Greece

During the last decades several lines of evidence reported the association of an adverse intrauterine environment, leading to intrauterine restriction, with future disease, such as obesity and metabolic syndrome, both leading to increased cardiovascular and cancer risk. The underlying explanation for this association has firstly been expressed by the Barker's hypothesis, the "*thrifty phenotype hypothesis*". According to *this hypothesis*, a fetus facing an adverse intrauterine environment adapts to this environment through a reprogramming of its endocrine-metabolic status, during the crucial window of developmental plasticity to save energy for survival, providing less energy and nutrients to the organs that are not essential for survival. This theory evolved to the concept of the developmental origin of health and disease (DOHaD). Thus, in the setting of an adverse, f. ex. protein restricted intrauterine environment, while the energy is mainly directed to the brain, the peripheral organs, f.ex. the muscles and the liver undergo an adaptation that is expressed through insulin resistance. The adaptation at the hepatic level predisposes to future dyslipidemia, the modifications at the vascular level to endothelial damage and future hypertension and, overall, through the insulin resistance to the development of metabolic syndrome. All these adaptations are suggested to take place through epigenetic modifications of the expression of genes without change of their amino-acid sequence. The epigenetic modifications leading to future obesity and cardiovascular risk are thought to induce appetite dysregulation, promoting food intake and adipogenesis, facilitating obesity development. The epigenetic modifications may even persist into the next generation even though the subsequent generation has not been exposed to an adverse intrauterine environment, a notion defined as the "transgenerational transfer of environmental information". As a consequence, if the increased

public health burden and costs of non-communicable chronic diseases such as obesity, hypertension, metabolic syndrome and type 2 diabetes have to be minimized, special attention should be laid to the healthy lifestyle habits of women of reproductive age, including healthy diet and physical activity to be established long before any pregnancy takes place in order to provide the best conditions for both somatic and mental health of future generations.

KEYWORDS

intrauterine growth restriction, IUGR, metabolic syndrome, obesity, offspring, small for gestational age, cardiovascular risk

Introduction

The definition of intrauterine growth restriction

Intrauterine growth restriction (IUGR) is a term attributed to the state of a fetus that was unable to achieve its growth potential due to an adverse intrauterine environment, based on serial fetal ultrasound measurements (1–4). The notion of IUGR should not be confused with the term "small for gestational age" (SGA), that is a descriptive term to characterize a neonate that is born with a birthweight and/or birth length below the -2 SDS for gestational age and sex. Although many neonates are fulfilling the criteria for both IUGR and SGA, it is important to differentiate between these two entities, since the first delineates an adverse intrauterine environment that forced the fetus to a re-adaptation of its metabolic and endocrine determinants, in order to spare energy for survival, that may affect also future growth and development, while the latter term of SGA is not obligatorily the result of an adverse intrauterine environment (2).

According to the expert Consensus of 2016, fetal growth restriction is defined by impaired biometric parameters as well as vascular abnormalities of the placenta such as an increased pulsatile index or an absent end-diastolic flow of the uterine artery (3). The expert Consensus of 2018 (4) proposed following variables as the definition of growth restriction in the newborn: "birth weight under the 3rd percentile or three out of five following points 1. Birth weight under the 10^{th} percentile, 2. Length under the 10^{nth} percentile, 3. Head circumference under the 10^{th} percentile, 4. Prenatal diagnosis of fetal growth restriction, 5. Maternal pregnancy information such as pre-eclampsia or hypertension."

The current narrative review article aims to highlight the difference between IUGR and SGA, as well as the causes and consequences of IUGR, with special emphasis on the mechanisms linking IUGR to future poor cardiometabolic outcome.

Differences between intrauterine growth restriction and small for gestational age

It is therefore important to differentiate between the terms fetal growth restriction (FGR) or Intrauterine growth restriction (IUGR) and small for gestational age (SGA). The term SGA defines a fetus whose size is below the 10^{th} percentile for gestational age. Reasons for this can be ethnicity, parental height, maternal weight, or age (5). However, the SGA-fetus may grow along the designated percentiles and there is no pathological condition. In contrast, the term FGR/IURG refers to a pathological condition in which the fetus grows below its expected percentiles. In this case the fetus can also grow over the 10^{th} percentile but does not reach its expected growth potential (6). In other words, a fetus exposed to inadequate protein intake or to increased psychosocial stress of the pregnant woman during gestation may adapt its endocrine-metabolic pathways for survival during the window of developmental plasticity, while a neonate born SGA can merely be born to an otherwise healthy woman of short stature, without being exposed to an adverse intrauterine environment.

The determinants of fetal growth

The adequate placental blood supply

A prerequisite for the appropriate growth of a fetus is the supply of nutrients and oxygen. This is guaranteed *via* the placenta. During pregnancy, the uterine vessels dilate to ensure sufficient blood flow to the placenta. This is regulated by hormones such as estrogens, progesterone, and human chorionic gonadotropin (7). In addition, the placenta grows by the formation of new villi. The number of intraplacental vessels increases so that the fetus is supplied with enough nutrients and oxygen (8). This procedure is regulated by growth factors, such as leukemia-inhibitory factor, epidermal growth factor and vascular endothelial growth factor, which are secreted by the uterine glandular cells (9).

The adequate blood supply through the placenta may therefore guarantee adequate nutrient supply to the fetus. In fetuses with FGR/IUGR the placenta is smaller and the number of villi as well as vessels within these villi are reduced (8). The decreased size of the placenta and the dysfunction of the trophoblasts lead to an undersupply of the fetus with oxygen and nutrients. Moreover, it has been described that both the velocity of the umbilical blood flow as well as the umbilical oxygen delivery are reduced in FGR pregnancies compared to normal pregnancies (10).

Therefore, causes of placental insufficiency may be of vascular origin, such as stenosis or inadequate vascular development/angiogenesis, but also the result of pre-existing chronic disease of the pregnant woman, such as arterial hypertension, predisposing to pre-eclampsia, poorly controlled pre-existing diabetes mellitus, or the influence of toxic agents, such as smoking due to the vasoconstrictive effect of nicotine or substance abuse, in the form of either excess alcohol consumption or drug abuse (7, 8, 11–14).

Nutrient supply

Adequate oxygen and nutrient supply through the placenta are therefore of paramount importance for normal fetal growth. During pregnancy, the need for energy increases by 69kcal/day in the first trimester, 266kcal/day in the second trimester and 496kcal/day in the last trimester. The main energy source of the fetus is glucose. Since the fetus cannot produce glucose during intrauterine life, it is dependent on the maternal supply (15). Studies in animals have shown that inappropriate supply of glucose during pregnancy, leading to fetal hypoglycemia, predisposes to growth retardation and to congenital malformations. Intellectual disability due to hypoglycemia has also been well documented as is the case in poorly controlled pregnant women with pre-existing type 1 diabetes mellitus or even gestational diabetes (16). Moreover, an adequate supply of amino acids and omega-3-fatty acids is particularly important for the growth of the fetus and the placenta. In addition, a sufficient intake of iron, iodide, and calcium is essential for normal fetal growth (17). Vitamin D is also indispensable since vitamin D deficiency has been shown to be associated with an increased risk of preeclampsia and the development of gestational diabetes, circumstances that, in turn, affect fetal growth (18). Furthermore, folate supplementation can prevent congenital malformations, especially neural tube defects, preeclampsia or IUGR, caused by folate deficiency. Vitamin B1 and B6 deficiency are also associated with pre-eclampsia and/or IUGR (19).

The hormonal determinants of normal fetal growth

Fetal growth is regulated by hormones mainly secreted by the placenta and the fetus (20, 21). The most important key regulator of fetal growth is fetal insulin, while insulin secreted by the beta-cells of the maternal pancreas does not cross the placenta (22). Thus, the passage of the maternal glucose to the fetal circulation drives the production of insulin by the fetal pancreas, leading to fetal hyperinsulinemia, which, in turn, promotes fetal growth.

During pregnancy, a state of inherent insulin resistance of the pregnant woman, the beta-cells of the maternal pancreas expand to secrete more insulin. In addition to that, the insulin-sensitive organs, such as skeletal muscle and adipose tissue, that in normal circumstances absorb glucose, through the development of this insulin-resistant state, allow more glucose to be available through the placenta to the fetus (23). The glucose of the maternal circulation, therefore, is passively diffused through the placenta to provide adequate fuel for fetal growth. Thus, in situations characterized by hyperglycemia of the mother, such as in case of pre-existing type 1 diabetes or gestational diabetes, the increased glucose induces hypertrophy of the fetal pancreatic beta cells and increased insulin production, leading thus to fetal macrosomia (24, 25). In other words, the paramount role of insulin during fetal development is highlighted by the macrosomia of newborns born to mothers with pre-existing diabetes, where maternal hyperglycemia drives overproduction of insulin by the fetal pancreatic beta cells. On the other hand, in cases of inadequate insulin production by the fetus or inadequate action, such as in the case for example of pancreatic agenesis of the fetus or leprechaunism, a genetic cause of extreme insulin resistance, the newborn is characterized by an extremely reduced birth weight, proving again the paramount role of fetal insulin on fetal growth (26, 27). Furthermore, fetal growth as well as placental development is stimulated by the insulin like growth-factors (IGF), mainly IGF-2 and, to a lesser degree, IGF-1 (28). The role of IGF-2 in fetal growth has been demonstrated in the context of the Silver-Russel syndrome, characterized by extreme intrauterine growth restriction due to disorders in the methylation pattern of the IGF-2 gene in affected fetuses. A further key regulator of fetal growth is the growth hormone variant (GHV) secreted by the placenta. From the 17th week of pregnancy, GHV replaces GH secreted by the pituitary gland in the maternal blood circulation. GHV promotes fetal growth by increasing blood and glucose supply in favor of the fetus (29). Furthermore, fetal growth is regulated by thyroid hormones (30). Thyroid hormones are secreted by both the maternal thyroid gland, especially in the first half of pregnancy, and the fetal thyroid gland, especially in the second half of pregnancy. Thyroid hormones are essential for neurogenesis and osteogenesis of the fetus. During pregnancy, the demand for thyroid hormones and, therefore, also for iodide increases by 20-50%. Consequently, the need-based supplementation of iodide is crucial for pregnant women (31).

All these growth-promoting hormonal factors are counteracted by the glucocorticoids. Glucocorticoids have mainly an inhibitory effect on growth, but are essential for the differentiation of fetal tissues and preparation for extrauterine life (28).

The causes of impaired fetal growth

Impaired fetal growth may thus be the result either of maternal, fetal, or placental causes (Table 1).

Maternal causes

The most common cause is preeclampsia in the pregnant woman (13). Preeclampsia is defined by maternal hypertension occurring after the 20th week of gestation with proteinuria and/or maternal organ dysfunction and/or placental insufficiency (5). Risk factors for preeclampsia are an antiphospholipid syndrome, history of previous preeclampsia, poorly controlled pre-existing type 1 diabetes mellitus, hypertension, positive family history for preeclampsia, multiple pregnancy, nulliparity, obesity and age over 40y (32). The pathogenesis of preeclampsia is not clearly understood. The hypothesis, however, is that there is an impaired development of the uterine spiral arteries. This leads to an undersupply of the placenta, and consequently, to placental ischemia. This results to the secretion of anti-angiogenic factors into the maternal circulation, which, in turn, induce endothelial damage (33). Preeclampsia is correlated with 5-23% lower birth weight in comparison to uneventful normal pregnancies (34).

A further placental abnormality, a circumvallate placenta is also associated with IUGR (35). In a circumvallate placenta the chorionic surface is smaller than the basal surface, leading to a folding of the membrane margin in an annular shape (35). Circumvallate placenta is associated with persistent vaginal bleeding in the first trimester and premature rupture of the membranes (36). In these cases, the fetus is undersupplied with blood and nutrients, which induces growth restriction. Furthermore, pregnancies with placenta previa are also accompanied by an increased risk for IUGR (37).

Concerning maternal factors leading to impaired fetal growth, inadequate control of maternal diabetes mellitus, as mentioned before, is also a risk factor for fetal growth restriction, as also witnessed by murine studies (38). This is caused by the vasculopathy existing in diabetes, as also observed in humans (39, 40). However, too tight glycemic control in pre-existing diabetes can also lead to growth retardation if the mother has prolonged hypoglycemia, and the fetus does not receive adequate glucose supply (41). On the other hand, a poor glycemic control of maternal diabetes can result to fetal macrosomia due to fetal insulin overproduction as a response to maternal hyperglycemia. The resulting glucose oversupply to the fetus during pregnancy may then be complicated by postnatal hypoglycemia, due to the interruption of the increased glucose supply from the maternal circulation after birth, while the insulin production of the offspring is still stimulated (42, 43). Furthermore, besides impairment of fetal growth, either in the sense of macrosomia or fetal growth restriction, preexisting diabetes mellitus of the pregnant woman correlates with an increased risk of congenital malformations, mainly congenital heart defects, neuronal, musculoskeletal and limb malformations (41, 44).

Another maternal cause of growth retardation is maternal hypertension (45, 46). Fetuses of women presenting gestational hypertension have an increased risk of IUGR as well as an increased risk of fetal morbidity and mortality (47). This can be explained by the vasculopathy and associated ischemia of the placenta, also leading to an oxygen undersupply of the fetus. In addition, gestational hypertension is also associated with a higher cardiovascular risk of the offspring (48).

Furthermore, the lack of micronutrients such as vitamins and minerals can lead to impaired fetal growth and/or congenital malformations (49). Therefore, maternal malnutrition but also

TABLE 1 Causes of intrauterine growth restriction.

Causes of Intrauterine Growth Restriction		
Maternal causes	Medical conditions	A. Arterial hypertension and Pre-eclampsia B. Poorly controlled Diabetes mellitus C. Hyperthyroidism D. Infections during pregnancy such as rubella, Toxoplasma etc.
	Lifestyle factors	A. Malnutrition B. Smoking C. Substance abuse
Placental causes	Inadequate placental blood supply	
	Placental abnormalities	A. Circumvallate placenta B. Placenta previa
	Chromosomal aberrations in the placental tissue	
Fetal causes	Genetic causes	A. Chromosomal aberrations B. Monogenic causes C. Imprinting disorders
	Congenital malformations	
	Metabolic causes	
	Twin/multiple pregnancies	
Varia		

restrictive diets in the pregnant woman, as is the case in vegan diets without supplementation of essential micronutrients and vitamins, such as vitamin B12 supplementation, constitute further causes of IUGR (45, 50, 51).

Moreover, impaired fetal growth can be the result of thyroid dysfunction, namely maternal hyperthyroidism (52–54). In studies, including that of Luewan et al., it has been shown that hyperthyroidism and thyrotoxicosis of the pregnant woman increases the risk of growth restriction and low birth weight of the fetus (55, 56). On the other hand, since thyroid hormones are vital for an appropriate fetal development, especially fetal brain development, in a state of thyroid hormone deficiency the fetus cannot develop properly. Hypothyroidism can therefore also lead to impaired growth and development of the fetus (57, 58). Furthermore, it has been observed that maternal hypothyroidism increases the risk of non-reversible intellectual disability of the offspring (31, 59–61).

Infections during pregnancy are also further causes of IUGR. There are numerous causative pathogens such as HIV, Zika virus, Rubellla virus, cytomegalovirus, Toxoplasma gondii, etc. (5).

Fetal and genetic causes

Growth retardation can also occur in the context of an underlying genetic disorder. The genetic causes can be classified into chromosomal aberrations (incl. aneuploidy and copy number variants etc.) as well as monogenic causes. Imprinting defects are also a known cause of growth retardation. The most common underlying abnormalities are chromosomal aneuploidies, such as trisomy 13, 18 and 21 (62). The probability of chromosomal aberration increases with the severity of fetal growth restriction (FGR) (63). Copy number variants can also be the cause of FGR (64). In a French multicenter study, a pathogenic or likely pathogenic copy number variant was detected in 7.5% of fetuses with isolated growth retardation diagnosed prenatally (65). Frequent copy number variants associated with FGR are the 22q11.2 duplication, the Xp22.3 deletion as well as the 7q11.23 deletion (66, 67). The 22q11.2 duplication, also called DiGeorge syndrome, is a disorder characterized by immunodeficiency, hypoparathyroidism, and congenital heart disease. Other features can be developmental delay, hypothyroidism, renal as well as skeletal abnormalities (68). Patients with a Xp22.3 deletion suffer from ichthyosis and can also have intellectual disability (69). The 7q11.23 deletion leads to the clinical phenotype of the Wiliams-Beuren syndrome. Williams-Beuren syndrome is characterized by a vascular stenosis, cardiac valve abnormalities, hypercalcemia, renal abnormalities, hypodontia and developmental delay (70). Monogenic disorders can also constitute causes of FGR. Examples of monogenic diseases associated with FGR are Cornelia de Lange syndrome, Smith Lemli Opitz syndrome, Bloom syndrome, 3M, Seckel syndrome (62). Furthermore, imprinting defects can be associated with FGR. Epigenetic modifications can lead to a silencing of certain alleles, so that only one allele, the paternal or maternal one, is expressed in a tissue-specific manner. This process is called genomic imprinting. Defects of this imprinting procedure can lead to imprinting disorders resulting in IUGR, as for example Silver-Russel syndrome (71). In most patients with Silver-Russel-syndrome there is a hypomethylation of the imprinting cluster region on the paternal chromosome 11p15.5, which leads to biallelic silencing of the IGF2-gene and a biallelic expression of the noncoding region H19. It can also be caused by maternal uniparental disomy of chromosome 7 (UPD 7) or other rarer molecular genetic causes. Silver-Russel syndrome is mainly characterized by FGR, postnatal growth restriction, body asymmetry and often developmental delay (72). Further imprinting disorders associated with IUGR are the Temple syndrome, the Kagami-Ogata syndrome, the Prader-Willi syndrome, the pseudohypoparathyroidism 1b and others (73).

In addition, a correlation between FGR and chromosomal aberrations in placental tissue has been described. A chromosomal aberration affecting only one cell line of the placenta, in which the fetus has a regular number of chromosomes, is called “confined placental mosaicism” (CPM). As presented in the review of Eggenhuizen et al., 71.7% of CPM cases resulted in FGR (74).

The consequences of being born IUGR

Short term consequences

There are both short- and long-term consequences of being born with IUGR concerning both somatic and mental health as highlighted below and listed in Table 2.

IUGR can be associated with complications during the neonatal period. Fetuses born with IUGR have an increased risk of morbidity and mortality. They are also at increased risk of developing hypoglycemia shortly after birth due to reduced glycogen and fat stores and limited ability of gluconeogenesis as well as fat oxidation (75). Moreover, they are prone to hypothermia due to lack of subcutaneous brown fat, disproportionate body mass and increased transdermal temperature loss (76).

Moreover, in the context of placental insufficiency, the fetuses often grow under chronic hypoxia. This leads to increased erythropoiesis resulting in high hematocrit values and subsequent hyperviscosity of the blood. This can result in acute neonatal adverse events such as necrotizing enterocolitis or thrombosis (5). Furthermore, neonates born IUGR are at increased risk for developing respiratory complications (77).

Besides the short-term complications of newborns born IUGR during the neonatal period, much more attention has been laid to the long-term consequences of inadequate fetal growth, in later life, as presented below.

TABLE 2 Consequences of intrauterine growth restriction.

Consequences of Intrauterine Growth Restriction		
Short term consequences	Increased risk of morbidity and mortality	
	Hypoglycemia	
	Hypothermia	
	Hyperviscosity	
	Respiratory complications	
Long term consequences	Metabolic derangements	Metabolic syndrome
		Cardiovascular disease
	Growth impairment	
	Endocrine disorders	Precocious adrenarche
		Polycystic ovarian syndrome
	Nephrological problems	Renal insufficiency
		Hypertension
	Cancer risk	Hepatoblastoma
		Retinoblastoma

Long term consequences

Adverse future outcomes concerning somatic health and disease

Metabolic derangements – cardiovascular risk

During the last decades numerous epidemiological studies have reported an association of being born IUGR with future non-communicable diseases in adolescence or adult life, namely an increased incidence of insulin resistance expressed as future obesity and metabolic syndrome with high risk of lipid abnormalities, endothelial dysfunction leading to arterial hypertension, fatty liver disease, glucose intolerance or even type 2 diabetes in adult life, all contributing to higher cardiovascular risk (75, 78, 79).

Furthermore, it has been reported that the increased risk of metabolic syndrome and cardiovascular disease exists not only during adulthood but also during childhood (78, 79). This phenomenon is accentuated in case of a rapid weight gain during infancy (80). The redistribution of weight gain in favor of abdominal fat mass accumulation takes place mainly between the 2nd and 4th year of life. At the age of 4 years, IUGR children show higher fat mass, insulin resistance and proinflammatory parameters (81). Furthermore, they present an increased risk of dyslipidemia and metabolic syndrome (5, 82). For example, Tenhola et al. studied a group of 55 children with low birth weight and 55 children with appropriate birth weight and found that children born with low birth weight were at increased risk of hypercholesterolemia. Predisposing factors were the female gender, poor catch up growth in height and early initiation of puberty (83).

Somatic growth- catch-up growth

Infants born with IUGR usually show a fast growth in the first years of life, called catch-up growth. This is accelerated in the first months of life and continues with a modest acceleration till the 7th year, although most IUGR born children are expected to present catch-up growth and enter the normal trajectories of weight and height until the age of 3-4 years (5, 6, 41). A study by de Ridder et al. showed that 91% of children born SGA reached a normal height during the catch-up growth phase (84). Children that cannot reach the normal height trajectory after their 4th anniversary are less probable to enter the normal height trajectories later. Moreover, studies in infants of diabetic mothers with nephropathy born with IUGR showed that in childhood they had lower height and weight even after the catch up-period (85).

Reproductive axis

Low birth weight is associated with insulin resistance and decreased IGF1 levels reminiscent of a state of multi-hormonal resistance (86). This hormonal constellation correlates with increased LH levels and reduced SHBG levels in prepubertal girls pointing to an underlying hormonal setting of PCOS-like phenotype. This may result to precocious adrenarche and increased androgen availability. The increased androgen levels in girls born IUGR predispose thus to the development of polycystic ovary syndrome (87), while no association has been described between low birth weight and disorders of adrenarche or puberty in male subjects (88). This association of former SGA with reproductive axis disturbance and functional hyperandrogenism in girls has been extensively studied by Lourdes Ibanez and Francis de Zegher in previous years (81, 89, 90). However, concerning pubertal initiation, most children born SGA, enter puberty slightly earlier but still in the normal range compared to children born with normal weight for gestational age, so called Adequate for Gestational Age (91).

Nephrological problems

A further complication of IUGR is kidney disease. According to the review of Ritz et al. IUGR can lead to a reduced number of

nephrons. A low number of nephrons predisposes to glomerular hypertrophy as well as tubular dysfunction, increasing the risk of developing hypertension in later life (92). This is also supported by the Brenner's hypothesis, according to which a low nephron number is associated with hypertension (93). Furthermore, IUGR is associated with an increased risk of impaired renal function and later development of end-stage renal disease (94–96).

Cancer risk

IUGR also appears to be a risk factor for the development of pediatric tumors. According to Spector et al. low birth weight strongly correlates with the risk of developing hepatoblastoma (97). Furthermore, low birth weight is associated with gliomas, with an odds ratio of 2.13 (95% CI: 0.71-6.39 for birth weight <1500g) as well as retinoblastomas with an odds ratio of 2.43 (95% CI: 1.00-5.89 for birth weight <1500g) (98). However, O'Neill et al. postulated that there is no correlation between birth weight and the risk of developing retinoblastoma (99). In addition, it is worth mentioning that high birth weight is also associated with higher tumor risk, namely with an increased risk of leukemia (99).

The etiological pathway highlighting the link between IUGR and impaired somatic future outcomes

The initial observations that intrauterine growth restriction can adversely influence health in adult life came from Hertfordshire in the UK, where former IUGR-born babies have been reported to have a significantly increased risk to develop Metabolic syndrome in later life in comparison to those born adequate for gestational age or even large for gestational age (LGA). These initial observations have been reported by David Barker in the late 80ies and early 90ies, who supported the notion of 'fetal origin of adult disease" and explained the reported association through the "Thrifty phenotype hypothesis" (100, 101).

The thrifty phenotype hypothesis

According to the thrifty phenotype hypothesis of Hales and Barker, the fetus in a nutrient-restricted environment redistributes energy for survival mainly directing nutrients supply to the vital organs such as the brain, which leads to an undersupply of other organs such as the pancreas. This brain-sparing effect prioritizes the energy supply to the brain and ensures fetal survival. However, it inevitably leads to a disturbance of insulin homeostasis by provoking insulin resistance of the peripheral organs such as the liver and the muscles. This insulin resistant state predisposes the individual to the development of metabolic syndrome and all its parameters such as dyslipidemia, fatty liver, arterial hypertension and type 2 diabetes mellitus later in life (102).

These initial observations from Hertfordshire, UK, have also been confirmed from data originated from the Dutch famine, where it became clear that children, whose mothers have been undernourished during pregnancy due to the famine developed arterial hypertension in later life, data also confirmed in other parts of the world (103).

The notion of developmental mismatches

In other words, in case of impaired intrauterine milieu, for example in the context of protein-restricted maternal nutrition during pregnancy, exaggerated stress of the pregnant woman, inadequate placental nutrient supply or blood underperfusion, the endocrine-metabolic modifications that took place during the important window of developmental plasticity ascertain offspring's survival on the short term, a notion that is known as Immediate adaptive response (IAR) (104). This also persist into the extrauterine life and confer an increased cardiometabolic risk to the offspring, especially when the restricted intrauterine milieu does not match to the nutrient-abundant extrauterine milieu, as Gluckman supported in his notion of the match-mismatch principle (105, 106) or otherwise reported as predictive adaptive response (PAR) (107).

The predictive-adaptive response

According to the notion of predictive adaptive response (PAR) as formulated by Gluckman et al, if the fetus has been exposed to an adverse intrauterine environment, that has induced an immediate adaptive response for survival, during the window of developmental plasticity, this adaptation has prepared the fetus to face the extrauterine environment through a prediction of an also nutrient restricted extrauterine environment. However, if the intrauterine nutrient-restricted environment does not really match to the extrauterine environment, since the nutrient-restricted intrauterine environment is followed by a nutrient- or calories abundant extrauterine environment, then this predictive adaptive response may have long-lasting consequences for future health and disease, supporting the notion of Developmental Origin of health and disease (DOHaD) (108, 109).

Developmental origin of health and disease - epigenetic modifications

According to the Developmental Origin of Health and Disease (DOHaD) concept, this increased future cardiometabolic risk of offspring born as IUGR is attributed to epigenetic modifications taking place during the crucial window of developmental plasticity in intrauterine life (109–111).

The probability of the occurrence of metabolic diseases during life can be therefore influenced by prenatal events. Adversaries in the prenatal environment can influence the metabolism of the fetus. These processes can be mainly caused by epigenetic modifications. Epigenetic modifications include

DNA methylation and histone modifications (111) that take place already prenatally. During early embryogenesis, the methylation patterns of the fetus are programmed and can be influenced by various factors, such as maternal nutrition (112). As Waterland et al. have shown in a murine model, maternal nutrition affects the phenotype of the fetus by modifying methylation pattern in the offspring (113). These methylation modifications induced by the maternal diet can thus increase the risk of developing metabolic diseases in the offspring (114), which may explain the increased incidence of metabolic syndrome and type 2 diabetes mellitus in IUGR born individuals. Moreover, factors such as maternal nutrition, hypoxia or other pathologies can alter the expression profile of amino acid transporters. This adaptive change in the expression of amino acid transporters in the trophoblast also regulates fetal growth (115, 116).

The transgenerational transfer of environmental clues

According to Gluckman et al., these epigenetic modifications that took place in the first generation that has been exposed to an adverse intrauterine environment may even be transferred and expressed in the offspring of the next generation, although this offspring has not been exposed to an adverse intrauterine milieu, suggesting the notion of transgenerational transfer of epigenetic modifications and highlighting the impact of avoiding intrauterine adversaries in one generation to ascertain a healthy outcome of future generations (104).

It is also worth mentioning that this transgenerational effect that has been reported for the female line of inheritance has also an impact from the paternal side affecting not only the immediate subsequent generation, but also the generation after that. Moreover, epigenetic modifications can take place not only during intrauterine life but also during adolescence. Therefore, not only the mother's nutrition and lifestyle are relevant for the health of the child, but also the grandparents' nutrition and lifestyle choices (117). In other words, the epigenetic changes apply not only to the maternal epigenome but also to the paternal (118). In this context it is important mentioning that the Avon Longitudinal Study of Parents and Children (ALSPAC), after appropriate adjustment, has demonstrated that early paternal smoking is associated with greater body mass index (BMI) at 9 years in sons, but not in daughters. Sex-specific effects have also been shown in the Overkalix data reporting that paternal grandfather's food supply was only linked to the mortality relative risk (RR) of grandsons, while paternal grandmother's food supply was only associated with the granddaughters' mortality RR. These transgenerational effects were observed with exposure during the slow growth period (concerning both grandparents) of fetal/infant life (grandmothers) but not during either grandparent's puberty. The authors concluded that sex-specific, male-line transgenerational responses exist in humans and have hypothesized that these transmissions are mediated by the sex chromosomes, X and Y. Such responses add an entirely new dimension to the study of gene-environment interactions in development and health and provide more data concerning the impact of healthy lifestyle choices through the lifespan for future generations (119–121).

Molecular mechanisms linking IUGR to insulin resistance and future obesity/disturbed appetite

As mentioned above, children born IUGR are prone to develop insulin resistance in later life (122). This was also demonstrated in a murine IUGR model (123). Long et al. proposed that this observation could be explained by an impaired LRP6-Wnt-signaling pathway, which regulates the expression of insulin -receptors, leading to insulin resistance (124). A further possible underlying mechanism for the insulin resistance in individuals born with IUGR is the upregulation of ACSL1 expression. ACSL1 is a gene which regulates lipid metabolism. The authors claim that the upregulation of ACSL1 could facilitate the catch-up growth. However, this could also lead to increased secretion of esterified fatty acids, which promote insulin resistance and dyslipidemia (125).

Animal studies investigating the link between IUGR and adipogenesis have demonstrated that the underlying mechanisms predisposing to future obesity include a dysregulation of appetite/satiety signals and abnormal adipogenesis (126). According to Ross and Desai (126) there is a developmental origin of adipogenesis and disturbed appetite signals in intra-uterine-restricted newborns. As observed in animal models of IUGR, maternal calorie restriction or ligation of the uterine artery led to increased adult adipogenesis, accentuated when the IUGR status has been followed by a rapid extrauterine catch-up growth. It has been demonstrated that gestational nutrient restriction led to a dysregulation of orexigenic neuronal circuits at the hypothalamic level. The predominant appetite regulatory site, the hypothalamic Arcuate nucleus (ARC) receive signals from peripheral circuits, such as the gastrointestinal tract, pancreas, and the adipocytes but also from central inputs such as the brain. The ARC contains the medial orexigenic neurons (NPY and Agouti-related peptide neurons) and the lateral anorexigenic neurons, the Pro-opiomelanocortin (POMC) and the Cocaine and amphetamine regulated transcripts (CART). During fetal development the hypothalamic neuronal stem cells (NSC) proliferate and ultimately differentiate into neurons. Among those, the ones destined to the ARC appetite center further differentiate to express either orexigenic or anorexigenic peptides. In their experimental animal setting the researchers have demonstrated that intrauterine growth restricted animals resulted in significantly increased food intake with resulting hyperphagia due to dysregulated satiety signals, as evidenced by reduced satiety signals to leptin or, on the contrary, increased responses to appetite stimulatory signals from ghrelin. Moreover, laboratory studies from the same research group

have demonstrated that IUGR male offspring have upregulated adipogenic signaling cascade evidenced by an increased expression of enzymes promoting adipocyte lipid storage and synthesis. IUGR adipocytes in culture retained this adipogenetic characteristics even when deprived from the hormonal milieu in which the IUGR offspring has been exposed *in utero* (126). It has therefore been postulated that the mechanisms that result in offspring obesity include the programming of the hypothalamic appetite pathway and adipogenic signals regulating lipogenesis. Processes include nutrient sensors, epigenetic modifications, and alterations in stem cell precursors of both appetite/satiety neurons and adipocytes which are modulated to potentiate offspring obesity.

Furthermore, in a more recent experimental study Gong et al. investigated the Bone marrow mesenchymal stem cells (BMSC) of the intrauterine growth-restricted rat offspring and demonstrated that they exhibited an enhanced adipogenic molecular profile at miRNA, mRNA and protein levels, with an overall up-regulated PPARγ (miR-30d, miR-103, PPARγ, C/EPBα, ADRP, LPL, SREBP1), but down-regulated Wnt (LRP5, LEF-1, β-catenin, ZNF521 and RUNX2) signaling profile (127).

Further experimental data point towards a sex-dimorphic impact of IUGR on future adipogenesis with male offspring exhibiting stronger adipogenic propensity than females, especially with advancing age, also highlighting both the sex dimorphism of such an effect but also the permissive effect of postnatal caloric intake on future obesity development (128).

The disturbance of the hypothalamic-pituitary axis could also predispose to an increased cardiovascular risk. Individuals born SGA show GH resistance, witnessed by increased GH and reduced IGF1 and IGFBP3 levels. Since reduced IGF1 levels are associated with increased cardiovascular risk, this could constitute a further underlying mechanism linking IUGR with increased future obesity and cardiovascular risk (129).

Adverse future outcomes concerning neurocognitive health and disease

According to the meta-analysis by Sacchi et al. (2), IUGR is also associated with cognitive impairment. Children born IUGR or SGA have lower cognitive scores than those born AGA. This is the case for both preterm and term babies (2). These cognitive abnormalities can also be verified by functional MRI (fMRI) studies, that have shown reduced para-hippocampal activity in SGA children compared to AGA children (130). Furthermore, children born preterm with IUGR show impaired fine and gross motor function and an increased risk of developing autistic traits in comparison to preterm children born AGA (131). Cognitive impairments are also described in children born with IUGR during their school years, namely presenting learning difficulties with reduced memory and concentration skills. The risk of developing cerebral palsy is also increased in children born IUGR (1). These observations could be explained by the reduced brain volume, observed in children born IUGR (132). In addition, thyroid dysfunction in IUGR children could also contribute to the cognitive impairment (5).

The etiological pathway highlighting the link between IUGR and impaired neurocognitive future outcomes

The notion of developmental plasticity

Children with low birth weight show neurocognitive abnormalities as described above. This could be explained by disturbed prenatal neuronal development. St. Pierre et al. were able to demonstrate in a murine IUGR model that IUGR is associated with impaired synaptic plasticity in the hippocampus (133). In addition, Brown et al. described that IUGR mice show a reduced number of neural stem cells in the hippocampus as well as a disturbed induction of neuronal differentiation. These processes could be caused by a downregulation of the Wnt pathway (134). Other parameters such as neuroinflammation, a disturbed blood-brain barrier and oxidative stress can also contribute to the pathogenesis of neuronal dysfunction (135).

Therefore, although future neurocognitive outcome is besides the scope of the current review article, these observations of impaired neurocognitive development in case of intrauterine restriction further highlight the importance of an optimal intrauterine milieu to ascertain all aspects of future health.

Treatment modalities

The role of breast feeding

Breastfeeding has beneficiary effects on the health of the child. Studies have shown that breastfed children showed a slower weight gain during the catch-up period and a reduced risk of obesity and hypertonia (17, 136, 137). According to the meta-analysis of Qiao et al. breast feeding is associated with decreased risk of childhood obesity. This positive effect also increases with increasing duration of breastfeeding (138). A positive effect of breastfeeding has also been described for children's cognitive performance (139). Belfort et al. studied 1224 3-years-old and 1037 7-years-old children and found that children with a longer breastfeeding duration showed higher language comprehension scores at age 3 and higher verbal and nonverbal IQ scores at age 7 (140). Furthermore, breastfeeding can have protective effects against necrotizing enterocolitis, an inflammatory disorder common in premature and IUGR neonates (141).

Appropriate nutrition during infancy, childhood, and adolescence

Optimal maternal nutrition not only during pregnancy but also before pregnancy is of paramount importance for adequate

nutrient supply to the fetus, as already presented in the previous sections of this review. During the last years it became moreover clear that the first 1000 days of life starting from conception until the end of the second year of life are critical for both future health and neurodevelopment. In other words, these 1000 days spanning the period of the 270 days of pregnancy plus the 365 days of the first year of life plus the next 365 days of the second year of life play a major role in future health outcome of the offspring (142). A lot of evidence has been accumulated for the importance of the 270 days of pregnancy in terms of healthy nutrition (micro- and macronutrient composition) and avoidance of noxious agents such as tobacco or alcohol abuse in the lifestyle pattern of the pregnant woman. Since special dietetic preferences have also been adopted by young women of reproductive age in modern societies, such as vegan or vegetarian diets that cannot cover micronutrients requirements of the pregnant woman, and several deficiencies emerge as a consequence of such diets like iron, folate of vitamin B12 deficiency, special attention should be laid to supplementation with micronutrients and vitamins, especially vitamin B12 in women following vegan diets (50, 143). Also, the change in lifestyle in modern societies with increasing indoor activities and decreasing sun exposure leads to higher rates of vitamin D deficiency among women. Supplementation of vitamin D3 during pregnancy leads to a decreased risk of preeclampsia and IUGR (144). Furthermore, since deficiency of maternal iron, calcium, magnesium, and selenium is associated with low birth weight/IUGR, the supplementation of these micronutrients in the pregnant woman can be beneficial for the proper development of the fetus (19).

Moreover, moving into the early extrauterine feeding environment, exclusive breastfeeding for the first 6 months of extrauterine life, as already mentioned, should be advocated to diminish the rates of future obesity and other negative health issues. After the first 6 months of extrauterine life, when exclusive breastfeeding can no more completely cover the nutritional needs of the infant, then complementary food should be introduced. It has been demonstrated that initiation of solid foods before the age of 4 months is associated with an increased risk for future obesity. Thus, promoting exclusive breastfeeding during the first 6 months of extrauterine life and avoiding the early introduction of solid foods before the age of 4 months during infancy are important components in the combat against obesity. The time until the completion of the second year of life is important for being exposed to new tasty, low fat, rich in fruits and vegetables nutrition, the so-called Mediterranean diet (145). Thus, promoting healthy food choices not only during infancy but also during childhood are main determinants of the strategy to prevent future obesity, as already reported by the importance of the first 1000 days for future metabolic health, especially when combined with physical activity and avoidance of sleep deprivation (142, 146, 147).

Nutrition also plays an important role during the rest of life, which can be partly explained by epigenetic modifications taking place also at later stages of life, mainly during adolescence. Therefore, epigenetic modifications are important during the lifespan both pre- and postnatally as well as during adolescence. According to Han et al. BMI and smoking during adolescence can influence the DNA methylation (148). Furthermore, studies have shown that exercise and weight loss can also change the methylation of certain genes and thus their expression (149–151). Besides nutrition, further environmental factors such as exposure to chemicals or metals can induce epigenetic modifications (152). Taken together, a balanced diet and a healthy lifestyle during adolescence are of paramount importance for preventing future non-communicable diseases of the current as well as the next generation (17).

Growth hormone treatment

Children born with SGA who do not show catch up growth up to the chronological age of 4 years are often treated with growth hormone (GH) therapy. The GH administration may start at 2 years of age according to the US Food and Drug Administration (FDA) and at 4 years of age according to the European Medicines Agency (EMA). Beginning the therapy at a young age has beneficial effects on the growth gain (153). For example, Al Shaikh et al. studied retrospectively the growth parameters of 26 patients with SGA. The patients received growth hormone replacement therapy at a dose of 0.025-0.05 mg/kg/day. After 3.5 years of therapy, they observed an increase in height of 1.46 SDS (154). Besides the improvement of growth parameters, GH administration has a favorable effect on metabolism as it reduces the risk of hypertension (155) and leads to a reduction in adipose tissue and lipids at the beginning of the treatment (156). At the end of therapy, individuals after GH treatment have similar amounts of adipose tissue and lower lipid levels compared to untreated ones (87, 157). SGA individuals who have received GH therapy exhibit bone-mineral density deficiencies shortly after cessation of therapy, but these normalize 5 years after cessation of therapy (158). Furthermore, GH treatment has positive effects on the kidney development since it leads to an increased renal length and volume. Moreover, after cessation of GH treatment the renal function has been shown to be comparable between SGA patients who received GH and healthy controls (159).

Metformin

Another therapeutic target is the dysfunctional metabolism in low-birth-weight children. As described above, IUGR children tend to be overweight and develop metabolic syndrome and type 2 diabetes mellitus. Besides healthy lifestyle habits and healthy nutrition there are not many therapeutic possibilities.

Therapeutically, metformin could be considered as a therapeutic option in children born with IUGR, although metformin is neither FDA- nor EMA approved for use in children. A randomized controlled trial by Diaz et al. showed that children treated with metformin had a lower weight and BMI than the control group. There was also an improvement in biochemical variables, with a reduction in glucose and triglyceride levels as well as fat mass (160). Furthermore, studies performed by Ibáñez et al. showed that treatment of low-birth-weight pubertal girls led to reduced fat gain, delayed pubertal development, and improved biochemical parameters (161–163).

Moreover, according to Garcia-Conteras et al., based on animal studies, maternal therapy with metformin could theoretically also be beneficial for fetal growth (164). In the study by Garcia-Conteras et al. IUGR pregnancies modelled by malnourished pigs were investigated. They were able to demonstrate that the weight of the internal organs and the brain was higher in the metformin-treated group than in the control group. Therefore, the authors claimed that metformin therapy of the pregnant woman could contribute to the prevention of IUGR (165). However, the results of such animal studies cannot safely be extrapolated to humans yet.

Preventive measures

In order to eliminate some of the risk factors for IUGR development, a healthy lifestyle of the woman before and during pregnancy is mandatory. Preeclampsia and gestational hypertension are associated with a higher BMI of the woman. Therefore, healthy diet and regular moderate exercise are recommended (166). According to Crovetto et al., in pregnancies with an increased risk of SGA, following a Mediterranean diet can lead to a risk reduction of SGA births (167). Furthermore, an adequate supply of micronutrients can also reduce the risk of growth restriction. If sufficient coverage is not provided by adequate food intake, such as is the case in restrictive diets, like vegan or vegetarian diets, then supplementation with iron, magnesium, folate, and iodine could be considered (166, 168). In the case of gestational hypertension which cannot be regulated by lifestyle modification, drug therapy is recommended. Methyldopa, calcium channel inhibitors and beta-blockers are allowed for pregnant women, while ACE inhibitors or AT1 antagonists are contraindicated during pregnancy (169). In addition, in pregnant women with increased risk of preeclampsia a low-dose aspirin treatment is indicated. A Doppler velocimetry screening of the uterine artery in the second trimester is also recommended in women with an increased risk of preeclampsia (170). As already reported, smoking, alcohol, and substance abuse are also associated with an intrauterine growth restriction (171–173). Therefore, education of young women of reproductive age to avoid such noxious agents is of paramount importance. However, if the young women used to adopt such unhealthy habits, then their consumption should be ceased long before pregnancy planning and conception.

Conclusion

There is accumulated evidence during the last decades pointing to the importance of an optimal intrauterine environment to provide the best chances for future health.

On the contrary, according to the developmental origin of health and disease, the susceptibility for future disease greatly depends on the developmental window, during which an adverse environmental cue, leading to intrauterine growth restriction, took place, making an individual more vulnerable to adverse future outcomes such as obesity, metabolic syndrome, hypertension, non-alcoholic fatty liver disease and type 2 diabetes mellitus. All these non-communicable diseases are interconnected ultimately leading to increased cardiovascular risk and mortality.

Moreover, it has been proven that intrauterine growth restriction may generally impair future health, increasing the risk for nephrological problems or even cancer risk. Furthermore, an adverse intrauterine environment leading to IUGR is further associated with future neurocognitive impairments of the offspring.

As a consequence, if the increased public health burden and costs of non-communicable chronic diseases but also mental and neurocognitive impairments have to be minimized, special attention should be laid to the healthy lifestyle habits of young people, especially women of reproductive age, including both avoidance of noxious agents, such as smoking or alcohol consumption, healthy diet and physical activity to be established long before any pregnancy is programmed or takes place and these healthy lifestyle patterns should be sustained through the whole duration of pregnancy in order to provide the best basis for both somatic and mental health of future generations. Furthermore, promotion of breastfeeding and healthy eating habits in infancy, childhood, and adolescence along with physical activity may further minimize the risk of future disease.

Author contributions

Both authors KVG and CK-G have a significant contribution to the manuscript, have read and approved the final version of the manuscript that has been submitted and affirm that no part of the present manuscript has been submitted elsewhere for publication. In detail, KVG has prepared the initial draft of the ms, has performed the extended literature search on the topic and provided suggestions on the presentation of the specific segments of the ms. CK-G has prepared the outline of the review

article and has edited the manuscript. Both authors contributed to the article and approved the submitted version.

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

References

1. Miller SL, Huppi PS, Mallard C. The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome. *J Physiol* (2016) 594(4):807–23. doi: 10.1113/JP271402

2. Sacchi C, Marino C, Nosarti C, Vieno A, Visentin S, Simonelli A. Association of intrauterine growth restriction and small for gestational age status with childhood cognitive outcomes: A systematic review and meta-analysis. *JAMA Pediatr* (2020) 174(8):772–81. doi: 10.1001/jamapediatrics.2020.1097

3. Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, et al. Consensus definition of fetal growth restriction: a Delphi procedure. *Ultrasound Obstet Gynecol* (2016) 48(3):333–9. doi: 10.1002/uog.15884

4. Beune IM, Bloomfield FH, Ganzevoort W, Embleton ND, Rozance PJ, van Wassenaer-Leemhuis AG, et al. Consensus based definition of growth restriction in the newborn. *J Pediatr* (2018) 196:71–76.e1. doi: 10.1016/j.jpeds.2017.12.059

5. Finken MJJ, van der Steen M, Smeets CCJ, Walenkamp MJE, de Bruin C, Hokken-Koelega ACS, et al. Children born small for gestational age: Differential diagnosis, molecular genetic evaluation, and implications. *Endocr Rev* (2018) 39 (6):851–94. doi: 10.1210/er.2018-00083

6. Bendix I, Miller SL, Winterhager E. Editorial: Causes and consequences of intrauterine growth restriction. *Front Endocrinol (Lausanne)* (2020) 11:205. doi: 10.3389/fendo.2020.00205

7. Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. *Am J Obstet Gynecol* (2018) 218(2s):S745–s761. doi: 10.1016/j.ajog.2017.11.577

8. Sun C, Groom KM, Oyston C, Chamley LW, Clark AR, James JL. The placenta in fetal growth restriction: What is going wrong? *Placenta* (2020) 96:10–8. doi: 10.1016/j.placenta.2020.05.003

9. Pollheimer J, Vondra S, Baltayeva J, Beristain AG, Knöfler M. Regulation of placental extravillous trophoblasts by the maternal uterine environment. *Front Immunol* (2018) 9:2597. doi: 10.3389/fimmu.2018.02597

10. Cetin I, Taricco E, Mandò C, Radaelli T, Boito S, Nuzzo AM, et al. Fetal oxygen and glucose consumption in human pregnancy complicated by fetal growth restriction. *Hypertension* (2020) 75(3):748–54. doi: 10.1161/HYPERTENSIONAHA.119.13727

11. Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. *Reproduction* (2009) 138(6):895–902. doi: 10.1530/REP-09-0092

12. Desoye G. The human placenta in diabetes and obesity: Friend or foe? *2017 Norbert Freinkel Award Lecture. Diabetes Care* (2018) 41(7):1362–9. doi: 10.2337/dci17-0045

13. Herzog EM, Eggink AJ, Reijnierse A, Kerkhof MA, de Krijger RR, Roks AJ, et al. Impact of early- and late-onset preeclampsia on features of placental and newborn vascular health. *Placenta* (2017) 49:72–9. doi: 10.1016/j.placenta.2016.11.014

14. Sastry BV. Placental toxicology: tobacco smoke, abused drugs, multiple chemical interactions, and placental function. *Reprod Fertil Dev* (1991) 3(4):355–72. doi: 10.1071/rd9910355

15. Hay WWJr. Placental-fetal glucose exchange and fetal glucose metabolism. *Trans Am Clin Climatol Assoc* (2006) 117:321–39.

16. ter Braak EW, Evers IM, Willem Erkelens D, Visser GH. Maternal hypoglycemia during pregnancy in type 1 diabetes: Maternal and fetal consequences. *Diabetes Metab Res Rev* (2002) 18(2):96–105. doi: 10.1002/dmrr.271

17. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet* (2013) 382(9890):427–51. doi: 10.1016/S0140-6736(13)60937-X

18. Marangoni F, Cetin I, Verduci E, Canzone G, Giovannini M, Scollo P, et al. Maternal diet and nutrient requirements in pregnancy and breastfeeding. an Italian consensus document. *Nutrients* (2016) 8(10):1–17. doi: 10.3390/nu8100629

19. Hovdenak N, Haram K. Influence of mineral and vitamin supplements on pregnancy outcome. *Eur J Obstet Gynecol Reprod Biol* (2012) 164(2):127–32. doi: 10.1016/j.ejogrb.2012.06.020

20. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr Rev* (2006) 27 (2):141–69. doi: 10.1210/er.2005-0011

21. Gonçalves BM, Graceli JB, da Rocha PB, Tilli HP, Vieira EM, de Sibio MT, et al. Placental model as an important tool to study maternal-fetal interface. *Reprod Toxicol* (2022) 112:7–13. doi: 10.1016/j.reprotox.2022.06.005

22. Alekseenkova EN, Selkov SA, Kapustin RV. Fetal growth regulation *via* insulin-like growth factor axis in normal and diabetic pregnancy. *J Perinat Med* (2022) 50(7):947–60. doi: 10.1515/jpm-2021-0510

23. Ladyman SR, Brooks VL. Central actions of insulin during pregnancy and lactation. *J Neuroendocrinol* (2021) 33(4):e12946. doi: 10.1111/jne.12946

24. Song X, Chen L, Zhang S, Liu Y, Wei J, Wang T, et al. Gestational diabetes mellitus and high triglyceride levels mediate the association between pre-pregnancy Overweight/Obesity and macrosomia: A prospective cohort study in central China. *Nutrients* (2022) 14(16):1–13. doi: 10.3390/nu14163347

25. Martín-Estal I, Castorena-Torres F. Gestational diabetes mellitus and energy-dense diet: What is the role of the Insulin/IGF axis? *Front Endocrinol (Lausanne)* (2022) 13:916042. doi: 10.3389/fendo.2022.916042

26. Bang P. Pediatric implications of normal insulin-GH-IGF-Axis physiology. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al, editors. *Endotext*. South Dartmouth (MA: MDText.com) (2000).

27. Ben Harouch S, Klar A, Falik Zaccai TC. INSR-related severe syndromic insulin resistance. In: Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, et al, editors. *GeneReviews®*. Seattle (WA: University of Washington, Seattle (1993).

28. Fowden AL, Forhead AJ. Endocrine regulation of feto-placental growth. *Horm Res* (2009) 72(5):257–65. doi: 10.1159/000245927

29. Liao S, Vickers MH, Stanley JL, Baker PN, Perry JK. Human placental growth hormone variant in pathological pregnancies. *Endocrinology* (2018) 159 (5):2186–98. doi: 10.1210/en.2018-00037

30. Carvalho DP, Dias AF, Sferruzzi-Perri AN, Ortiga-Carvalho TM. Gaps in the knowledge of thyroid hormones and placental biology†. *Biol Reprod* (2022) 106 (6):1033–48. doi: 10.1093/biolre/ioac006

31. Springer D, Jiskra J, Limanova Z, Zima T, Potlukova E. Thyroid in pregnancy: From physiology to screening. *Crit Rev Clin Lab Sci* (2017) 54 (2):102–16. doi: 10.1080/10408363.2016.1269309

32. English FA, Kenny LC, McCarthy FP. Risk factors and effective management of preeclampsia. *Integr Blood Press Control* (2015) 8:7–12. doi: 10.2147/IBPC.S50641

33. Phipps E, Prasanna D, Brima W, Jim B. Preeclampsia: Updates in pathogenesis, definitions, and guidelines. *Clin J Am Soc Nephrol* (2016) 11 (6):1102–13. doi: 10.2215/CJN.12081115

34. Bokslag A, van Weissenbruch M, Mol BW, de Groot CJ. Preeclampsia; short and long-term consequences for mother and neonate. *Early Hum Dev* (2016) 102:47–50. doi: 10.1016/j.earlhumdev.2016.09.007

35. Celik OY, Obut M, Keles A, Calık MG, Dagdeviren G, Yucel A, et al. Outcomes of pregnancies diagnosed with circumvallate placenta, and use of uterine artery pulsatility index and maternal serum alpha-fetoprotein for prediction of adverse outcomes. *Eur J Obstet Gynecol Reprod Biol* (2021) 262:57–61. doi: 10.1016/j.ejogrb.2021.05.008

36. Taniguchi H, Aoki S, Sakamaki K, Kurasawa K, Okuda M, Takahashi T, et al. Circumvallate placenta: Associated clinical manifestations and complications-

a retrospective study. *Obstet Gynecol Int.* (2014) 2014:986230. doi: 10.1155/2014/986230

37. Balayla J, Desilets J, Shrem G. Placenta previa and the risk of intrauterine growth restriction (IUGR): A systematic review and meta-analysis. *J Perinat Med* (2019) 47(6):577–84. doi: 10.1515/jpm-2019-0116

38. Golic M, Stojanovska V, Bendix I, Wehner A, Herse F, Haase N, et al. Diabetes mellitus in pregnancy leads to growth restriction and epigenetic modification of the Srebf2 gene in rat fetuses. *Hypertension* (2018) 71(5):911–20. doi: 10.1161/HYPERTENSIONAHA.117.10782

39. Langmia IM, Kräker K, Weiss SE, Haase N, Schütte T, Herse F, et al. Cardiovascular programming during and after diabetic pregnancy: Role of placental dysfunction and IUGR. *Front Endocrinol (Lausanne)* (2019) 10:215. doi: 10.3389/fendo.2019.00215

40. Huynh J, Yamada J, Beauharnais C, Wenger JB, Thadhani RI, Wexler D, et al. Type 1, type 2 and gestational diabetes mellitus differentially impact placental pathologic characteristics of uteroplacental malperfusion. *Placenta* (2015) 36 (10):1161–6. doi: 10.1016/j.placenta.2015.08.004

41. Ornoy A, Becker M, Weinstein-Fudim L, Ergaz Z. Diabetes during pregnancy: A maternal disease complicating the course of pregnancy with long-term deleterious effects on the offspring. a clinical review. *Int J Mol Sci* (2021) 22 (6):2965. doi: 10.3390/ijms22062965

42. Kc K, Shakya S, Zhang H. Gestational diabetes mellitus and macrosomia: A literature review. *Ann Nutr Metab* (2015) 66(Suppl 2):14–20. doi: 10.1159/000371628

43. Persson M, Shah PS, Rusconi F, Reichman B, Modi N, Kusuda S, et al. Association of maternal diabetes with neonatal outcomes of very preterm and very low-Birth-Weight infants: An international cohort study. *JAMA Pediatr* (2018) 172 (9):867–75. doi: 10.1001/jamapediatrics.2018.1811

44. Macintosh MC, Fleming KM, Bailey JA, Doyle P, Modder J, Acolet D, et al. Perinatal mortality and congenital anomalies in babies of women with type 1 or type 2 diabetes in England, Wales, and northern Ireland: Population based study. *Bmj* (2006) 333(7560):177. doi: 10.1136/bmj.38856.692986.AE

45. Muhammad T, Khattak AA, Shafiq ur R, Khan MA, Khan A, Khan MA. Maternal factors associated with intrauterine growth restriction. *J Ayub Med Coll Abbottabad* (2010) 22(4):64–9.

46. Mohammad N, Sohaila A, Rabbani U, Ahmed S, Ahmed S, Ali SR. Maternal predictors of intrauterine growth retardation. *J Coll Physicians Surg Pak* (2018) 28 (9):681–5. doi: 10.29271/jcpsp.2018.09.681

47. Villar J, Carroli G, Wojdyla D, Abalos E, Giordano D, Ba'aqeel H, et al. Preeclampsia, gestational hypertension and intrauterine growth restriction, related or independent conditions? *Am J Obstet Gynecol* (2006) 194(4):921–31. doi: 10.1016/j.ajog.2005.10.813

48. Davis EF, Lewandowski AJ, Aye C, Williamson W, Boardman H, Huang RC, et al. Clinical cardiovascular risk during young adulthood in offspring of hypertensive pregnancies: Insights from a 20-year prospective follow-up birth cohort. *BMJ Open* (2015) 5(6):e008136. doi: 10.1136/bmjopen-2015-008136

49. Santander Ballestín S, Giménez Campos MI, Ballestín Ballestín J, Luesma Bartolomé MJ. Is supplementation with micronutrients still necessary during pregnancy? A review. *Nutrients* (2021) 13(9):3134, 1–30. doi: 10.3390/nu13093134

50. Benham AJ, Gallegos D, Hanna KL, Hannan-Jones MT. Intake of vitamin B (12) and other characteristics of women of reproductive age on a vegan diet in Australia. *Public Health Nutr* (2021) 24(14):4397–407. doi: 10.1017/S1368980021001695

51. Reischl-Hajiabadi AT, Garbade SF, Feyh P, Weiss KH, Mütze U, Kölker S, et al. Maternal vitamin B(12) deficiency detected by newborn screening-evaluation of causes and characteristics. *Nutrients* (2022) 14(18):3767, 1–17. doi: 10.3390/nu14183767

52. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, et al. Guidelines of the American thyroid association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid* (2017) 27 (3):315–89. doi: 10.1089/thy.2016.0457

53. Alves Junior JM, Bernardo WM, Ward LS, Villagelin D. Effect of hyperthyroidism control during pregnancy on maternal and fetal outcome: A systematic review and meta-analysis. *Front Endocrinol (Lausanne)* (2022) 13:800257. doi: 10.3389/fendo.2022.800257

54. Andersen SL, Andersen S. Hyperthyroidism in pregnancy: evidence and hypothesis in fetal programming and development. *Endocr Connect* (2021) 10(2): R77–r86. doi: 10.1530/EC-20-0518

55. Luewan S, Chakkabut P, Tongsong T. Outcomes of pregnancy complicated with hyperthyroidism: a cohort study. *Arch Gynecol Obstet* (2011) 283(2):243–7. doi: 10.1007/s00404-010-1362-z

56. Harn AMP, Dejkhamron P, Tongsong T, Luewan S. Pregnancy outcomes among women with graves' hyperthyroidism: A retrospective cohort study. *J Clin Med* (2021) 10(19):4495, 1–8. doi: 10.3390/jcm10194495

57. Idris I, Srinivasan R, Simm A, Page RC. Maternal hypothyroidism in early and late gestation: Effects on neonatal and obstetric outcome. *Clin Endocrinol (Oxf)* (2005) 63(5):560–5. doi: 10.1111/j.1365-2265.2005.02382.x

58. Derakhshan A, Peeters RP, Taylor PN, Bliddal S, Carty DM, Meems M, et al. Association of maternal thyroid function with birthweight: a systematic review and individual-participant data meta-analysis. *Lancet Diabetes Endocrinol* (2020) 8 (6):501–10. doi: 10.1016/S2213-8587(20)30061-9

59. Henrichs J, Bongers-Schokking JJ, Schenk JJ, Ghassabian A, Schmidt HG, Visser TJ, et al. Maternal thyroid function during early pregnancy and cognitive functioning in early childhood: The generation r study. *J Clin Endocrinol Metab* (2010) 95(9):4227–34. doi: 10.1210/jc.2010-0415

60. Li Y, Shan Z, Teng W, Yu X, Li Y, Fan C, et al. Abnormalities of maternal thyroid function during pregnancy affect neuropsychological development of their children at 25-30 months. *Clin Endocrinol (Oxf)* (2010) 72(6):825–9. doi: 10.1111/j.1365-2265.2009.03743.x

61. Huget-Penner S, Feig DS. Maternal thyroid disease and its effects on the fetus and perinatal outcomes. *Prenat Diagn* (2020) 40(9):1077–84. doi: 10.1002/pd.5684

62. Meler E, Sisterna S, Borrell A. Genetic syndromes associated with isolated fetal growth restriction. *Prenat Diagn* (2020) 40(4):432–46. doi: 10.1002/pd.5635

63. Peng R, Yang J, Xie HN, Lin MF, Zheng J. Chromosomal and subchromosomal anomalies associated to small for gestational age fetuses with no additional structural anomalies. *Prenat Diagn* (2017) 37(12):1219–24. doi: 10.1002/pd.5169

64. Shaffer LG, Rosenfeld JA, Dabell MP, Coppinger J, Bandholz AM, Ellison JW, et al. Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound. *Prenat Diagn* (2012) 32 (10):986–95. doi: 10.1002/pd.3943

65. Monier I, Receveur A, Houfflin-Debarge V, Goua V, Castaigne V, Jouannic JM, et al. Should prenatal chromosomal microarray analysis be offered for isolated fetal growth restriction? A French multicenter study. *Am J Obstet Gynecol* (2021) 225(6):676.e1–676.e15. doi: 10.1016/j.ajog.2021.05.035

66. Borrell A, Grande M, Pauta M, Rodriguez-Revenga L, Figueras F. Chromosomal microarray analysis in fetuses with growth restriction and normal karyotype: A systematic review and meta-analysis. *Fetal Diagn Ther* (2018) 44 (1):1–9. doi: 10.1159/000479506

67. An G, Lin Y, Xu LP, Huang HL, Liu SP, Yu YH, et al. Application of chromosomal microarray to investigate genetic causes of isolated fetal growth restriction. *Mol Cytogenet* (2018) 11:33. doi: 10.1186/s13039-018-0382-4

68. McDonald-McGinn DM, Sullivan KE, Marino B, Philip N, Swillen A, Vorstman JA, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Primers* (2015) 1:15071. doi: 10.1038/nrdp.2015.71

69. Ben Khelifa H, Soyah N, Ben-Abdallah-Bouhjar I, Gritly R, Sanlaville D, Elghezal H, et al. Xp22.3 interstitial deletion: A recognizable chromosomal abnormality encompassing VCX3A and STS genes in a patient with X-linked ichthyosis and mental retardation. *Gene* (2013) 527(2):578–83. doi: 10.1016/j.gene.2013.06.018

70. Pober BR. Williams-beuren syndrome. *N Engl J Med* (2010) 362(3):239–52. doi: 10.1056/NEJMra0903074

71. Elhamamsy AR. Role of DNA methylation in imprinting disorders: an updated review. *J Assist Reprod Genet* (2017) 34(5):549–62. doi: 10.1007/s10815-017-0895-5

72. Saal HM, Harbison MD, Netchine I. Silver-Russell syndrome. In: Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, et al, editors. *GeneReviews*(®). Seattle (WA: University of Washington, Seattle (1993).

73. Eggermann T, Davies JH, Tauber M, van den Akker E, Hokken-Koelega A, Johansson G, et al. Growth restriction and genomic imprinting-overlapping phenotypes support the concept of an imprinting network. *Genes (Basel)* (2021) 12(4):585, 1–16. doi: 10.3390/genes12040585

74. Eggenhuizen GM, Go A, Koster MPH, Baart EB, Galjaard RJ. Confined placental mosaicism and the association with pregnancy outcome and fetal growth: a review of the literature. *Hum Reprod Update* (2021) 27(5):885–903. doi: 10.1093/humupd/dmab009

75. Longo S, Bollani L, Decembrino L, Di Comite A, Angelini M, Stronati M. Short-term and long-term sequelae in intrauterine growth retardation (IUGR). *J Matern Fetal Neonatal Med* (2013) 26(3):222–5. doi: 10.3109/14767058.2012.715006

76. Rosenberg A. The IUGR newborn. *Semin Perinatol* (2008) 32(3):219–24. doi: 10.1053/j.semperi.2007.11.003

77. Bernstein IM, Horbar JD, Badger GJ, Ohlsson A, Golan A. Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction. The Vermont Oxford network. *Am J Obstet Gynecol* (2000) 182(1 Pt 1):198–206. doi: 10.1016/s0002-9378(00)70513-8

78. Hennington BS, Alexander BT. Linking intrauterine growth restriction and blood pressure: Insight into the human origins of cardiovascular disease. *Circulation* (2013) 128(20):2179–80. doi: 10.1161/CIRCULATIONAHA.113.006323

79. Eriksson J, Forsén T, Tuomilehto J, Osmond C, Barker D. Fetal and childhood growth and hypertension in adult life. *Hypertension* (2000) 36(5):790–4. doi: 10.1161/01.hyp.36.5.790

80. Stettler N, Stallings VA, Troxel AB, Zhao J, Schinnar R, Nelson SE, et al. Weight gain in the first week of life and overweight in adulthood: A cohort study of European American subjects fed infant formula. *Circulation* (2005) 111(15):1897–903. doi: 10.1161/01.CIR.0000161797.67671.A7

81. Ibáñez L, Ong K, Dunger DB, de Zegher F. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. *J Clin Endocrinol Metab* (2006) 91(6):2153–8. doi: 10.1210/jc.2005-2778

82. Ornoy A. Prenatal origin of obesity and their complications: Gestational diabetes, maternal overweight and the paradoxical effects of fetal growth restriction and macrosomia. *Reprod Toxicol* (2011) 32(2):205–12. doi: 10.1016/j.reprotox.2011.05.002

83. Tenhola S, Martikainen A, Rahiala E, Herrgård E, Halonen P, Voutilainen R. Serum lipid concentrations and growth characteristics in 12-year-old children born small for gestational age. *Pediatr Res* (2000) 48(5):623–8. doi: 10.1203/00006450-200011000-00012

84. de Ridder MA, Engels MA, Stijnen T, Hokken-Koelega AC. Small for gestational age children without early catch-up growth: spontaneous growth and prediction of height at 8 years. *Horm Res* (2008) 70(4):203–8. doi: 10.1159/000137660

85. Biesenbach G, Grafinger P, Zazgornik J, Helmut, Stöger. Perinatal complications and three-year follow up of infants of diabetic mothers with diabetic nephropathy stage IV. *Ren Fail* (2000) 22(5):573–80. doi: 10.1081/jdi-100100898

86. Nawathe AR, Christian M, Kim SH, Johnson M, Savvidou MD, Terzidou V. Insulin-like growth factor axis in pregnancies affected by fetal growth disorders. *Clin Epigenet* (2016) 8:11. doi: 10.1186/s13148-016-0178-5

87. Mericq V, Martinez-Aguayo A, Uauy R, Iñiguez G, van der Steen M, Hokken-Koelega A. Long-term metabolic risk among children born premature or small for gestational age. *Nat Rev Endocrinol* (2017) 13(1):50–62. doi: 10.1038/nrendo.2016.127

88. Jensen RB, Vielwerth S, Larsen T, Greisen G, Veldhuis J, Juul A. Pituitary-gonadal function in adolescent males born appropriate or small for gestational age with or without intrauterine growth restriction. *J Clin Endocrinol Metab* (2007) 92(4):1353–7. doi: 10.1210/jc.2006-2348

89. de Zegher F, Reinehr T, Malpique R, Darendeliler F, López-Bermejo A, Ibáñez L. Reduced prenatal weight gain and/or augmented postnatal weight gain precedes polycystic ovary syndrome in adolescent girls. *Obes (Silver Spring)* (2017) 25(9):1486–9. doi: 10.1002/oby.21935

90. Ibáñez L, de Zegher F. Puberty and prenatal growth. *Mol Cell Endocrinol* (2006) 254-255:22–5. doi: 10.1016/j.mce.2006.04.010

91. Verkauskiene R, Petraitiene I, Albertsson Wikland K. Puberty in children born small for gestational age. *Horm Res Paediatr* (2013) 80(2):69–77. doi: 10.1159/000353759

92. Ritz E, Amann K, Koleganova N, Benz K. Prenatal programming-effects on blood pressure and renal function. *Nat Rev Nephrol* (2011) 7(3):137–44. doi: 10.1038/nrneph.2011.1

93. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. less of one, more the other? *Am J Hypertens* (1988) 1(4 Pt 1):335–47. doi: 10.1093/ajh/1.4.335

94. Hallan S, Euser AM, Irgens LM, Finken MJ, Holmen J, Dekker FW. Effect of intrauterine growth restriction on kidney function at young adult age: the nord trøndelag health (HUNT 2) study. *Am J Kidney Dis* (2008) 51(1):10–20. doi: 10.1053/j.ajkd.2007.09.013

95. Vikse BE, Irgens LM, Leivestad T, Hallan S, Iversen BM. Low birth weight increases risk for end-stage renal disease. *J Am Soc Nephrol* (2008) 19(1):151–7. doi: 10.1681/ASN.2007020252

96. Doan TNA, Akison LK, Bianco-Miotto T. Epigenetic mechanisms responsible for the transgenerational inheritance of intrauterine growth restriction phenotypes. *Front Endocrinol (Lausanne)* (2022) 13:838737. doi: 10.3389/fendo.2022.838737

97. Spector LG, Johnson KJ, Soler JT, Puumala SE. Perinatal risk factors for hepatoblastoma. *Br J Cancer* (2008) 98(9):1570–3. doi: 10.1038/sj.bjc.6604335

98. Spector LG, Puumala SE, Carozza SE, Chow EJ, Fox EE, Horel S, et al. Cancer risk among children with very low birth weights. *Pediatrics* (2009) 124(1):96–104. doi: 10.1542/peds.2008-3069

99. O'Neill KA, Murphy MF, Bunch KJ, Puumala SE, Carozza SE, Chow EJ, et al. Infant birthweight and risk of childhood cancer: international population-based case control studies of 40 000 cases. *Int J Epidemiol* (2015) 44(1):153–68. doi: 10.1093/ije/dyu265

100. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* (1986) 1(8489):1077–81. doi: 10.1016/s0140-6736(86)91340-1

101. Barker DJ. The fetal and infant origins of adult disease. *Bmj* (1990) 301(6761):1111. doi: 10.1136/bmj.301.6761.1111

102. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* (2001) 60:5–20. doi: 10.1093/bmb/60.1.5

103. Painter RC, Roseboom TJ, Bleker OP. Prenatal exposure to the Dutch famine and disease in later life: An overview. *Reprod Toxicol* (2005) 20(3):345–52. doi: 10.1016/j.reprotox.2005.04.005

104. Gluckman PD, Hanson MA, Low FM. Evolutionary and developmental mismatches are consequences of adaptive developmental plasticity in humans and have implications for later disease risk. *Philos Trans R Soc Lond B Biol Sci* (2019) 374(1770):20180109. doi: 10.1098/rstb.2018.0109

105. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of *in utero* and early-life conditions on adult health and disease. *N Engl J Med* (2008) 359(1):61–73. doi: 10.1056/NEJMra0708473

106. Ong YY, Sadananthan SA, Aris IM, Tint MT, Yuan WL, Huang JY, et al. Mismatch between poor fetal growth and rapid postnatal weight gain in the first 2 years of life is associated with higher blood pressure and insulin resistance without increased adiposity in childhood: The GUSTO cohort study. *Int J Epidemiol* (2020) 49(5):1591–603. doi: 10.1093/ije/dyaa143

107. Spencer HG, Pleasants AB, Gluckman PD, Wake GC. A model of optimal timing for a predictive adaptive response. *J Dev Orig Health Dis* (2022) 13(1):101–7. doi: 10.1017/S2040174420001361

108. Wadhwa PD, Buss C, Entringer S, Swanson JM. Developmental origins of health and disease: Brief history of the approach and current focus on epigenetic mechanisms. *Semin Reprod Med* (2009) 27(5):358–68. doi: 10.1055/s-0029-1237424

109. Goyal D, Limesand SW, Goyal R. Epigenetic responses and the developmental origins of health and disease. *J Endocrinol* (2019) 242(1):T105–t119. doi: 10.1530/JOE-19-0009

110. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: Physiology or pathophysiology? *Physiol Rev* (2014) 94(4):1027–76. doi: 10.1152/physrev.00029.2013

111. Gluckman PD, Hanson MA, Buklijas T, Low FM, Beedle AS. Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nat Rev Endocrinol* (2009) 5(7):401–8. doi: 10.1038/nrendo.2009.102

112. Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* (2007) 447(7143):425–32. doi: 10.1038/nature05918

113. Waterland RA, Jirtle RL. Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition* (2004) 20(1):63–8. doi: 10.1016/j.nut.2003.09.011

114. Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *Br J Nutr* (2007) 97(6):1064–73. doi: 10.1017/S000711450769196X

115. Edwards AK, McKnight SM, Askelson K, McKnight JR, Dunlap KA, Satterfield MC. Adaptive responses to maternal nutrient restriction alter placental transport in ewes. *Placenta* (2020) 96:1–9. doi: 10.1016/j.placenta.2020.05.002

116. Myatt L. Placental adaptive responses and fetal programming. *J Physiol* (2006) 572(Pt 1):25–30. doi: 10.1113/jphysiol.2006.104968

117. Breton CV, Landon R, Kahn LG, Enlow MB, Peterson AK, Bastain T, et al. Exploring the evidence for epigenetic regulation of environmental influences on child health across generations. *Commun Biol* (2021) 4(1):769. doi: 10.1038/s42003-021-02316-6

118. Rando OJ, Simmons RA. I'm Eating for two: parental dietary effects on offspring metabolism. *Cell* (2015) 161(1):93–105. doi: 10.1016/j.cell.2015.02.021

119. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjöström M, et al. Sex-specific, male-line transgenerational responses in humans. *Eur J Hum Genet* (2006) 14(2):159–66. doi: 10.1038/sj.ejhg.5201538

120. Vågerö D, Pinger PR, Aronsson V, van den Berg GJ. Paternal grandfather's access to food predicts all-cause and cancer mortality in grandsons. *Nat Commun* (2018) 9(1):5124. doi: 10.1038/s41467-018-07617-9

121. Kaati G, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. *Eur J Hum Genet* (2002) 10(11):682–8. doi: 10.1038/sj.ejhg.5200859

122. Thorn SR, Rozance PJ, Brown LD, Hay WWJr. The intrauterine growth restriction phenotype: fetal adaptations and potential implications for later life insulin resistance and diabetes. *Semin Reprod Med* (2011) 29(3):225–36. doi: 10.1055/s-0031-1275516

123. Berends LM, Dearden L, Tung YCL, Voshol P, Fernandez-Twinn DS, Ozanne SE. Programming of central and peripheral insulin resistance by low birthweight and postnatal catch-up growth in male mice. *Diabetologia* (2018) 61(10):2225–34. doi: 10.1007/s00125-018-4694-z

124. Long W, Zhou T, Xuan X, Cao Q, Luo Z, Qin Y, et al. IUGR with catch-up growth programs impaired insulin sensitivity through LRP6/IRS-1 in male rats. *Endocr Connect* (2022) 11(1):e210203. doi: 10.1530/EC-21-0203

125. Joseph R, Poschmann J, Sukarieh R, Too PG, Julien SG, Xu F, et al. ACSL1 is associated with fetal programming of insulin sensitivity and cellular lipid content. *Mol Endocrinol* (2015) 29(6):909–20. doi: 10.1210/me.2015-1020

126. Ross MG, Desai M. Developmental programming of offspring obesity, adipogenesis, and appetite. *Clin Obstet Gynecol* (2013) 56(3):529–36. doi: 10.1097/GRF.0b013e318299c39d

127. Gong M, Antony S, Sakurai R, Liu J, Iacovino M, Rehan VK. Bone marrow mesenchymal stem cells of the intrauterine growth-restricted rat offspring exhibit enhanced adipogenic phenotype. *Int J Obes (Lond)* (2016) 40(11):1768–75. doi: 10.1038/ijo.2016.157

128. Sreekantha S, Wang Y, Sakurai R, Liu J, Rehan VK. Maternal food restriction-induced intrauterine growth restriction in a rat model leads to sex-specific adipogenic programming. *FASEB J* (2020) 34(12):16073–85. doi: 10.1096/fj.202000985RR

129. Saenger P, Czernichow P, Hughes I, Reiter EO. Small for gestational age: short stature and beyond. *Endocr Rev* (2007) 28(2):219–51. doi: 10.1210/er.2006-0039

130. de Bie HM, de Ruiter MB, Ouwendijk M, Oostrom KJ, Wilke M, Boersma M, et al. Using fMRI to investigate memory in young children born small for gestational age. *PLoS One* (2015) 10(7):e0129721. doi: 10.1371/journal.pone.0129721

131. Sacchi C, O'Muircheartaigh J, Batalle D, Counsell SJ, Simonelli A, Cesano M, et al. Neurodevelopmental outcomes following intrauterine growth restriction and very preterm birth. *J Pediatr* (2021) 238:135–44.e10. doi: 10.1016/j.jpeds.2021.07.002

132. Chen J, Chen P, Bo T, Luo K. Cognitive and behavioral outcomes of intrauterine growth restriction school-age children. *Pediatrics* (2016) 137(4): e20153868. doi: 10.1542/peds.2015-3868

133. St Pierre M, Rastogi N, Brown A, Parmar P, Lechner C, Fung C, et al. Intrauterine growth restriction disrupts the postnatal critical period of synaptic plasticity in the mouse dorsal hippocampus in a model of hypertensive disease of pregnancy. *Dev Neurosci* (2021) 44(4-5):214–32. doi: 10.1159/000521611

134. Brown AS, Wieben M, Murdock S, Chang J, Dizon MLV, St Pierre M, et al. Intrauterine growth restriction causes abnormal embryonic dentate gyrus neurogenesis in mouse offspring that leads to adult learning and memory deficits. *eNeuro* (2021) 8(5):1–16. doi: 10.1523/ENEURO.0062-21.2021

135. Wan L, Luo K, Chen P. Mechanisms underlying neurologic injury in intrauterine growth restriction. *J Child Neurol* (2021) 36(9):776–84. doi: 10.1177/0883073821999896

136. Hochberg Z. Developmental plasticity in child growth and maturation. *Front Endocrinol (Lausanne)* (2011) 2:41. doi: 10.3389/fendo.2011.00041

137. Ma J, Qiao Y, Zhao P, Li W, Katzmarzyk PT, Chaput JP, et al. Breastfeeding and childhood obesity: A 12-country study. *Matern Child Nutr* (2020) 16(3): e12984. doi: 10.1111/mcn.12984

138. Qiao J, Dai LJ, Zhang Q, Ouyang YQ. A meta-analysis of the association between breastfeeding and early childhood obesity. *J Pediatr Nurs* (2020) 53:57–66. doi: 10.1016/j.pedn.2020.04.024

139. Rao MR, Hediger ML, Levine RJ, Naficy AB, Vik T. Effect of breastfeeding on cognitive development of infants born small for gestational age. *Acta Paediatr* (2002) 91(3):267–74. doi: 10.1080/08035250252833905

140. Belfort MB, Rifas-Shiman SL, Kleinman KP, Guthrie LB, Bellinger DC, Taveras EM, et al. Infant feeding and childhood cognition at ages 3 and 7 years: Effects of breastfeeding duration and exclusivity. *JAMA Pediatr* (2013) 167(9):836–44. doi: 10.1001/jamapediatrics.2013.455

141. Barone G, Maggio L, Saracino A, Perri A, Romagnoli C, Zecca E. How to feed small for gestational age newborns. *Ital J Pediatr* (2013) 39:28. doi: 10.1186/1824-7288-39-28

142. Pietrobelli A, Agosti M. Nutrition in the first 1000 days: Ten practices to minimize obesity emerging from published science. *Int J Environ Res Public Health* (2017) 14(12):1491, 1–14. doi: 10.3390/ijerph14121491

143. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. *Cochrane Database Syst Rev* (2017) 4(4):Cd004905. doi: 10.1002/14651858.CD004905.pub5

144. Ali AM, Alobaid A, Malhis TN, Khattab AF. Effect of vitamin D3 supplementation in pregnancy on risk of pre-eclampsia - randomized controlled trial. *Clin Nutr* (2019) 38(2):557–63. doi: 10.1016/j.clnu.2018.02.023

145. Gantenbein KV, Kanaka-Gantenbein C. Mediterranean Diet as an antioxidant: The impact on metabolic health and overall wellbeing. *Nutrients* (2021) 13(6):1951, 1–18. doi: 10.3390/nu13061951

146. Larqué E, Labayen I, Flodmark CE, Lissau I, Czernin S, Moreno LA, et al. From conception to infancy - early risk factors for childhood obesity. *Nat Rev Endocrinol* (2019) 15(8):456–78. doi: 10.1038/s41574-019-0219-1

147. Mameli C, Mazzantini S, Zuccotti GV. Nutrition in the first 1000 days: The origin of childhood obesity. *Int J Environ Res Public Health* (2016) 13(9):838, 1–9. doi: 10.3390/ijerph13090838

148. Han L, Zhang H, Kaushal A, Rezwan FI, Kadalayil L, Karmaus W, et al. Changes in DNA methylation from pre- to post-adolescence are associated with pubertal exposures. *Clin Epigenet* (2019) 11(1):176. doi: 10.1186/s13148-019-0780-4

149. Huang YT, Maccani JZJ, Hawley NL, Wing RR, Kelsey KT, McCaffery JM. Epigenetic patterns in successful weight loss maintainers: a pilot study. *Int J Obes (Lond)* (2015) 39(5):865–8. doi: 10.1038/ijo.2014.213

150. Lindholm ME, Marabita F, Gomez-Cabrero D, Rundqvist H, Ekström TJ, Tegnér J, et al. An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. *Epigenetics* (2014) 9(12):1557–69. doi: 10.4161/15592294.2014.982445

151. van Dijk SJ, Tellam RL, Morrison JL, Muhlhausler BS, Molloy PL. Recent developments on the role of epigenetics in obesity and metabolic disease. *Clin Epigenet* (2015) 7:66. doi: 10.1186/s13148-015-0101-5

152. Goodrich JM, Dolinoy DC, Sánchez BN, Zhang Z, Meeker JD, Mercado-Garcia A, et al. Adolescent epigenetic profiles and environmental exposures from early life through peri-adolescence. *Environ Epigenet* (2016) 2(3):dvw018. doi: 10.1093/eep/dvw018

153. Houk CP, Lee PA. Early diagnosis and treatment referral of children born small for gestational age without catch-up growth are critical for optimal growth outcomes. *Int J Pediatr Endocrinol* (2012) 2012(1):11. doi: 10.1186/1687-9856-2012-11

154. Al Shaikh A, Daftardar H, Alghamdi AA, Jamjoom M, Awidah S, Ahmed ME, et al. Effect of growth hormone treatment on children with idiopathic short stature (ISS), idiopathic growth hormone deficiency (IGHD), small for gestational age (SGA) and turner syndrome (TS) in a tertiary care center. *Acta BioMed* (2020) 91(1):29–40. doi: 10.23750/abm.v91i1.9182

155. Saggese G, Fanos M, Simi F. SGA children: auxological and metabolic outcomes - the role of GH treatment. *J Matern Fetal Neonatal Med* (2013) 26(Suppl 2):64–7. doi: 10.3109/14767058.2013.832870

156. van der Steen M, Kerkhof GF, Smeets CCJ, Hokken-Koelega ACS. Cardiovascular risk factors and carotid intima media thickness in young adults born small for gestational age after cessation of growth hormone treatment: A 5-year longitudinal study. *Lancet Diabetes Endocrinol* (2017) 5(12):975–85. doi: 10.1016/S2213-8587(17)30311-X

157. van der Steen M, Smeets CC, Kerkhof GF, Hokken-Koelega AC. Metabolic health of young adults who were born small for gestational age and treated with growth hormone, after cessation of growth hormone treatment: A 5-year longitudinal study. *Lancet Diabetes Endocrinol* (2017) 5(2):106–16. doi: 10.1016/S2213-8587(16)30422-3

158. Smeets CCJ, van der Steen M, Renes JS, Hokken-Koelega ACS. Bone mineral density after cessation of GH treatment in young adults born SGA: A 5-year longitudinal study. *J Clin Endocrinol Metab* (2017) 102(9):3508–16. doi: 10.1210/jc.2017-00269

159. Goedegebuure WJ, Kerkhof GF, Hokken-Koelega ACS. Glomerular filtration rate, blood pressure and microalbuminuria in adults born SGA: A 5-year longitudinal study after cessation of GH treatment. *Clin Endocrinol (Oxf)* (2019) 91(6):892–8. doi: 10.1111/cen.14095

160. Díaz M, Bassols J, López-Bermejo A, de Zegher F, Ibáñez L. Metformin treatment to reduce central adiposity after prenatal growth restraint: A placebo-controlled pilot study in prepubertal children. *Pediatr Diabetes* (2015) 16(7):538–45. doi: 10.1111/pedi.12220

161. Ibáñez L, López-Bermejo A, de Zegher F. Pubertal adiposity after fetal growth restraint: Toward a calorie restriction mimetic approach. *Metabolism* (2008) 57(5):672–5. doi: 10.1016/j.metabol.2008.01.002

162. Ibáñez L, Valls C, Ong K, Dunger DB, de Zegher F. Metformin therapy during puberty delays menarche, prolongs pubertal growth, and augments adult height: a randomized study in low-birth-weight girls with early-normal onset of puberty. *J Clin Endocrinol Metab* (2006) 91(6):2068–73. doi: 10.1210/jc.2005-2329

163. Ibáñez L, López-Bermejo A, Díaz M, Marcos MV, de Zegher F. Metformin treatment for four years to reduce total and visceral fat in low birth weight girls with precocious pubarche. *J Clin Endocrinol Metab* (2008) 93(5):1841–5. doi: 10.1210/jc.2008-0013

164. Garcia-Contreras C, Vazquez-Gomez M, Pesantez-Pacheco JL, Heras-Molina A, Encinas T, Astiz S, et al. The effects of maternal metformin treatment on late prenatal and early postnatal development of the offspring are modulated by sex. *Pharm (Basel)* (2020) 13(11):1–11. doi: 10.3390/ph13110363

165. Garcia-Contreras C, Vazquez-Gomez M, Pesantez-Pacheco JL, Torres-Rovira L, Heras-Molina A, Encinas T, et al. Maternal metformin treatment improves developmental and metabolic traits of IUGR fetuses. *Biomolecules* (2019) 9(5):1–10. doi: 10.3390/biom9050166

166. Grivell R, Dodd J, Robinson J. The prevention and treatment of intrauterine growth restriction. *Best Pract Res Clin Obstet Gynaecol* (2009) 23 (6):795–807. doi: 10.1016/j.bpobgyn.2009.06.004

167. Crovetto F, Crispi F, Casas R, Martín-Asuero A, Borràs R, Vieta E, et al. Effects of Mediterranean diet or mindfulness-based stress reduction on prevention of small-for-Gestational age birth weights in newborns born to At-risk pregnant individuals: The IMPACT BCN randomized clinical trial. *JAMA* (2021) 326 (21):2150–60. doi: 10.1001/jama.2021.20178

168. Georgieff MK. Iron deficiency in pregnancy. *Am J Obstet Gynecol* (2020) 223(4):516–24. doi: 10.1016/j.ajog.2020.03.006

169. Agrawal A, Wenger NK. Hypertension during pregnancy. *Curr Hypertens Rep* (2020) 22(9):64. doi: 10.1007/s11906-020-01070-0

170. McCowan LM, Figueras F, Anderson NH. Evidence-based national guidelines for the management of suspected fetal growth restriction: comparison, consensus, and controversy. *Am J Obstet Gynecol* (2018) 218(2s):S855–s868. doi: 10.1016/j.ajog.2017.12.004

171. Martín-Estal I, Castilla-Cortázar I, Castorena-Torres F. The placenta as a target for alcohol during pregnancy: The close relation with IGFs signaling pathway. *Rev Physiol Biochem Pharmacol* (2021) 180:119–53. doi: 10.1007/112_2021_58

172. Banderali G, Martelli A, Landi M, Moretti F, Betti F, Radaelli G, et al. Short and long term health effects of parental tobacco smoking during pregnancy and lactation: A descriptive review. *J Transl Med* (2015) 13:327. doi: 10.1186/s12967-015-0690-y

173. Hetea A, Cosconel C, Stanescu AAM, Simionescu AA. Alcohol and psychoactive drugs in pregnancy. *Maedica (Bucur)* (2019) 14(4):397–401. doi: 10.26574/maedica.2019.14.4.397

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 16 November 2022
DOI 10.3389/fendo.2022.1031884

OPEN ACCESS

EDITED BY
Malgorzata Wojcik,
Jagiellonian University Medical College, Poland

REVIEWED BY
Agnieszka Kozioł-Kozakowska,
Jagiellonian University Medical College, Poland
Thomas Pixner,
Salzkammergut Klinikum Vöcklabruck, Austria

*CORRESPONDENCE
Grzegorz Sobek
g.sobek@wp.pl

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 30 August 2022
ACCEPTED 31 October 2022
PUBLISHED 16 November 2022

CITATION
Sobek G and Dąbrowski M (2022) The importance of the taste preferences and sensitivity of mothers and their children in the aspect of excessive body weight of children. *Front. Endocrinol.* 13:1031884. doi: 10.3389/fendo.2022.1031884

The importance of the taste preferences and sensitivity of mothers and their children in the aspect of excessive body weight of children

Grzegorz Sobek[1]* and Mariusz Dąbrowski[2]

[1]Institute of Health Sciences, College of Medical Sciences, University of Rzeszow, Rzeszów, Poland, [2]Institute of Medical Sciences, College of Medical Sciences, University of Rzeszow, Rzeszów, Poland

Introduction: Food selection among adults and mostly children depends mainly on the taste of a dish. Poor taste sensitivity as well as strong preferences for sweet and fat taste may be the factors predisposing children to become overweight and/or develop obesity. Family environment, including mothers' eating habits and preferences, may affect children's taste perception and preferences. The aim of the study was to assess taste perception and preferences in children and their mothers in relation to their weight status.

Methods: Sensory tests were carried out using puddings with different sugar and fat content. In all study participants anthropometric measurements (weight and height with BMI calculation) were performed.

Results: The study results did not reveal any differences in the taste sensitivity of overweight/obese and normative body weight children. Similarity was found in the perception of different levels of sweet/fat flavors among children and parents. Overweight/obese children were two times more likely to choose a very fat and very sweet taste compared to normal weight children. The results showed that children prefer a sweet taste more often than their mothers. Mothers' fat taste preferences were important - the fatter the taste they selected, the greater the percentage of children with obesity.

Discussion: Mothers' taste sensitivity may affect children's perception of the quality (intensity) of flavors. Normal-weight children chose a low fat and low sweet taste more frequently than those with excess of body weight. The role of parents in shaping taste preferences is of utmost importance and should be based on limiting the consumption of products rich in sugar and/or fat.

KEYWORDS

children, mothers, taste preferences, taste perception, overweight and obesity

Introduction

Human food intake is regulated by a complex physiological mechanism associated not only with the feeling of hunger and satiety, but also with the perception of sensory stimuli. Food assessment is largely based on sensory impressions such as look, smell and finally taste. All basic flavors, i.e. sweet, bitter, salty, sour, umami, as well as fat play the role of food quality markers (1–3). Studies clearly show that sensory performance of the taste recognition apparatus is an individual feature that can affect the perception of food products and daily choices of food consumed (4, 5). The reasons for different sensitivity are complex and are the result of numerous factors including genetic, physiological and environmental ones. Factors that affect sensory perception can also be associated with age, sleep, body mass index, anxiety level and neurotransmitters, hormonal factors, and habitual diet (6). It is hypothesized that those less able to detect fatty acids (hyposensitive) appear to have, a higher body mass index (BMI) (7, 8). There are no clear data to confirm this, but several testable theory may be proposed. A frequently investigated hypothesis is that overweight and obese individuals are less sensitive to palatable fatty texture (mouthfeel) and therefore need a greater concentration to detect fatty mouthfeel (9).

Given that food preferences are an extremely important factor influencing diet quality, understanding how they change and how they can be modified can help to promote a healthy diet for both children and adults. Taste preferences can therefore be defined as making a food selection based on one's own subjective, hedonic ("pleasant", "unpleasant") perceptions. In practice, due to the strong relationship between taste preferences and food selection, the term food preferences is often used, which means that we like some food products more or less, and we have an aversion towards others (10). Current research results indicate that children perceive some flavors differently than adults. Preferences of the youngest for higher levels of salty and sweet taste can be justified by evolutionary factors (11). The first reports describing the relationship between taste preferences, food consumption and obesity suggested that a greater intensity of taste contributes to the improvement of the palatability of food products, and thus to over-consumption and possible obesity (12). Lack of conclusive evidence in a few subsequent studies is associated with the heterogeneity of methods for measuring taste preferences and food consumption. Doubts also relate to the use of flavors in the form of laboratory samples in sensory tests due to their insufficient relation to consumption in real conditions (13).

Parents play a significant role in shaping the taste preferences of their children and adolescents. Their participation in developing sensory experiences is not limited to "setting an example," but can have a much broader context. Parents can consciously or unconsciously control the availability of food products, and thus affect the exposure of different flavors (14).

Several reports are available in the literature analyzing the impact of children's taste perception preferences in the context of overweight and/or obesity as well as adult taste preferences and perceptions separately (15–17). So far, there are no reports in the literature comparing the taste preferences and taste sensitivity of children and their parents with the state of body weight.

Materials and methods

Subject

The study is a preliminary assessment of the relationship between the taste perception and taste preferences of mothers and their children in relation to the BMI of mothers and children. 239 children aged 8-15 years were recruited for the study from two schools selected *via* an randomized algorithm, one in an urban (Stanisław Wyspiański Secondary School Complex No. 3. in Rzeszów, Poland) and one in a rural area (Complex of Schools in Kosina, Poland). The study group consisted of 75 children with overweight or obesity aged from 8 to 15 years. The control group consisted of 75 children with normal weight aged from 8 to 15 years, strictly matched to the study group regarding sex and age (the nearest date of birth for each comparator from the study group, in 1:1 ratio). The remaining 89 children not strictly matching to the study group regarding sex and age were excluded. All mothers (n=150) of the pediatric subjects wereincluded in the study. Mothers were chosen because of their impact on the development of childhood eating habits. 69 of them were overweight and/or obesity (BMI ≥25 kg/m^2) (18). The inclusion criteria for children were age 8–15, the attendance of one of two selected schools, and parents' acceptance to participate in the study. The requirement for the child's participation in the study was the simultaneous participation of his/her mother. The exclusion criteria for children included suffering from chronic diseases affecting body weight, being underweight (<5th percentile), inability to consume food samples used in the study, implanted pacemaker and pregnancy (contraindications for bioimpedance testing). The children participating in the study were a representative sample of the population. The inclusion and exclusion criteria for mothers were the same, except for the age and body weight. During the parents meeting with teachers at schools, the main goals of the study were presented. Attending mothers were asked to participate in the study by themselves and to permit their children to participate. It was noted that the research is voluntary and has only a scientific purpose. The study was conducted after obtaining written consent from the participating children's parents and the children themselves. All participants and parents were fully informed in writing and verbally about the nature of the study.

Assessments of preferences and sensitivity

The study was conducted according to the protocol used to assess sweet and fat taste perception in the "I Family" study (funded by the EC FP7 project No 266044) using puddings with different sugar content and with different fat content (SOP - Carrying out a taste intensity test with children, adolescents and their parents to assess sweet and fat taste perception of different puddings) (19). The subjects task was to assess the intensity of three pudding samples with different fat content and three pudding samples with different sugar content. The evaluators were to rank three samples according to increasing fat content and another three according to increasing sugar content. In addition, the evaluators had to indicate which of the samples with different fat content and samples with different sugar content they like best. Between each test sequence the participants rinsed their mouth with demineralised water to avoid adaptation and took a two to three minutes break. The test continued with the second block (taste) following the same procedure. The pudding samples were presented under a red light in order to mask colour differences. Cold whipped vanilla pudding (RUF Schlemmer Crème, vanilla flavour) was a carrier of taste with different concentrations of sugar and fat. Base samples for both flavors were identical and contained 14.5% sugar and 3.1% fat. Modified samples to examine the perception of fat taste contained sugar base amount and increased fat concentration, i.e. 6.8% and 14.1%. In case of samples for the assessment of sweet taste, the base amount of fat was retained, and the amount of sugar was modified to 24.1% and 36.2%. All participants were given a template on which they had to complete a scale of taste perception intensity rating for each taste and concentration. The scale consisted of 3 intensity values (from 1 to 3), 1 meaning "low fat/sweet" and 3 "high fat/sweet".

The interpretation of the results consisted in assessing the accuracy of ordering the intensity of the fat/sweet taste from the least fat/sweet to the high fat/sweet. In the case when all (three) samples were correctly ranked by participant, the test result was marked as maximum accuracy. When one or two errors were made, the test result was classified as average accuracy, while in the case when all the samples were incorrectly ranked, the test result was rated as lack of accuracy. For the fat taste the term "creamy" was used to avoid negative associations with the word fat.

Anthropometric measurements of the studied group

Height measurements were made three times with the SECA 213 portable stadiometer, with an accuracy of 5 mm, in a standing position, upright, without footwear. The average figure of the three measurements was used in the analyzes. Body weight was assessed with an accuracy of 0.1 kg using a body composition analyzer (BC-420, Tanita, Tokyo, Japan). According to the instructions for the Tanita BC 420 device for accurate measurements, the machine was positioned as horizontally as possible. Participants stood on the platform barefoot, upright, on straight legs and made sure that the front of the feet touched the front electrodes and rear parts of the rear electrodes. The height and weight of all participants (children, mothers) were measured in fasting status wearing underwear. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Based on BMI values, the BMI percentile of individual childrens was calculated. BMI percentile charts specific for age, sex, and body height were used. Percentile charts which were developed within the framework of the Polish project entitled "Developing standards of blood pressure in children and adolescents in Poland, OLAF" were used (20). Based on the BMI percentile values, underweight (<5th percentile), normal weight (between 5th and 85th percentile), overweight (BMI ≥85th percentile and < 95th percentile), or obesity (≥95th percentile) were determined. BMI classification for mothers' was carried out according to the WHO guidelines: underweight (<18.5kg/m^2), normal weight (between 18.5-24.99kg/m^2), overweight (between 25-29.99kg/m^2), and obesity (≥30kg/m^2) (18).

Statistical analysis

The statistical analysis was performed using the Statistica v.12.0 Software (StatSoftPolska Sp. z o.o., Kraków, Poland). Differences between groups were analyzed using χ^2 test or McNemar's test where appropriate, with Yates correction applied. For associations between taste preferences and body mass categories odds ratios (OR) with 95% confidence interval (CI) were calculated. A P value <0.05 was considered statistically significant.

Results

Perception for fat and sweet taste among children

Test results provide information on the level of taste perception for fat and sweet taste (Table 1). The differences in fat content in puddings were imperceptible for nearly 30% of the subjects. Another approx. 30% have correctly assessed the differences in the levels of fat taste. The remaining individuals, i.e. about 40%, had some problems with the exact ranking of fat content in the pudding samples tested, which is why they can be included in the group with average sensitivity to fat taste. The

TABLE 1 Accuracy of children's taste perception according to body mass category.

Accuracy of fat/sweet taste ratings	Body mass classification **			P value
	normal	overweight + obese	all	
high (fat)	23 (30,7%)	21 (28,0%)	44 (29,3%)	p = 0,6573
average (fat)	34 (45,3%)	31 (41,3%)	65 (43,3%)	
low (fat)	18 (24,0%)	23 (30,7%)	41 (27,3%)	
high (sweet)	48 (64,0%)	45 (60,0%)	93 (62,0%)	p = 0,7976
average (sweet)	22 (29,3%)	23 (30,7%)	45 (30,0%)	
low (sweet)	5 (6,7%)	7 (9,3%)	12 (8,0%)	

** data are expressed as n (%).

important information is that the assessments were not associated with the BMI of the tested children.

Mothers' taste perception vs. children' taste perception

Although the accuracy of recognizing the intensity of fat and sweet taste was roughly similar in both groups, the disconcordance between the groups reached the statistical significance level, which was more pronounced with regards to sweet taste (Table 2).

Children's taste preferences

Taste preferences are significantly different for children with normal and excessive body weight (Table 3).The individuals with normal body weight choose a low fat and low sweet taste relatively more frequently than those with an overweightand obesity. High sweet compared to low sweet taste preference was associated with significantly higher probability of development of excess body weight, OR 3.77 (1.66-8.55), P=0.002. In case of high and low fat taste preference, this relationship appeared to be borderline insignificant, OR 2.42 (1.03-5.69), P=0.067.

Taste preferences of children and mothers – comparison

There were no significant differences in fat content preferences between mothers and their children, while children significantly more frequently preferred sweet or high sweet taste compared to mothers, OR 2.26 (1.42-3.58), P<0.001 (Table 4).

Mothers' taste preferences and their body mass

A sweet taste preferences were significantly different between normal-weight, overweight and obese mothers, P=0.002.Obesity was significantly associated with sweet and high sweet taste preferences compared to normal-weight mothers. These preferences were not significantly different between normal-weight and overweight mothers (Table 5). No similar relationship was found for the fat taste, P=0.091.

Parental taste preferences and the incidence of overweight and obesity among children

Sensitivity to fat taste among mothers is not associated with the incidence of weight disorders among children, while fat taste

TABLE 2 Accuracy of recognizing the intensity of fat and sweet taste by mothers and children, and concordance between them.

Mothers		Fat			Sweet		
Children		low	average	high	low	average	high
Fat	low	17 (33,3%)	20 (29,4%)	7 (22,6%)	–	–	–
	average	28 (54,9%)	25 (36,8%)	12 (38,7%)	–	–	–
	high	6 (11,8%)	23 (33,8%)	12 (38,7%)	–	–	–
Sweet	low	–	–	–	68 (68,7%)	23 (50,0%)	2 (40,0%)
	average	–	–	–	26 (26,3%)	18 (39,1%)	1 (20,0%)
	high	–	–	–	5 (5,1%)	5 (10,9%)	2 (40,0%)
P value for concordance			p = 0,0371*			p = 0,0171*	

** data are expressed as n (%).,* statistically significant.

TABLE 3 Relationship between taste preferences and body weight category among children.

Taste Preference Test	Body mass classification**		P value
	normal	overweight + obese	
low (fat)	23 (30,7%)	19 (25,3%)	p = 0,0173*
average (fat)	36 (48,0%)	24 (32,0%)	
high (fat)	16 (21,3%)	32 (42,7%)	
low (sweet)	37 (49,3%)	19 (25,3%)	p = 0,0046*
average (sweet)	22 (29,3%)	25 (33,3%)	
high (sweet)	16 (21,3%)	31 (41,3%)	

** data are expressed as n (%).,*statistically significant.

preferences are of great significance - the fatter the taste preferred by mothers, the greater the percentage of children with obesity (17% in case of choosing low-fat taste, and 37% and 41% in case of fat or high fat) (Figure 1). No similar relationship was found for the sweet taste.

Discussion

Sensory sensations have a significant impact on people's nutritional behavior (21). In everyday contact with food, the most pleasant is such stimulus intensity to which the consumer is accustomed. Sensitivity to each of the flavors varies widely from person to person (22). Some studies confirm that people who are sensory insensitive prefer higher concentrations of specific tastes (sour, bitter, sweet, salty, umami) (23, 24). The consequence of individual differences in the perception of tastes may be disproportions in the consumption of some dietary ingredients, especially sugar or fat, which, as a result, in those who are less sensory sensitive may lead to an increase in caloric content of the diet.The diet of people who liked to consume more sweet products was characterized by a higher energy supply and a higher consumption of carbohydrates (starch, fructose, glucose, total sugars) (25). Lim et al. suggests that the tendency towards higher sweet consumption may be due to lower sensory sensitivity (26). Most of reliable studies also point out the positive relationship between acceptance of high fat content and BMI or body weight (27–29).At the same time, obese people may be less sensitive to some unpleasant sensory fatty acids, so their appetite in some products or fatty foods is not diminished (30).Most of the available publications check the level of sensory sensitivity by comparing thresholds for individual flavors on standardized aqueous solutions. It is worth noting, however, that taste sensitivity is not always associated with preferences for stimuli at the threshold level or suprathreshold level so the lack of correlation with body weight is not necessarily surprising (31, 32). The study of such factors as the intensity of taste, hedonic sensation ("liking"), and not the threshold of sensation, are more representative for sensory measurements, because people are in contact with food every day in which flavors occur at suprathreshold levels (33).

During the experiment it was evident that the evaluators had a significant problem with the correct assessment of the fat content of various puddings. The obtained results (Table 1) confirmed this fact, because over 70% of the respondents rated the intensity of the fat taste incorrectly, while in the case of sweet taste as much as 62% of the respondents had no problem with the proper ranking of the intensity. Low sensitivity to fat taste is confirmed by some authors, who suggested that fat taste is not well perceived by humans and this may be a problem in sensory perception measurements (34). Despite the use of food samples instead of standardized aqueous solutions, the study did not confirm differences in the taste perception of children with proper body weight and obese children. There were also no significant differences in the perception of fat taste between parents and their children.

TABLE 4 Fat and sweet taste preferences for children and mother's – comparison.

	Preference test**					
	fat taste			sweet taste		
	low	average,	high	low	average,	high
Children	42 (28,0%)	60 (40,0%)	48 (32.0%)	56 (37,4%)	47 (31.3%)	47 (31.3%)
Mothers	35 (23,3%)	49 (32,7%)	66 (44.0%)	86 (57,3%)	36 (24.0%)	28 (18.7%)
P value		0.101			0.002*	

**data are expressed as n (%).,*statistically significant.

TABLE 5 Association between mother's sweet taste preferences and their body mass index category.

BMI classification mother (M)	Sweet taste preference									
	low sweet		sweet				high sweet			
	N	%	*N*	%	*OR*	*p*	*N*	%	*OR*	*p*
Normal weight	52	64.2	17	21.0	Ref.	–	12	14.8	Ref.	–
Overweight	29	63.0	7	15.2	0.74 ((0.27-1.99)	0.721	10	21.7	1.49 (0.58-3.88)	0.562
Obese (M)	5	21.7	12	52.2	7.34 (2.26-23.85)*	<0.001	6	26.1	5.20 (1.36-19.91)*	0.019*

OR, odds ratio; N, quantity; * statistically significant.

However, the study confirmed strong preferences for sweet taste in children. Sweet or very sweet puddings were more often chosen by children and youth (63%) than their mothers (43%) (Table 4) Mennella et al. (35), who also used puddings in her research presents the same conclusions regarding sweet taste preferences in children aged 5-10. Children preferred a higher concentration of sugar in both puddings and aqueous solutions. According to other reports, children are predisposed to prefer foods rich in energy, sugar and salt (36, 37).There was also a significant difference in the ability to rank samples relative to the intensity of sweet taste. Children more often did it incorrectly, as in our study, where the difference between children and mothers in this aspect was not so significant.It might be due to the higher average age of children involved in the experiment compared to Mennella's study. In the same study it was observed that children were less willing than parents to choose puddings with a higher fat content. In our study it was noted as some literature sources suggested that preferences for sweet/fat taste were associated with overweight and obesity of children (Table 3) (14). Also in the case of mothers, similarly to the study by Ettinger et al. (38), preferences for sweet taste were associated with overweight and obesity, and particularly clearly with obesity (Table 5). Undoubtedly, many facts prove that taste preferences are related to sensory perception, food consumption and people's body weight. However, it is not obvious how the mechanism works, which causes people with different sensitivity to flavors, different taste preferences react differently to consumed food. Ambiguous results regarding the impact of taste sensitivity on consumer choices of children withdo not help to draw objective conclusions. Sensitivity to fat taste identified as NEFA (non-esterified fatty acids) was the subject of a meta-analysis by Tucker et al. (15). It showed no differences between lean and obese people in threshold sensitivity and in assessing the suprathreshold intensity. Other such studies using NEFA provide different results. If a relationship is already detected, it is usually negative, i.e. as the body mass increases, sensitivity to NEFA decreases (39, 40). Available literature also gives examples of fat sensitivity tests using oleic acid (41), or linoleic acid as in Marinez - Ruiz et al. study (42) which confirms the inverse correlation between fat perception and BMI of the participants. Alternatively, it has been suggested that stimuli of considerable intensity, especially if they are exposed for a long time, can cause changes consisting in a permanent increase in the sensitivity threshold. Changes in stimulus sensitivity may be the greater, the longer the exposure time and the higher the stimulus concentration. A high-fat diet can lead to habituation and the need to increase stimulus levels to the desired quality in the mouth, leading to an increase in dietary fat intake and weight gain (29, 43). In Di Patrizio studies, both slim and obese people on a 4-week low-fat diet

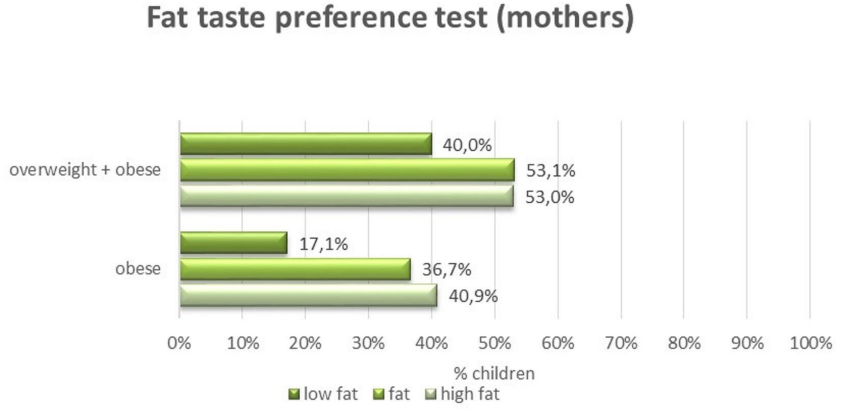


FIGURE 1
Preferences of the fat taste among mothers and the occurrence of overweight and obesity among children.

showed a higher taste sensitivity to fatty acids compared to the state at the beginning of the experiment (44). In the case of using a high-fat diet, the level of sensitivity in overweight and obese people has not changed, which may indicate that the subjects easily adapted to the permanent high-fat diet.

Parents, through their eating habits as well as everyday decisions, influence the child's environment and influence the shaping of their sensory preferences (14, 45). The role of early nutritional experience, breastfeeding, early exposure to a wide range of products varied in taste, determine later preferences and dietary habits (46, 47). The phase of introducing a complementary feeding is the most important period of learning the taste preferences and the control of appetite in human life. Infants discover sensory impressions (texture, taste, smell) and nutritional properties (energy density) of food that is part of the diet of adults (48). From this perspective, comparing the results regarding the perception and taste preferences of children and parents is very interesting. However, in our study, comparing the results of taste preferences of children and mothers did not show significant differences. It is obvious that the newborn's "primary" taste preferences are significantly modified in childhood by various environmental factors and may change over time as the child develops (49). Parents' taste preferences are the result of their extensive nutritional experience related to their age and are determined more strongly by such factors as age, gender, health status, level of education or income (50). The obtained results showed the dependence of preferences regarding the taste of fat in mothers andBMI of children. This relationship may be explained by the influence of preferences - the parents' preference for high fat content in the type and method of preparing meals prepared for the whole family (including children). Jilani et al. showed that children's sensitivity to sweet and salty tastes is related to weight status (51). The comparison of the results of the taste sensitivity (accuracy of recognizing the intensity of taste) of children and their mothers indicates that the level of taste perception of children is correlated with the level of taste perception of mothers. It can therefore be argued that taste perception (level of taste sensitivity) can be genetically determined to a large extent. It has been established that the taste sensitivity associated with a particular gene variant has an impact on the feeling of satisfaction with food and certain eating behaviors (52, 53). It would be valuable for the results of the study to include also the fathers of participants. Our results were also limited by the small number of examined children. It should be noted that other factors like socio-cognitive determinants (e.g., parental diet, the availability of healthy food at home, income) were not included in the study. Apart from these limitations our study has several strengths. The use of a daily product (pudding) with different sugar and fat content is a strong point of the study. The protocol for testing preferences and taste perception was simple and understandable for both children and mothers, which also limited potential errors.

Proper eating patterns are especially important as there are more and more arguments indicating that impaired fat taste perception/sensitivity in obesity may result from excessive activation of the brain's reward system, leading to an increase in the consumption of foods rich in lipids, carbohydrates and energy, and consequently overweight and obesity (54).

Conclusions

In this study, an attempt was made to compare the preferences and taste sensitivity of children and their mothers. Puddings with different sugar and fat content were used in the research. The obtained results showed that children of mothers who preferred the more fatty products (puddings) have a higher BMI than other children. Moreover, children preferred a higher concentration of sugar in the pudding compared to theirs mothers. The result also showed that there were no significant differences in the taste perception of fat between parents and their children, although parents preferred fatty foods. However, there was no correlation between sensitivity to sweet and fat tastes and BMI of children. Our results confirm that mothers, through their eating behavior, can influence the diet of their children and ultimately their BMI. Children with normal body weight chose a low fat and low sweet taste relatively more frequently than those with an overweight. The role of parents in shaping taste preferences is very important and should be based on limiting the consumption of products rich in sugar or fat.

Data availability statement

The raw data supporting the conclusions of this article are available on reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Bioethics Committee at the University of Rzeszow on 02.06.2015 (Resolution No. 15/06/2015) and by all appropriate administrative bodies. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

GS, MD: study design. GS: data collection. GS, MD: data analysis and interpretation. GS, MD: manuscript preparation and critical revision.

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

References

1. Mennella JA, Bobowski NK. The sweetness and bitterness of childhood: Insights from basic research on taste preferences. *Physiol Behav* (2015) 152(0 0):502–7. doi: 10.1016/j.physbeh.2015.05.015

2. Besnard P, Passilly-Degrace P, Khan NA. Taste of fat: A sixth taste modality? *Physiol Rev* (2016) 96:151–76. doi: 10.1152/physrev.00002.2015

3. Costanzo A, Orellana L, Nowson C, Duesing K, Keast R. Fat taste sensitivity is associated with short-term and habitual fat intake. *Nutrients* (2017) 9:781. doi: 10.3390/nu9070781

4. Kovesby CP, Furth SL, Zoccali C. Obesity and kidney disease: Consequences of the epidemic. *Blood Purif* (2017) 43:346–54. doi: 10.1159/000458481

5. Wilk K, Korytek W, Pelczyńska M, Moszak M, Bogdański P. The effect of artificial sweeteners use on sweet taste perception and weight loss efficacy: A review. *Nutrients* (2022) 14(6):1261. doi: 10.3390/nu14061261

6. Feeney EL, Leacy L, O'Kelly M, Leacy N, Phelan A, Crowley L, et al. Sweet and umami taste perception differs with habitual exercise in males. *Nutrients* (2019) 11:155. doi: 10.3390/nu11010155

7. Haryono RY, Sprajcer MA, Keast RSJ. Measuring oral fatty acid thresholds, fat perception, fatty food liking, and papillae density in humans. *J Vis Exp* (2014) 88):51236. doi: 10.3791/51236

8. Tucker RM, Edlinger C, Craig BA, Mattes RD. Associations between BMI and fat taste sensitivity in humans. *Chem Senses* (2014) 39(4):349–57. doi: 10.1093/chemse/bju006

9. Cox DN, Hendrie GA, Carty D. Sensitivity, hedonics and preferences for basic tastes and fat amongst adults and children of differing weight status: A comprehensive review. *Food Qual Preferences* (2016) 48:359–67. doi: 10.1016/j.foodqual.2015.01.006

10. Drewnowski A. Taste preferences and food intake. *Annu Rev Nutr* (1997) 17:237–53. doi: 10.1146/annurev.nutr.17.1.237

11. Beauchamp GK, Cowart BJ. Congenital and experiential factors in the development of human flavor preferences. *Appetite* (1985) 6:357–72. doi: 10.1016/S0195-6663(85)80004-0

12. Nasser J. Taste, food intake and obesity. *Obes Rev* (2001) 2(4):213–8. doi: 10.1046/j.1467-789X.2001.00039.x

13. Rozin P, Tuorola H. Simultaneous and temporal contextual influences on food acceptance. *Food Qual Preference* (1993) 4:11–20. doi: 10.1016/0950-3293(93)90309-T

14. Paroche MM, Samantha J, Caton SJ, Vereijken CM, Weenen H, Houston-Price C. How infants and young children learn about food: A systematic review. *Front Psychol* (2017) 8:1046. doi: 10.3389/fpsyg.2017.01046

15. Tucker RM, Kaiser KA, Parman MA, George BJ, Allison DB, Mattes RD. Comparisons of fatty acid taste detection thresholds in people who are lean vs. overweight or obese: A systematic review and meta-analysis. *PloS One* (2017) 12(1):e0169583. doi: 10.1371/journal.pone.0169583

16. Low JYQ, Lacy KE, McBride R, Keast RSJ. The association between sweet taste function, anthropometry, and dietary intake in adults. *Nutrients* (2016) 8:241. doi: 10.3390/nu8040241

17. Lanfer A, Knof K, Barba G, Veidebaum T, Papoutsou S de Henauw S, Soós T, et al. Taste preferences in association with dietary habits and weight status in European children: results from the IDEFICS study. *Int J Obes* (2012) 36:27–34. doi: 10.1038/ijo.2011.164

18. World Health Organization. *BMI classification* . Available at: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html (Accessed April 21, 2021).

19. Jilani H, Peplies J, Buchecker K. On behalf of the IDEFICS and I.Family consortia. assessment of sensory taste perception in children. In: Bammann K, Lissner L, Pigeot I, Ahrens W, editors. *Instruments for health surveys in children and adolescents*. Cham: Springer Nature Switzerland (2019). p. 257–75.

20. Kułaga Z, Różdżyńska A, Palczewska I. Percentile charts of height, body mass and body mass index in children and adolescents in Poland – results of the OLAF study. *Stand Med* (2010) 7:690–700.

21. Feeney EL, O'Brien SA, Scannell AG, Markey A, Gibney ER. Suprathreshold measures of taste perception in children - association with dietary quality and body weight. *Appetite* (2017) 113:116–23. doi: 10.1016/j.appet.2017.02.026

22. Cicerale S, Riddell LJ, Keast RS. The association between perceived sweetness intensity and dietary intake in young adults. *J Food Sci* (2012) 77:H31–5. doi: 10.1111/j.1750-3841.2011.02473.x

23. Kato Y, Roth R. Relationship between taste sensitivity and eating style in Japanese female university students. *Food Nutr Sci* (2012) 3:302–9. doi: 10.4236/fns.2012.33044

24. Puputti S, Aisala H, Hoppu U, Sandell M. Factors explaining individual differences in taste sensitivity and taste modality recognition among Finnish adults. *J Sens Stud* (2019) 34:4. doi: 10.1111/joss.12506

25. Jayasinghe S, Kruger R, Walsh DC, Cao G, Rivers S, Richter M, et al. Is sweet taste perception associated with sweet food liking and intake. *Nutrients* (2017) 9:750. doi: 10.3390/nu9070750

26. Lim AJ, Sze Teo P, Wei Kee Tan V, Forde CG. Associations between psycho-hedonic responses to sweet and savoury tastes with diet and body composition in a sample of Asian females. *Foods* (2020) 9(9):1318. doi: 10.3390/foods9091318

27. Drewnowski A, Kurth CL, Rahaim JE. Taste preferences in human obesity: Environmental and familial factors. *Am J Clin Nutr* (1991) 54(4):635–41. doi: 10.1093/ajcn/54.4.635

28. HarnischfegerF, DandoR. Obesity-induced taste dysfunction, and its implications for dietary intake. *Int J Obes* (2021) 45(8):1644–55. doi: 10.1038/s41366-021-00855-w

29. Stewart JE, Feinle-Bisset C, Golding M, Delahunty C, Clifton PM, Keast RS. Oral sensitivity to fatty acids, food consumption and BMI in human subjects. *Br J Nutr* (2010) 104(1):145–52. doi: 10.1017/S0007114510000267

30. Stewart JE, Keast RS. Recent fat intake modulates fat taste sensitivity in lean and overweight subjects. *Int J Obes* (2012) 36(6):834–42. doi: 10.1038/ijo.2011.155

31. Bartoshuk LM. Methodological problems in psychophysical testing of taste and smell. In: Han SS, Coons DH, editors. *Special senses in aging: A current biological assessment*. Ann Arbor: University of Michigan (1979).

32. Wise PM, Hansen JL, Reed DR, Breslin PAS. Twin study of the heritability of recognition thresholds for sour and salty taste. *Chem Sense* (2007) 32:749–54. doi: 10.1093/chemse/bjm042

33. Hartman-Petrycka M, Klimacka-Nawrot E, Ziora K, Suchecka W, Gorczyca P, Rojewska K, et al. Sweet, salty, and umami taste sensitivity and the hedonic perception of taste sensations in adolescent females with anorexia nervosa. *Nutrients* (2022) 14(5):1042. doi: 10.3390/nu14051042

34. Bouhlal S, Issanchou S, Nicklaus S. The impact of salt, fat and sugar levels on toddler food intake. *Br J Nutr* (2011) 105(4):645–53. doi: 10.1017/S0007114510003752

35. Mennella JA, Finkbeiner S, Reed DR. The proof is in the pudding: Children prefer lower fat but higher sugar than do mothers. *Int J Obes (Lond)* (2012) 36:1285–91. doi: 10.1038/ijo.2012.51

36. Iatridi V, Armitage RM, Yeomans MR, Hayes JE. Effects of sweet-liking on body composition depend on age and lifestyle: A challenge to the simple Sweet-Liking–obesity hypothesis. *Nutrients* (2020) 12(9):2702. doi: 10.3390/nu12092702

37. Fuchs-Neuhold B, Staubmann W, Peterseil M, Rath A, Schweighofer N, Kronberger A, et al. Investigating new sensory methods related to taste sensitivity, preferences, and diet of mother-infant pairs and their relationship with body composition and biomarkers: Protocol for an explorative study. *JMIR Res Protoc* (2022) 11(4):e37279. doi: 10.2196/37279

38. Ettinger L, Duizer L, Caldwell T. Body fat, sweetness sensitivity, and preference: determining the relationship. *Can J Dietetic Pract Res* (2012) 73(1):45–8. doi: 10.3148/73.1.2012.45

39. Stewart JE, Seimon RV, Otto B, Keast RSJ, Clifton PM, Feinle-Bisset C. Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men. *Am J Clin Nutr* (2011) 93:703–11. doi: 10.3945/ajcn.110.007583

40. Daoudi H, Plesnik J, Sayed A, Sery O, Rouabah A, Rouabah L, et al. Oral fat sensing and CD36 gene polymorphism in Algerian lean and obese teenagers. *Nutrients* (2015) 7:9096–104. doi: 10.3390/nu7115455

41. Kindleysides S, Beck KL, Walsh DC, Henderson L, Jayasinghe SN, Golding M, et al. Fat sensation: Fatty acid taste and olfaction sensitivity and the link with disinhibited eating behaviour. *Nutrients* (2017) 9(8):879. doi: 10.3390/nu9080879

42. Martínez-Ruiz NR, López-Díaz JA, Wall-Medrano A, Jiménez-Castro JA, Angulo O. Oral fat perception is related with body mass index, preference and consumption of high-fatfoods. *Physiol Behav* (2014) 129:36–42. doi: 10.1016/j.physbeh.2014.02.010

43. Lim SXL, Höchenberger R, Busch NA, Bergmann M, Ohla K. Associations between taste and smell sensitivity, preference and quality of life in healthy aging-the NutriAct family study examinations (NFSE) cohort. *Nutrients* (2022) 14 (6):1141. doi: 10.3390/nu14061141

44. Di Partizio NF. Is fat taste ready for primetime? *Physiol Behav* (2014) 136:145–54. doi: 10.1016/j.physbeh.2014.03.002

45. Strączek K, Horodnicka-Józwa A, Szmit-Domagalska J, Petriczko E, Safranow K, Walczak M. The influence of maternal nutritional behavior on the nutritional behavior of children with excess body weight. *Pediatr Endocrinol Diabetes Metab* (2021) 27(3):159–69. doi: 10.5114/pedm.2021.107721

46. Story M, Neumark-Sztainer D, French S. Individual and environmental influences on adolescent eating behaviours. *J Am Diet Assoc* (2002) 102:40–51. doi: 10.1016/s0002-8223(02)90421-9

47. Yuan WL, Lange C, Schwartz C, Martin C, Chabanet C, de Lauzon-Guillain B, et al. Infant dietary exposures to sweetness and fattiness increase during the first year of life and are associated with feeding practices. *J Nutr* (2016) 146:2334–42. doi: 10.3945/jn.116.234005

48. Nicklaus S. The role of dietary experience in the development of eating behavior during the first years of life. *Ann Nutr Metab* (2017) 70:241–5. doi: 10.1159/000465532

49. Davison KK, Birch LL. Obesigenic families: parents' physical activity and dietary intake patterns predict girls' risk of overweight. *Int J Obes Relat Metab Disord* (2002) 26:1186–93. doi: 10.1038/oby.2005.243

50. Lampure A, Castetbon K, Deglaire A, Schlich P, Péneau S, Hercberg S, et al. Associations between liking for fat, sweet or salt and obesity risk in French adults: a prospective cohort study. *Int J Behav Nutr Phys Activity* (2016) 13:74. doi: 10.1186/s12966-016-0406-6

51. Jilani H, Intemann T, Buchecker K, Charalambos H, Gianfagna F, De Henauw S, et al. Correlates of bitter, sweet, salty and umami taste sensitivity in European children: Role of sex, age and weight status - the IDEFICS study. *Appetite* (2022) 175:106088. doi: 10.1016/j.appet.2022.106088

52. Feeney E, O'Brien S, Markey A, Scannell A, Gibney E. Genetic variation in taste perception. does it have a role in healthy eating? *Proc Nutr Soc* (2011) 70 (1):135–43. doi: 10.1017/S0029665110003976

53. Chamoun E, Mutch DM, Allen-Vercoe E, Buchholz AC, Duncan AM, Spriet LL, et al. Guelph family health study. a review of the associations between single nucleotide polymorphisms in taste receptors, eating behaviors, and health. *Crit Rev Food Sci Nutr* (2018) 58(2):194–207. doi: 10.1080/10408398.2016.1152229

54. Brondel L, Quilliot D, Mouillot T, Khan NA, Bastable P, Boggio V, et al. Taste of fat and obesity: Different hypotheses and our point of view. *Nutrients* (2022) 14(3):555. doi: 10.3390/nu14030555

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 01 December 2022
DOI 10.3389/fendo.2022.1034148

OPEN ACCESS

EDITED BY
Aneta Monika Gawlik,
Medical University of Silesia, Poland

REVIEWED BY
Piotr Fichna,
Poznan University of Medical Sciences, Poland
Marie-Laure Frelut,
Independent Researcher, Albi, France
Ewa Barg,
Wroclaw Medical University, Poland

*CORRESPONDENCE
Kamilla Strączek
kamillastraczek@wp.pl

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 01 September 2022
ACCEPTED 04 November 2022
PUBLISHED 01 December 2022

CITATION
Strączek K, Horodnicka-Józwa A, Szmit-Domagalska J, Jackowski T, Safranow K, Petriczko E and Walczak M (2022) Familial dietary intervention in children with excess body weight and its impact on eating habits, anthropometric and biochemical parameters. *Front. Endocrinol.* 13:1034148. doi: 10.3389/fendo.2022.1034148

Familial dietary intervention in children with excess body weight and its impact on eating habits, anthropometric and biochemical parameters

Kamilla Strączek[1]*, Anita Horodnicka-Józwa[1], Justyna Szmit-Domagalska[1], Tomasz Jackowski[1], Krzysztof Safranow[2], Elżbieta Petriczko[1] and Mieczysław Walczak[1]

[1]Department of Paediatrics, Endocrinology, Diabetology, Metabolic Diseases and Cardiology of the Developmental Age, Pomeranian Medical University, Szczecin, Poland, [2]Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland

Introduction: Obesity is considered a civilisation disease which increases mortality and impairs quality of life, also among children and adolescents. The prevalence of overweight and obesity is steadily increasing in the developmental age population. Environmental factors are responsible for the main reason of excessive adipose tissue accumulation. Among these, poor eating habits and lack of exercise play the largest role. Familial prevalence of obesity and family dietary patterns also receive significant attention. Many specialists believe that the treatment of obesity should be multidirectional, effective and minimally invasive. Therefore, effective and safe methods are being investigated to effectively reduce body weight and improve eating habits. Dietary education programmes are an alternative to improve the health status of obese and overweight children and adolescents. To be fully effective, these programmes should involve the whole family.

Aim of the study: In the face of constantly increasing prevalence of overweight and obesity in the developmental age population and the lack of effective methods to combat its occurrence, it seems appropriate to try to assess the effectiveness of a one-year-long dietary education of children and adolescents with excess body weight on their eating habits and the eating habits of their mothers, as well as selected anthropometric and biochemical parameters in these children using a simple educational tool, the Healthy Food Pyramid.

Patients and methods: The study group consisted of 68 children with overweight and obesity, patients of the Department of Paediatrics, Endocrinology, Diabetology, Metabolic Diseases and Cardiology of the Developmental Age of the Pomeranian Medical University in Szczecin. The study used a proprietary questionnaire to assess dietary habits. Patients participated in six individual educational meetings over a twelve-month

10.3389/fendo.2022.1034148

period. Eating habits were assessed in children and mothers before and after dietary intervention. Sixty-seven questionnaires before and after the dietary intervention were used for analysis.

Results: Sixty-eight children completed the study. Those who did not complete the study came from families living in rural areas and their mothers mostly had primary or vocational education. One-year dietary education resulted in significant improvements in body weight, waist and hip circumference, WHtR and selected measured carbohydrate and lipid metabolism parameters with the exception of total cholesterol. The one-year dietary intervention did not have the same effect on the change in dietary habits in children and in their mothers.

KEYWORDS

childhood obesity, lipid profile, nutrition, behavioral intervention, glucose metabolism

Introduction

Obesity has accompanied humans since prehistoric times. It was considered a sign of prosperity, successful social status and health (1). Nowadays, it is recognised as a civilisation disease that increases mortality and diminishes quality of life, also among children and adolescents (2). Among 5-19 year olds, the prevalence of overweight and obesity has increased dramatically from 4.0% to 22.0% over the last four decades (3, 4).

Overweight and obesity in childhood carry many health risks. It is a risk for the development of musculoskeletal disorders, type 2 diabetes, liver disease, as well as cardiovascular disease and emotional and psychosocial problems (5).

environmental factors are responsible for the main reason for the accumulation of excessive adipose tissue. Among these, poor dietary habits and lack of exercise play the greatest role (6). The family prevalence of obesity (7) and family dietary patterns (8) have also received considerable attention. The parents' diet acts as a pattern for the child's nutrition. Parents shape dietary habits, especially in the early years of a child's life. The family diet determines what habits the child will adopt and how their diet will be shaped in adulthood (9). One of the most common incorrect dietary habits in overweight children is not eating breakfast regularly or skipping it altogether (10).

Additionally, it is worrying that the frequency of eating decreases with the age of the child (11). Another abnormal eating habit is snacking between meals, mainly on sweets, the consumption of which at least once a day was declared by as many as 60.0% of primary school children (12). Highly undesirable, and commonly reported among school children, is the consumption of fast-foods. Consumption of this type of food at least once a month was declared by 30.0% of 8–10 year olds (13). A very worrying trend is the increasing consumption of this type of food in the youngest age groups. After the age of 12 months, 2.5% of children ate this type of food at least once a month, and at pre-school age 11.5% at least once a week. In the same age group, eating two dinners is equally common. This results in overfeeding and a consequent imbalance in the body's energy intake, which, in the absence of sufficient physical activity, results in increased body weight (14). A major controversy in terms of child and adolescent health is the amount of sweetened, carbonated and non-carbonated drinks consumed. Among 10–17 year olds, coloured fizzy drinks are listed as the second most consumed liquid of the day (15). It has also been shown that the incidence of overweight was higher in children who consumed more than 350 ml of juice per day (16). The habit of consuming sufficient amounts of fruit and vegetables is still unsatisfactory (17). Children mainly consume them in the form of salads with lunch (18) and only 1 in 10 preschool children meet the recommended intake of 4-5 portions of vegetables per day (19). Furthermore, the lack of acceptance of cereal products containing whole grains is worrying. Approximately 90.0 per cent of adolescents do not eat whole grain cereal products, such as whole grain bread, on a daily basis, and the consumption of groats or rice less than once a week was declared by almost 50.0 per cent of adolescents. Lack of the habit of eating fish is still a major problem. Their occasional consumption is declared by 50.0–70.0% of schoolchildren (20). The reluctance to consume fish does not change even in those with unrestricted access to it. Only less than 45.0% of adolescents from schools at the seaside meet WHO expert recommendations for regular consumption of fish at least 1–2 times a week (21). In addition to poor dietary habits, low

physical activity is an important factor increasing the risk of obesity and overweight in children. In recent years, physical activity patterns of school-aged children, have changed dramatically. Industrialisation, technical progress and modern means of transport have created ideal conditions for the development of obesity. Television, computers, limited access to playgrounds and additional activities at school have contributed to a reduction in physical activity (22). On average, 5–10 hours of physical activity per week were declared by only about 28.0% 6–10 year olds, and about 27.0% reported only 3-5 hours of physical activity/week (23).

Many specialists believe that the treatment of obesity should be multidirectional, effective and, if possible, as minimally invasive as possible. In addition to the use of restrictive diets, pharmacotherapy and bariatric surgery, nutrition education programmes are an alternative to improve the health of obese and overweight children and adolescents. To be fully effective, these programmes should involve the whole family, or at least those most involved in perpetuating healthy eating patterns, usually the mother. One such model is the "Healthy Food Pyramid", created by a group of experts from the Institute of Food and Nutrition in Warsaw. "The Healthy Food Pyramid" is a graphic illustration of the principles of proper nutrition, which forms the basis of dietary recommendations in Poland (24).

Considering the constant increase in the prevalence of overweight and obesity in the developmental age population and the lack of effective methods to combat its prevalence, it seems advisable to attempt to assess the effect of one-year-long dietary education of children and adolescents with excessive body weight on their eating habits and the eating habits of their mothers, as well as selected anthropometric and biochemical parameters in these children, using a simple educational tool, which is the Food Pyramid.

Patients and methods

Eligibility

Children aged 3–18 years and their parents, referred to the Department of Paediatrics were invited to the study for planned diagnostics of the causes of excessive body weight. Ultimately, only mothers participated in the study with their children.

Participation in the study was voluntary. Each mother and child over 13 years of age were given written information about the purpose of the study. Informed consent to participate in the study was obtained from each mother, as well as children over 13 years of age.

Approval for the study was obtained from the Bioethics Committee at the (decision number KB-0012/34/11, dated 16th May 2011).

Children with underlying conditions were excluded, such as:

- congenital diseases predisposing to obesity, e.g. Down syndrome, Prader-Willi syndrome,
- thyroid disorders,
- adrenal gland dysfunction,
- gonadal disorders,
- intellectual disabilities,
- chronically ill children whose treatment may have influenced weight gain, such as steroid therapy.

Ninety-four children with excess body weight, The study was completed by 68 (72.3%) children, aged 4-17 years ($\overline{x}$=12.4 ± 3.7), including 37 (54.4%) girls and 31 (45.6%) boys.

Anthropometric parameters, biochemical test and dietary habits were assessed in this group. All measurements were made before and after the dietary intervention.

Methods of anthropometric measurements and adipose tissue measurement

The following anthropometric parameters were measured in the children: body height, with an accuracy of 0.01 cm, using a stadiometer (type Harpenden 602VR, UK); body weight, with an accuracy of 0.01 kg, on a medical scale (Radwag WPT 60/150 OW, Poland); waist circumference, with an accuracy of 0.5 cm, with a centimetre tape.

The obtained results of individual measurements were related to the norms for the population of Polish children, developed in the OLA and OLAF project (25, 26). The cut-off points defining overweight and obesity within this project are consistent with the criteria of the International Obesity Task Force (IOTF) (27, 28).

Based on the measurements taken, body mass index (BMI) was calculated, according to the formula: BMI = body weight (kg)/ body height (m2). Waist-height ratio (WHtR), according to the formula: WHtR = waist circumference(cm)/body height (cm). Abdominal obesity was diagnosed when waist circumference was ≥90th percentile for sex and age and WHtR >0.5 (29).

A standard deviation-score (SDS) was calculated to eliminate the effect of age and sex of the studied children on the measured anthropometric parameters. To calculate the SDS, reference values for the population of Polish children from the OLA and OLAF project were used (25, 26). According to the adopted criteria, BMI ≥+1SDS and <+2 SDS was considered overweight, and BMI ≥2 SDS was considered obesity. Body fat and lean muscle content were measured using an electrical bioimpedance analyser (Jawon IOI-353, Selvas Healthcare, South Korea). The test was performed according to the manufacturer's instructions. As specified by the manufacturer, the test was not performed in children under 5 years of age.

Methods of anthropometric measurements in mothers of the studied children

The data collected during the interview and available medical records were used to calculate the body mass index in the mothers of the studied children. BMI was calculated from these data. WHO criteria were used to assess the nutritional status of the mothers (30).

Methods for biochemical measurements

Carbohydrate metabolism was assessed by measuring glucose and insulin levels at fasting and 120 min after an oral glucose tolerance test with load of 1.75 g glucose/kg body weight (maximum 75 g). Serum glucose and insulin concentrations were determined using Cobas C501 device (Roche, Germany).

For the assessment of carbohydrate metabolism, the guidelines of the Polish Diabetes Association (31) were used, assuming the following values for fasting measurements: normal fasting glycaemia: 70–99 mg/dl; impaired fasting glucose (IFG): 100–125 mg/dl; fasting glycaemia ≥126 mg/dl – diabetes mellitus. The following glycaemic values were assumed at 120 minutes of the OGTT: normal glucose tolerance (NGT): glycaemia <140 mg/dl; impaired glucose tolerance (IGT): glycaemia 140–199 mg/dl, diabetes mellitus: glycaemia ≥200 mg/dl.

Hyperinsulinaemia at 120 min of the OGTT test was diagnosed at insulin levels >75 μIU/ml (32).

To assess serum lipid metabolism in the study group, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride (TG) concentrations were measured using Cobas C501 device (Roche, Germany). The National Cholesterol Education Program (NCEP) expert guidelines (33) were used to assess lipid metabolism, adopting the following reference values: total cholesterol – <170 (mg/dl); LDL-cholesterol – <110 (mg/dl); HDL-cholesterol – >45 (mg/dl); triglycerides: <75 (mg/dl) (0–9 years of age); <90 (mg/dl) (10–19 years of age).

Using the measured biochemical parameters, HOMA insulin resistance index (HOMA-IR, Homeostasis Model Assessment – Insulin Resistance) was calculated, using the formula: HOMA-IR index= fasting glucose concentration (mg/dl) x fasting insulin concentration (μIU/ml)/405 (34).

The literature shows that there is no established value for determining insulin resistance in children. It has been shown that HOMA-IR increases during adolescence (35). Due to the heterogeneity of the study group in terms of age and sex, HOMA-IR centiles developed for the Caucasian population were used, and insulin resistance was diagnosed at HOMA-IR ≥97th percentile for age and sex [33/39/].

Diet analysis methods

The study used the dietary survey method and the research tool of a proprietary questionnaire to assess dietary habits and frequency of consumption. The nutrition part of the questionnaire was preceded by questions relating to the socio-demographic characteristics of the subjects and the anthropometric parameters of mothers and children. The dietary questionnaire for mother and child consisted of 30 questions. In the questions concerning frequency of product intake, a 7-point scale was used to assess it, where the answer, several times a day scored 6 points, once a day – 5 points, several times a week – 4 points, once a week – 3 points, several times a month – 2 points, once a month – 1 point, does not eat – 0 points, and a 3-point scale of answers, where the answers were scored: eats for every meal – 2 points, eats but not for every meal – 1 point, does not eat – 0 points. Children under 13 years of age completed the questionnaire with their mother, whereas children over 13 years of age completed the questionnaire on their own or with the mother's help.

The "Healthy Food Pyramid" and the 10 Principles of Healthy Eating of Children and Adolescents developed by the Institute of Food and Nutrition in Warsaw, 2009, were used to change eating habits and improve nutrition (24). In education, particular emphasis was placed on: reducing the intake of saturated fat in the diet, by: eliminating snacks such as crisps, nuts, salty sticks and fast-foods from the diet, limiting fried meals in favour of stewed, boiled and steamed ones, increasing the consumption of fish and lean meat and dairy products; increasing the amount of fruit and vegetables consumed; reducing the intake of sugar, sweets and sweetened dairy products; eliminating sweetened drinks and fruit juices; introducing whole grain cereal products; drinking water daily and eating an adequate amount of food regularly; increasing control over nutritional behaviours and informed decisions considering choosing the right product – the ability to analyse the products' labels; encouraging daily physical activity.

Dietary education

During the study, children and their mothers participated in 6 individual educational meetings during which dietary education and correction of dietary errors were carried out. During the first 3 months, follow-ups took place at a frequency of 1 meeting per month, follow-up 4, 5 and 6 – 1× every 3 months. On the 6th visit, the children and their mothers were invited back to the Clinic for follow-up examinations and measurement of anthropometric parameters and evaluation of the dietary habits acquired during the dietary education.

Statistical analysis

Quantitative and rank variables were analysed with non-parametric tests: Mann-Whitney test for comparisons between groups and Wilcoxon signed-rank test for comparisons between two time points (before and after dietary intervention) within one group. Dichotomous variables (yes/no) were compared between groups with Fisher exact test, and between time points with McNemar's $\chi 2$ test. Differences with $p<0.05$ were considered statistically significant. Statistical analysis was performed with Statistica 13 software.

Results

Study group characteristics

Out of the 94 children who qualified for the study, 26 (27.7%) opted out from further participation in education, at various stages. Those who did not complete the study came from families living in rural areas, and their mothers mostly had primary or vocational education. In terms of comparison of anthropometric parameters, the children who completed the dietary intervention did not differ from those who dropped out of the intervention. Sixty-eight children were further analysed. Based on BMI SDS, 59 (86.8%) children were found to be obese and 9 (13.2%) overweight. In 41 children (60,3%) BMI exceeded +3 SDS and in almost one in 10 subjects +6 SDS. Due to the relatively small size of the study population, children with overweight and obesity were combined into one group.

The education levels of the studied children's mothers varied. Almost half (47.8%) of them had secondary education, more than a third (35.8%) had higher education, and primary or vocational education 8.9% and 7.5% respectively. The vast majority of mothers (83.6%) were professionally active. In the study group, 2/3 (64.2%) of the mothers lived in urban areas and 1/3 (35.8%) in rural areas. Nearly 3/4 (68.7%) of the mothers were also characterized by excessive body weight.

Before the dietary intervention, the relationship between mothers' nutritional status and the prevalence of overweight and obesity in their children was also analysed. No such relationship was observed (p=0.08). However, it was found that the prevalence of overweight decreased and the prevalence of obesity increased in children following an increase in maternal BMI.

In addition, the BMI of the mothers of children with overweight and obesity was assessed before the dietary intervention. It was found that the BMI of mothers of children with obesity was significantly higher compared to mothers of children with overweight ($\bar{x}$=28.28 ± 4.98 vs. $\bar{x}$=23.46 ± 5.71, respectively; p=0.02).

The effect of one-year dietary intervention on measured anthropometric parameters of the studied children and their mothers

For some measurements, the differences in the number of measured biochemical parameters were caused by insufficient material collected for the assay, haemolysis of the blood and problems related to the consumption of the recommended amount of glucose in the OGTT by the subjects.

One year of dietary education resulted in a significant (p<0.00001) improvement in body weight, waist and hip circumference and WHtR (Table 1). There was also a significant increase in the amount of lean muscle tissue and a decrease in the percentage of body fat in the studied children.

After dietary intervention, it was additionally shown that the children of mothers with normal BMI were significantly more likely to have reduced body weight than children of mothers with overweight and obesity (p=0.02). The difference in BMI of

TABLE 1 Changes in measured anthropometric parameters in the studied children before and after the dietary intervention.

Measured parameter	n	Before the dietary intervention		After the dietary intervention		Difference	p
		$\bar{x}$ ± SD	Me (min.-max.)	$\bar{x}$ ± SD	Me (min.-max.)	$\bar{x}$ ± SD	
Weight SDS	68	3.47 ± 1.57	3.53 (0.05-7.25)	2.83 ± 1.62	2.57(-0.19-7.94)	−0.64 ± 082	<0.00001
BMI SDS	68	3.70 ± 1.68	3.56 (1.12-9.68)	2.90 ± 1.70	2.76 (0.09-8.57)	−0.80 ± 0.96	<0.00001
Waist circumference SDS	68	4,27 ± 1.79	3.91 (1.60-9.76)	3.50 ± 1.92	3.15 (0.07-9,22)	−0,.77 ± 1.23	<0.00001
Hip circumference SDS	68	2.85 ± 1.37	2.74 (0.77-7.14)	2.07 ± 1.48	1.83 (-0.32-8.38)	−0.78 ± 0.97	<0.00001
WHtR	68	0,60 ± 0.06	0.58 (0.50-0.82)	0.56 ± 0.07	0.55 (0.43-0.77)	−0.04 ± 0.04	<0.00001
Adipose tissue [kg]	66*	24.62 ± 12.56	2.22 (6.20-61,60)	22.86 ± 12.23	19.55 (4.20-65.20)	−1.76 ± 4.85	0.03
Adipose tissue percentage	66*	32.27 ± 5.8	33.3 (18.4-42,20)	30.13 ± 7.02	30.05 (12.4-42,20)	−2.14 ± 3.54	<0.00001
Lean muscle tissue [kg]	66*	43.89 ± 4.47	44.6 (18.4-76.30)	45.23 ± 14.01	45.85 (19.80-81.10)	1.34 ± 2.83	<0.00001

BMI, body mass index; WHtR, waist-height ratio; SDS, standard deviation score; SD, standard deviation; p, probability; $\bar{x}$, mean value; n, sample size; * in 2 children adipose and muscle tissue were not assessed due to their young age (<5).

mothers of children with obesity and mothers of children with overweight after the dietary intervention was further assessed. On average, the BMI of mothers of children with obesity was significantly higher compared to the BMI of mothers of children with overweight (28.9 ± 4.8 vs. 25.4 ± 5.5, respectively; p=0.01).

Effect of one-year dietary education on measured parameters of lipid metabolism in the studied children

After one year of dietary education, significant improvement in almost all measured parameters of lipid metabolism was observed in the children who completed the education programme, with the exception of total cholesterol concentrations (Table 2). There was a significant increase of HDL-cholesterol fraction (p<0.00001) and a significant reduction of LDL-cholesterol and TG fraction (p=0.02).

Effect of one-year dietary education on selected parameters of carbohydrate metabolism in the studied children

As shown in Table 2, there was a significant effect of annual dietary education on the improvement of all measured carbohydrate parameters in the study group.

Effect of one-year dietary education on the change in frequency of consumption of selected food groups in the children and their mothers included in the study

Sixty-eight questionnaires from children and 67 questionnaires from mothers before and after the one-year dietary intervention were eligible for detailed analysis. 2 mothers did not agree to complete their dietary questionnaire – one before the intervention and the other after the dietary intervention. Two children did not complete the questionnaires – one regarding the consumption of sweetened dairy products and the other regarding the use of cooking techniques.

The one-year dietary education did not have the same effect on the change in eating habits of children and their mothers (Table 3). Mothers significantly increased the number of meals consumed, from three to four per day (p=0.02). The children, on the other hand, significantly decreased the frequency of sweets consumption (p=0.0001). Before the intervention, they consumed sweets most commonly several times a week, while after the intervention they consumed sweets once a week on average.

In contrast, the nutritional intervention had a negative effect on the frequency of children's vegetable consumption. After the intervention, children consumed vegetables significantly less often (p=0.002). Mothers, on the other hand, significantly increased the frequency of their consumption (p<0.00001) and ate them with every meal of the day. The mothers also significantly improved the frequency of consumption of whole grain cereal products, up to once per day (p=0.04).

In addition, the dietary intervention resulted in a significant improvement in children's and their mothers' self-assessment of correct dietary habits (p=0.0008; p=0.01, respectively). However, there was no effect of the dietary intervention on changing the frequency of fruit, fish and fast-foods consumption by either the children or their mothers.

As seen in Table 4, the dietary intervention resulted in a more frequent change in the assessed eating habits in children than in their mothers. There was a significant 17.7% reduction in the frequency of snacking between meals (p=0.009) and a significant 20.6% reduction in the frequency of after-dinner snacking in the children studied (p=0.007). The educational meetings significantly influenced the water drinking habit, primarily in children. There was a significant 20.6% increase

TABLE 2 Changes in lipid and glucose metabolism parameters before and after the dietary intervention.

Measured parameter	n	Before the intervention $\bar{x}$ ± SD	After the intervention $\bar{x}$ ± SD	Difference $\bar{x}$ ± SD	p
Total cholesterol [mg/dl]	67	166.11 ± 27.62	161.91 ± 29.9	−4.20 ± 20.39	0.22
HDL-CH [mg/dl]	67	46.19 ± 10.98	51.5 ± 13.8	5.31 ± 7.72	<0.00001
TG [mg/dl]	67	114.45 ± 60.59	97.5 ± 49.4	−16.95 ± 53.98	0.02
LDL-CH [mg/dl]	66	108.89 ± 26.12	102.6 ± 29.4	−6.29 ± 19.23	0.02
Fasting glucose [mg/dl]	67	88.44 ± 7.87	86.25 ± 8.58	−2.19 ± 9.39	0.04
Glucose in 120' of OGTT [mg/dl]	65	110.90 ± 20.32	102.80 ± 17.67	−8.10 ± 27.43	0.01
Fasting insulin [μIU/ml]	66	21.37 ± 10.86	16.46 ± 8.35	−4.91 ± 11.56	0.001
Insulin in 120' of OGTT [μIU/ml]	64	125.02 ± 105.09	86.42 ± 68.19	−38.60 ± 98.08	0.005
HOMA –IR	65	4.74 ± 2.49	3.53 ± 1.87	-1.21 ± 2.72	0.002

HDL, CH-HDL-cholesterol; TG, triglycerides; LDL-CH, LDL-cholesterol; OGTT, oral glucose tolerance test; HOMA-IR, insulin resistance index ;SD, standard deviation; p, probability; $\bar{x}$, mean value; n, sample size.

10.3389/fendo.2022.1034148

TABLE 3 Effect of dietary intervention on chosen eating habits in children and their mothers.

Eating habits	R	Children n = 68		Mothers n = 66	
		Before intervention	After intervention	Before intervention	After intervention
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Number of meals consumed daily		4.48 ±0.90	4.64 ±0.76	3.77 ±0.89	4.03 ±0.78
	6				
	5				
	4				
	3				
	2	p=0.12		p=0.02	
Frequency of sweets consumption		3.95±1.25	3.19±1.11	3.54±1.42	3.19±1.08
	several times a day 6				
	once a day 5				
	several times a week 4				
	once a week 3				
	several times a month 2				
	once a month 1				
	doesn't eat 0	p=0.0001		p=0.057	
Frequency of vegetables consumption		1.13 ±0.59	0.79 ±0.56	0.74 ±0.88	1.57 ±0.49
	eats with every meal 2				
	eats. not with every meal 1				
	doesn't eat 0	p= 0.002		p< 0.00001	
Frequency of whole grain cereal products consumption		3.85±1.63	4.20±1.63	4.21±1.35	4.62±1.38
	several times a day 6				
	once a day 5				
	several times a week 4				
	once a week 3				
	several times a month 2				
	once a month 1				
	doesn't eat 0	p=0.10		p=0.04	
Self-assesment – do they consider their nutrition correct?		0.89±0.58	1.26±0.61	0.87±0.41	1.04±0.44
	yse 2				
	puts an effort 1				
	no 0	p=0.0008		p=0.01	

R, ranks; SD, standard deviation; $\bar{x}$, mean value; p, probability; n, sample size.

in the frequency of water consumption in the children studied (p=0.003). The nutritional intervention also influenced the resignation from the consumption of sweet drinks, not only by the children, but also by their mothers. A 26.5% significant reduction in the frequency of sweetened beverage consumption was observed in the studied children (p=0.0002) and a 13.6% significant reduction in the frequency of sweetened beverage consumption (p=0.03) in the studied mothers. The dietary

10.3389/fendo.2022.1034148

TABLE 4 Effect of one-year dietary intervention on changes in dietary habits in studied children and their mothers.

Assesed habit		Assessed habit – before intervention							
		Children n = 68				Mothers n = 66			
		No n (%)	Yes n (%)	Total n (%)	p	No n (%)	Yes n (%)	Total n (%)	p
Assessed habit – after intervention	Do they snack between meals								
	No	0 (0%)	15(22.1%)	15 (22.1%)	0.009	1 (1.5%)	3 (4.5%)	4 (6%)	0.61
	Yes	3 (4.4%)	50 (73.5%)	53(77.9%)		1 (1.5%)	61 (92.5%)	62 (94%)	
	Total	3 (4.4%)	65(95.6%)	68 (100%)		2 (3.0%)	64 (97.0%)	66 (100%)	
	Do they snack after dinner								
	No	29(42.7%)	19(27.9%)	48 (70.6%)	0.007	25 (37.9%)	14 (21,2%)	39(59.1%)	0.06
	Yes	5 (7.3%)	15 (22.1%)	20(29.4%)		5 (7.6%)	22 (33,3%)	27(40.9%)	
	Total	34(50.0%)	34(50.0%)	68 (100%)		30 (45.5%)	36 (54,5%)	66 (100%)	
	Do they like vegetables								
	No	4 (5.9%)	4 (5.9%)	8 (11.8%)	<0.00001	1 (1.5%)	7 (10.6%)	8 (12.1%)	0.02
	Yes	52(76.4%)	8 (11.8%)	60(88.2%)		0 (0%)	58 (87.9%)	58(87.9%)	
	Total	56(82.3%)	12(17.7%)	68 (100%)		1(1.5%)	65(98.5%)	66 (100%)	
	Do they drink water								
	No	4 (5.9%)	3 (4.4%)	7 (10.3%)	0.003	7 (10.6%)	4 (6.1%)	11(1.,7%)	0.75
	Yes	17 (25%)	44 (64.7%)	61(89.7%)		6 (9.1%)	49 (74.2%)	55(83.3%)	
	Total	21(30.9%)	47(69.1%)	68 (100%)		13 (19.7%)	53 (80.3%)	66 (100%)	
	Do they drink sweet beverages								
	No	10 (14.8%)	20(29.4%)	30 (44.2%)	0.0002	14 (21.2%)	12(18.2%)	26(39.4%)	0.03
	Yes	2 (2.9%)	36 (52.9%)	38(55.8%)		3 (4.6%)	37 (56.1%)	40(60.6%)	
	Total	12 (17.7%)	56(82.3%)	68 (100%)		17 (25.8%)	49(74.2%)	66 (100%)	

– habit habit declared before intervention, – habit seen only before intervention, – habit declared after intervention, – habit seen only after intervention, p- probability, n – sample size.

intervention resulted in a positive change in the acceptance of vegetables by children, but not by mothers. There was a 70.5% significant increase in the frequency of vegetable acceptance in children, while there was a 10.6% significant decrease in mothers. (p=0,02). Unfortunately, acceptance of vegetable intake did not result in realistically higher vegetable intake. Furthermore, there was no effect of the dietary intervention on the other dietary habits assessed, such as first and second breakfast consumption, and acceptance of fruit. Table 5 shows the effect of one year of dietary education on the consumption preferences of selected dairy products. The dietary intervention significantly increased the consumption of plain dairy products by the study children, but not by their mothers. A significant 27.9% increase in the frequency of consumption of plain dairy products (p=0.005) by the studied children was observed. Moreover, the effect of the one-year intervention was a significant 26.9% reduction in the frequency of consumption of sweetened dairy products by the children (p=0.001). The opposite effect of the dietary intervention was observed in mothers. There was a 24.3% significant increase in the frequency of mothers' consumption of sweetened dairy products (p=0.001). On the other hand, no significant effect of the dietary intervention was observed on the consumption of milk by the studied children. Half of the children did not consume milk both before and after the one-year dietary intervention.

Another diet component assessed was cereal products. As shown in Table 6, the one-year dietary intervention had a significant effect of reducing sweetened breakfast cereal by 36.8% in children (p=0.00003). However, no such effect was observed in mothers.

Dietary education significantly reduced the frequency of children's consumption of wheat bread by 26.5% (p=0.001), but had no effect on increasing the consumption of whole-grain bread. No effect was observed in mothers. One-year dietary education significantly influenced the replacement of wheat pasta with wholemeal pasta in the studied children. There was a 19.1% significant decrease in the frequency of wheat pasta consumption (p=0.02) in favour of a 25.0% significant increase in the frequency of wholemeal pasta consumption (p=0.002). Additionally, dietary education significantly increased the frequency of brown rice consumption by 25.0% in children (p=0.002) and by 16.6% in mothers (p=0.02). No such effect was observed for white rice and buckwheat kasha consumption.

The effect of the one year dietary intervention on the type of meat consumed was also analyzed (Table 7). The dietary

10.3389/fendo.2022.1034148

TABLE 5 Effect of one-year dietary intervention on consumption of selected dairy products consumed by studied children and their mothers.

Assesed habit		Before intervention – dairy products consumed daily							
		Children n = 68				Mothers n = 66			
		No n (%)	Yes n (%)	Total n (%)	p	No n (%)	Yes n (%)	Total n (%)	P
After intervention- dairy products consumed daily	Plain dairy products								
	No	30 (44.1%)	4 (5.9%)	34 (50.0%)	0,005	11(16.7%)	16 (24.2%)	27 (40.9%)	1.0
	Yes	23(33.8%)	11 (16.2%)	34(50.0%)		15(22.7%)	24 (36.4%)	39 (59.1%)	
	Total	53 (77.9%)	15(22.1%)	68 (100%)		26(39.4%)	40 (60.6%)	66 (100%)	
		Children n=67				Mothers n=66			
	Sweetened dairy products								
	No	19 (28.3%)	23(34.4%)	42 (62.7%)	0,001	44(66.7%)	3 (4.5%)	47 (71.2%)	0.001
	Yes	5 (7.5%)	20 (2.,8%)	25(37.3%)		19(28.8%)	0 (0%)	19(28.8%)	
	Total	24 (35.8%)	43(64.2%)	67 (100%)		63(95.5%)	3 (4.5%)	66 (100%)	

– habit declared before intervention, – habit seen only before intervention, – habit declared after intervention, – habit seen only after intervention, p – probability, n – sample size.

intervention significantly, by 16.1%, reduced the frequency of children's consumption of pork meat (p=0.02). For the other two assessed meat varieties and for the three assessed types of meat consumed by the mothers, no such effect was observed.

The last food group analysed was the type of fat and the preferred cooking techniques used. As shown in Table 8, one year's dietary education had a notable effect only on mothers.

There was a 19.7% significant increase in the frequency of fat-free cooking techniques used (p=0.001) and a 15.2% significant increase in the frequency of use of plant-based fat by the mothers surveyed (p=0.01).

Discussion

Overweight and obesity in the developmental age population remains an unresolved problem that is increasing worldwide (36). It is necessary to take measures to prevent new and eliminate existing disorders. One such action is a change in lifestyle, an important component of which, alongside physical activity, is a change in eating habits. Sisson et al. (37), in a systematic review of interventions conducted in children with obesity, showed that out of 45 dietary interventions, 87.0% resulted in the desired outcome.

The dietary intervention we conducted was based on the principles of healthy nutrition and the 2009 'Healthy Food Pyramid' model developed by experts from the Institute of Food and Nutrition (24). In addition, it was conducted in the presence of one of the caregivers – the mother. These efforts showed a significant effect on improving both somatic development parameters, biochemical indices and nutritional habits in the studied children.

One year of dietary education in the studied children resulted in significant differences in the measured parameters of somatic development (respectively: BMI SDS −0.80; waist circumference SDS −0.77; WHtR −0.04; −1.76 kg body fat; −2.14 body fat percentage and +1.34 kg lean muscle tissue). The effects obtained in our study may be due to several reasons including the fact that each educational activity took place in the presence of one of the parents. In a similar study, called WATCH IT (38), researchers found smaller differences in reduction of waist circumference, −0.08 SDS. In contrast, unfavourable results were observed for BMI SDS and body fat percentage, (by +0.03 SDS BMI and +1.4% body fat, respectively). The cited authors explain this result, among other things, by not being able to involve the child's caregiver in the study. Conversely, in the study by Savoye et al. (39), a 4.0% reduction in body fat (−3.7kg) was achieved. Greater therapeutic success in these studies was achieved by involving families who attended meetings with a dietician and physical activity specialist. In a study evaluating one of the risk factors for cardiovascular disease, expressed as the ratio of waist circumference to body height in a group of 5–12 and 13–17 year-olds, Ranucci et al. (40) obtained a significant reduction in WHtR, from 0.63 to 0.61, in a group of 5-12 year-old children, and from 0.65 to 0.63 in a group of adolescents. In the above studies, active physical activity was an additional intervention. It is known from the literature that moderate exercise alone does not cause weight loss, but when combined with changes in dietary habits, significant weight loss can be achieved and maintained. Therefore, one element of education in our study was to encourage children and their parents to undertake additional physical activity. However, at the end of the one-year dietary intervention, no significant increase in time spent on additional physical activity was observed. In our study, children with excessive body weight as a result of dietary education and with maternal involvement achieved significantly greater differences in measured parameters of somatic development than in the work presented above. The

10.3389/fendo.2022.1034148

TABLE 6 Effect of one-year dietary intervention on cereal products consumption in studied children and their mothers.

Assessed habit		Before intervention – cereal product consumed daily							
		Children n = 68				Mothers n = 66			
		No n (%)	Yes n (%)	Total n (%)	p	No n (%)	Yes n (%)	Total n (%)	P
After intervention – cereal product consumed daily	Oat flakes								
	No	34 (50.0%)	7 (10.3%)	41 (60.3%)	0.02	33 (50%)	5 (7.5%)	38 (57.5%)	0.21
	Yes	20(29.4%)	7 (10.3%)	27(39.7%)		11 (17%)	17 (25.5%)	28 (42.5%)	
	Total	54 (79.4%)	14 (20.6%)	68 (100%)		44 (67%)	22 (33%)	66 (100%)	
	Sweetened breakfast cereal								
	No	23 (33.8%)	29 (42.7%)	52 (76.5%)	0.00003	48(72.7%)	5 (7.6%)	53 (80.3%)	1.0
	Yes	4 (5.9%)	12 (17.6%)	16(23.5%)		4 (6.1%)	9 (13.6%)	13 (19.7%)	
	Total	27 (39.7%)	41 (60.3%)	68 (100%)		52 (78.8%)	14 (21.2%)	66 (100%)	
	Wheat bread								
	No	25 (36.8%)	23 (33.8%)	48 (70.6%)	0.001	34 (51.5%)	13 (19.7%)	47 (71.2%)	0.26
	Yes	5 (7.3%)	15 (22.1%)	20(29.4%)		7 (10,.%)	12 (18.2%)	19 (28.8%)	
	Total	30 (44.1%)	38 (55.9%)	68 (100%)		41 (62.1%)	25 (37.9%)	66 (100%)	
	Wheat pasta								
	No	18 (26.5%)	20 (29.4%)	38 (55.9%)	0.02	19 (28.8%)	16 (24.2%)	35 (53.0%)	0.32
	Yes	7 (10.3%)	23 (33.8%)	30(44.1%)		10 (15.2%)	21 (31.8%)	31 (47.0%)	
	Total	25 (36.8%)	43 (63.2%)	68 (100%)		29 (44.0%)	37 (56.0%)	66 (100%)	
	Wholemeal pasta								
	No	38 (55.9%)	5 (7.3%)	43 (63.2%)	0.002	41 (62.1%)	5 (7.6%)	46 (69.7%)	0.06
	Yes	22(32.3%)	3 (4.5%)	25(36.8%)		14 (21.2%)	6 (9.1%)	20 (30.3%)	
	Total	60 (88.2%)	8 (11.8%)	68 (100%)		55 (83.3%)	11 (16.7%)	66 (100%)	
	Brown rice								
	No	37 (54.4%)	6 (8.8%)	43 (63.2%)	0.002	40 (60.6%)	4 (6.1%)	44 (66.7%)	0.02
	Yes	23(33.8%)	2 (3.0%)	25(36.7%)		15(22.7%)	7 (10.6%)	22(33.3%)	
	Total	60 (88.2%)	8 (11.7%)	68 (100%)		55 (83.3%)	11(16.7%)	66 (100%)	

– habit declared before intervention, – habit seen only before intervention, – habit declared after intervention, – habit seen only after intervention, p – probability, n – sample size.

available literature shows that parents, especially mothers, participate in the formation of the child's eating habits by providing appropriate products in the diet that they consider healthy. The mother is also a role model and controls food intake (41). A Cochrane review of erspectiv clinical trials of children in two age groups, 6–11 and 12–17 years, on "Interventions for the treatment of childhood obesity", found that behavioural interventions related to lifestyle changes, combined with parental involvement, are most effective (42). These interventions have been called the "gold standard for the management of childhood obesity" (43). Moreover, in our study, significant improvements were observed in body composition, not only in terms of fat reduction, but also in the percentage of muscle mass. The results obtained appear to be significant, as in some studies a reduction in muscle mass has also been reported in parallel with a reduction in fat mass (44). A reduction in lean body mass results in a "reduced metabolic rate" and consequently "slower weight loss" (45). In addition, it has been shown that the type of dairy consumed plays an important role in the prevention of overweight and abdominal obesity. In a study by Bradllee et al. (46), evaluating the association between intake of different foods and abdominal obesity in children and adolescents, conducted as part of NHANES III, the cited authors showed that dairy intake presents a negative correlation with central obesity. An identical association, but only in a group of girls, was found by Abreu et al. (47) and in a cross-sectional study by Hirschler et al. (48). This is explained by the protective role of calcium, which, through its effect on the regulation of energy metabolism, reduces lipogenesis in adipocytes and increases both faecal fat excretion and fat oxidation. In addition, whey proteins contained in dairy products cause a greater feeling of satiety (49) and are characterized by their high content of branched-chain amino acids, which are involved in muscle protein synthesis. Therefore, the energy consumed with dairy products is used to build muscle mass, at the expense of body fat (50). In our study, a significant increase in dairy consumption in the form of plain yoghurt after a one-year dietary intervention may have contributed to this outcome.

TABLE 7 Effect of one-year dietary education on the types of meat consumed by studied children and their mothers.

Assessed habit		Before intervention – type of meat consumed							
		Children n = 68				Mothers n = 66			
		No n (%)	Yes n (%)	Total n (%)	p	No n (%)	Yes n (%)	Total n (%)	p
After intervention – type of meat consumed	Pork								
	No	31 (45.6%)	15 (22.0%)	46 (67.6%)	0.02	25(37.9%)	13(19.7%)	38(57.6%)	0.38
	Yes	4 (5.9%)	18 (26.5%)	22 (32.4%)		8 (12.1%)	20(30.3%)	28(42.4%)	
	Total	35 (51.5%)	33 (48.5%)	68 (100%)		33(50.0%)	33(50.0%)	66 (100%)	
	Beef								
	No	51 (75.0%)	7 (10.4%)	58 (85.4%)	0.77	39(59.1%)	8 (12.1%)	47(71.2%)	0.80
	Yes	5 (7.3%)	5 (7.3%)	10 (14.6%)		8 (12.1%)	11(16.7%)	19(28.8%)	
	Total	56 (82.3%)	12 (17.7%)	68 (100%)		47(71.2%)	19(28.8%)	66 (100%)	

– habit declared before intervention, – habit seen only before intervention, – habit declared after intervention, – habit seen only after intervention, p – probability, n – sample size.

The changes in anthropometric parameters found in our study were accompanied by changes in measured biochemical parameters. The one-year dietary intervention significantly improved the lipid profile and the carbohydrate metabolism parameters.

The one-year dietary education applied in our study resulted in a significant reduction in LDL-cholesterol (by 6.29 mg/dl), triglycerides (by 16.95 mg/dl) and a significant increase in HDL-cholesterol (by 5.31 mg/dl). There was also a significant effect of the dietary intervention on the reduction of all measured parameters of carbohydrate metabolism, i.e.: fasting glucose and insulin levels (by 2.19 mg/dl and 4.91 μIU/ml, respectively) and in the 2h point of OGTT (glucose by 8.10 mg/dl and insulin by 38.60 μIU/ml). This effect may be related to both an improvement in anthropometric parameters and a change in dietary habits. The link between changes in BMI SDS and cardiovascular system and body composition has been analysed in several studies (51, 52). These publications noted that a reduction of 0.25 BMI SDS could be considered clinically significant for improving fasting insulin sensitivity and improving the total cholesterol/HDL-cholesterol ratio. However, greater benefits were observed with a reduction of 0.5 in BMI SD (53), and reduced markers of insulin resistance were indeed found when an even more significant reduction in BMI (≥0.5 BMI SDS) was achieved (54). In our study, after a one-year dietary intervention, the change in SDS BMI was −0.80. This change may therefore have contributed to an improved lipid profile and reduced insulin resistance.

As mentioned, diet is directly and indirectly related to cardiovascular risk factors, so improvements in lipid metabolism parameters depend not only on reducing body weight and body fat percentage, but also on diet composition (55). In the current study, the reduction in LDL-cholesterol levels may have been further associated with a reduction in pork consumption during the one-year dietary intervention. In contrast, the reduction in triglycerides may have been

TABLE 8 Effect of one-year dietary intervention on cooking techniques and type of cooking fats used by studied children and their mothers. .

Assessed habit		Children n = 67				Mothers n = 66			
		No n (%)	Yes n (%)	Total n (%)	p	No n (%)	Yes n (%)	Total n (%)	p
After intervention – cooking technique used and type of fat chosen	Cooking, steaming, grilling								
	No	3 (4.5%)	6 (8.9%)	9 (13.4%)	0.60	6 (9.1%)	1 (1.5%)	7 (10.6%)	0.001
	Yes	9 (13.4%)	49 (73.2%)	58 (86.6%)		14(21.2%)	45 (68.2%)	59(89.4%)	
	Total	12 (17.9%)	55 (82.1%)	67 (100%)		20 (30.3%)	46(69.7%)	66 (100%)	
	Plant-based								
	No	2 (3.0%)	8 (11.9%)	10 (14.9%)	0.38	4 (6.1%)	2 (3.0%)	6 (9.1%)	0.01
	Yes	13 (19.4%)	44 (65.7%)	57 (85.1%)		12(18.2%)	48 (72.7%)	60(90.9%)	
	Total	15 (22.4%)	52 (77.6)	67 (100%)		16 (24.3%)	50(75.7%)	66 (100%)	

– habit declared before intervention, – habit seen only before intervention, – habit declared after intervention, – habit seen only after intervention, p – probability, n – sample size.

associated with significantly lower sweets intake and more frequent choice of fat-free cooking techniques. In a study by Wengle et al. (56), a change in dietary habits through an increase in the consumption of whole-grain bread and fruit and vegetables was associated with a reduction in LDL-cholesterol levels. In our study, children significantly increased their intake of whole-grain cereal products after a one-year dietary intervention. In contrast, other researchers attributed the improvement in lipid and carbohydrate parameters not only to an increase in intake of whole-grain cereal products, but also to a reduction in fast-foods consumption. Gingras et al. (57) showed that consumption of fast food less than 1× per week was associated with less severe obesity in girls and less insulin resistance in boys. In our one-year dietary intervention, children's fast-food intake did not change much and they consumed these foods with similar frequency (on average 1 time per month), both before and after the one-year dietary intervention. Investigating the association of different dietary patterns with the prevalence of insulin resistance in children, Karatzi et al. (58) found that increased consumption of margarine, sweets and salty snacks was positively correlated with insulin resistance, while breakfast consumption showed a negative correlation. In our study, the one-year dietary intervention did not result in a significant change in the habit of eating the first breakfast. Before the dietary intervention, 60 (88.2%) and after the intervention 66 (97.1%) children consumed first breakfast. The improvement in measured parameters of carbohydrate metabolism can therefore be attributed to a significant improvement in other dietary habits, particularly the elimination of the consumption of sweets, sugar-sweetened beverages, sweetened, flavoured dairy products and sweetened, flavoured cereals, and the inclusion of plain yoghurt, oatmeal, brown rice and wholemeal pasta into the diet. Due to their favourable composition, i.e. the predominance of complex carbohydrates over simple carbohydrates, their consumption is followed by a gradual increase in blood glucose concentration, which "slows down" and reduces insulin secretion, preventing significant glycaemic fluctuations, prolonging the feeling of satiety and reducing the desire to snack between meals. As a matter of fact, in the dietary intervention we carried out, the children significantly reduced snacking between meals and after dinner. Our results are similar to those obtained by other authors with a low glycaemic index diet (59, 60). These data may indicate that proper dietary management, based on healthy eating principles, is important, and that the use of a dietary pattern such as the "Healthy Food Pyramid" (24) significantly improved the metabolic profile of the children studied. Meta-analyses (61) on dietary treatment of people at increased risk of cardiovascular disease and the effect of dietary treatment on lipid and carbohydrate metabolism are mainly concerned with adults. Few papers are devoted to the developmental age population. Therefore, the results we obtained can be used to develop dietary recommendations for this population. In addition, the reduction of disease risk factors in children in our study has major clinical implications. The improvements in lipid and carbohydrate metabolism in the present study are therefore as significant as those that can be achieved with pharmacological treatment, but without the fear of adverse effects from the medications used (62).

Analysing the dietary habits of children and their mothers in our studied population after a one-year dietary intervention showed that dietary education did not affect all children and their caregivers in the same way. Greater change in abnormal eating behaviour was observed only in children. An explanation for this could be sought, for example, in the way the intervention was conducted. This is because the children were actively involved in the training, kept food diaries which were analysed together and any errors were modified on an ongoing basis, which further enables the children to take control of their eating habits. In contrast, parental feeding errors and their replication are most often the result of parents' reluctance to change their own personal attitudes. The effectiveness of treatment of a child with excessive body weight is therefore increased if their parents also decide to change their diet. In the current study, it appears that only a small number of mothers' eating habits was modified by appropriate health-promoting education. This modification only addressed the correct number of meals consumed per day, more frequent consumption of vegetables, whole grain cereal products including brown rice, drinking sugary drinks, using fat-free cooking techniques and consuming vegetable fats.

Regardless of how the dietary modification plan is formulated, what tools are used and how long we plan to intervene, in the case of children it is important that their parents are also involved in the programme. This is because a child has limited capacity to make changes, such as eating habits. Numerous studies also indicate a lack of understanding on the part of parents on both how to form proper eating habits in their children and how to implement these recommendations. Therefore, a reasonable suggestion would be to provide parents with suitable education programmes, with opportunities for extended contact with nutrition professionals (63).

The study we conducted had some limitations. The lack of a control group made it impossible to compare the effect and strength of the interventions undertaken. On the other hand, all children, both with overweight and normal weight, and their families should have the same opportunity to obtain correct information on proper nutrition and current guidelines. Another limitation may be the size of the sample population that was subjected to the intervention. This is because the intervention was only targeted at the group of children referred to the Clinic for diagnosis of the causes of obesity. Therefore, this group may not be fully representative of all children with obesity, as children seeking treatment were more motivated to reduce their weight. Furthermore, the size of the group was limited, due to the time-consuming form of individual education. Additionally, due to the small number of children reaching the study's endpoint, the group was not

divided by sex and age for statistic analysis. During the 12-month dietary intervention, 26 (27.7%) participants dropped out at various stages of the intervention. The manner in which the questionnaires were completed and the under- or overestimation of foods that may be relevant to the prevention and treatment of excess body weight also need to be considered, as underestimation of food intake is a major problem in childhood obesity research.

In spite of the above limitations, it has been shown that educational measures aimed at family-based change in dietary behaviour have a positive effect on improving measured biochemical parameters. The plethora of health education methods means that an optimal model is constantly being pursued that will, in a meaningful way, help patients to sustainably accept the information provided. In the presented study, a simple model of the Healthy Eating Pyramid (24) was used, supported by the principles of healthy eating. As a result of its application, many children achieved not only a change in eating habits, but also an improvement in measured anthropometric and biochemical parameters without the use of pharmacological intervention.

However, further research is needed to assess the cause and effect of the nutritional interventions that were carried out.

Conclusions

1. In the studied group of children, the one-year dietary intervention contributed to a significant reduction in body weight, waist and hip circumference and body fat percentage. Moreover, a significant improvement in the measured parameters of carbohydrate metabolism, lipid metabolism, with the exception of total cholesterol concentration, was shown.
2. The applied nutrition pattern, based on the Healthy Food Pyramid, is an effective tool for eliminating or at least reducing incorrect eating habits in children with excess body weight.

As a result of the educational activities carried out, children significantly reduced their consumption of sweets and sugary drinks, sweetened dairy products and sugary breakfast cereals. They also reduced their consumption of wheat pasta and bread and their consumption of pork meat. They also significantly reduced "snacking" between meals and after dinner. However, they significantly increased their intake of water, natural dairy products and whole grain cereals.

3. The value of longitudinal dietary education, with constant monitoring of the effectiveness of the diet and the full involvement of the family, especially the mothers of children with excess body weight, was also demonstrated.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Bioethics Committee at the Pomeranian Medical University in Szczecin decision number KB-0012/34/11, dated 16th May 2011. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Conceptualization, KSt and MW; Methodology KSt, AH-J, KSa, EP, and MW; Visualization KSt, TJ, JS-D, KSa, EP, and MW; Writing—original draft preparation, KSt, AH-J, TJ, and MW; Writing—review and editing, KSt, AH-J, TJ, JS-D, and MW; Project administration, MW; Supervision, MW; All authors have read and agreed to the published version of the manuscript.

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 handling editor AMG declared a past co-authorship with the author EP.

Publisher's note

References

1. Galic S, Oskhill JS, Steinberg GR, Korek E. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol [online]* (2010) 316:129–39. doi: 10.1016/j.mce.2009.08.018

2. Gawlik A, Zachurzok-Buczyńska A, Małecka-Tendera E. Complications of obesity in children and adolescents. *Endokrynol Otyłość* (2009) 5(1):19–27.

3. Obesity and overweight: Key facts. *World Health Organ* (2018).

4. Ratajczak J, Petriczko E. The predictors of obesity among urban girls andBoys aged 8–10 years–a cross-sectional study inNorth-Western Poland. *Int J Environ Res Public Health* (2020) 17:1–13. doi: 10.3390/ijerph17186611

5. Turchiano M, Sweat V, Fierman A, Convit A. Obesity, metabolic syndrome, and insulin resistance in minority urban high school students. *Arch Pediatr Adolesc Med* (2012) 166(11):1030–6. doi: 10.1001/archpediatrics.2012.1263

6. Jarosz M. *Nutrition standards for the polish population*. Warsaw, Poland: Institute of Food and Nutrition (2017) p. 21–39.

7. Melzer RMTF, Magrini IM, Domene SMA, Martins PA. Factor associated with abdominal obesity in children. *Rev Paul Pediatr* (2015) 33(4):347–444. doi: 10.1016/j.rppede.2015.08.002

8. Maćkowiak-Lewandowicz K. Childhood obesity in historical 13Erspective. *Acta Medicorum Polonorum* (2017) 7:37–48.

9. Jarosz M. *A practical dietetic guide. institute of food and nutrition*. Warsaw, Poland: Institute of Food and Nutrition (2010).

10. Hoyland A, McWilliams KA, Duff RJ, Walton JL. Breakfast consumption in UK schoolchildren and provision of school breakfast clubs. *Nutr Bull* (2012) 37:232–40. doi: 10.1111/j.1467-3010.2012.01973.x

11. Smetanina N, Albaviciute E, Babinska V, Karinauskiene L, Albertsson-Wikland K, Petrauskiene A. Prevalence of overweight/obesity in relation to dietary habits and lifestyle among 7–17 years old children and adolescents in lithuani. *BMC Public Health* (2015) 15:1–9. doi: 10.1186/s12889-015-2340-y

12. Stefańska E, Falkowska A, Ostrowska L. Selected nutritional habits of children and teenagers aged 10 – 15 years. *Rocz Panstw Zakl Hig* (2012) 63 (1):91–8.

13. Krenc Z, Wosik-Erenbek M. Analysis of nutrition, nutritional habits and physical activity in younger school children in relation to prevention of civilization diseases. *Nowa Pediatr* (2011) 2:23–7.

14. Łoś-Rycharska E, Niecławska A. Fast-food consumption by 2–3 years old and preschool children. *Pediatr Pol* (2010) 85:345–52.

15. Jarosz A, Jarosz M. The structure of consumption of beverages in a group of adolescents aged 10-17 years. *Żyw Człow* (2015) 42(4):239–42.

16. Johnson L, Mander AP, Jones LR, Emmett PM, Jebb SA. Is sugar-sweetened beverage consumption associated with increased fatness in children? *Nutrition* (2007) 23(7–8):557–63. doi: 10.1016/j.nut.2007.05.005

17. Kolarzyk E, Janik A, Kwiatkowski J. Evaluation of the risk of metabolic syndrome among overweight and obese children. part i. anthropometric and biochemical factors of the risk of metabolic syndrome. *Probl Hig Epidemiol* (2011) 4:741–6.

18. Kostecka M. Environmental factors increasing the risk of overweight andobesity in children. *Med Metab* (2014) 3:40–6.

19. Harton A, Florczak J, Myszkowska-Ryciak J, Gajewska D. Fruit and vegetable consumption by preschool children. *Probl Hig Epidemiol* (2015) 96 (4):732–6.

20. Cieślik E, Filipiak-Florkiewicz A, Topolska K. The frequency of eating chosen groups of food products and the nutritional state among children 13-15 years old. *Żyw Człow* (2007) 36:846–51.

21. Dymkowska-Malesa M, Walczak Z, Zakrzewski J. Evaluation of the level of fish consumption among students in grades 4-6 of primary schools in koszalin. *Probl Hig Epidemiol* (2014) 95(1):182–5.

22. Jarosz M, Wolnicka K, Kłosowska J. Environmental factors associated with prevalence of overweight and obesity among children and adolescents. *Post Nauk Med* (2011) 9:770–7.

23. Woynarowska B, Mazur J, Oblacińska A. Participation of students in physical education lessons in schools in Poland. *Hygeia Public Health* (2015) 50 (1):183–90.

24. Jarosz M. *Nutrition standards for the polish population - amendment*. Warsaw, Poland: Institute of Food and Nutrition (2012).

25. Kułaga Z, Różdżyńska-Świątkowska A, Grajda A, Gurzkowska B, Wojtyło M, Góźdź M. Percentile charts for growth and nutritional status assessment in polish children and adolescents from birth to 18 year of age. *Stand Med Pediatr* (2015) 12:119–35.

26. Świąder-Leśniak A, Kułaga Z, Grajda A, Gurzkowska B, Góźdź M, Wojtyło M. References for waist and hip circumferences in polish children and adolescents 3-18 year of age. *Stand Med Pediatr* (2015) 12:137–50.

27. Kułaga Z, Różdżyńska A, Palczewska I, Grajda A, Gurzkowska B, Napieralska E. Percentile charts of height, body mass and body mass index in children and adolescents in Poland – results of the OLAF study. *Stand Med Pediatr* (2010) 7:690–700.

28. Kułaga Z, Grajda A, Gurzkowska B, Góźdź M, Wojtyło M, Swiąder A. Polish 2012 growth references for preschool children. *Eur J Pediatr* (2013) 172:753–61. doi: 10.1007/s00431-013-1954-2

29. Całyniuk B, Łukasik A, Szczepańska E. Grochowska-niedworok e. *Obes overweight complications Children youth. Piel Zdr Publ* (2014) 4(3):201–7.

30. NICE. *Obesity identification, assessment and management of overweight and obesity in children, young people and adults*. London: National Institute for Health and Care Excellence (UK (2014). Available at: https://www.ncbi.nlm.nih.gov/books/NBK264165/.

31. Polish Diabetes Association. 2021 guidelines on the management of patients with diabetes. *Diabetol Klin* (2021) 7:7–8. doi: 10.5603/dk.2021.0001

32. Stąpor N, Beń-Skowronek I. Insulin resistance in children. *Pediatr Endocrino Diabetes Metab* (2014) 21:3: 107–115. doi: 10.18544/PEDM-20.03.0010

33. Kwiterovich POJ. Recognition and management of dyslipidemia in children and adolescents. *J Clin Endocrinol Metab* (2008) 93(11):4200–9. doi: 10.1210/jc.2008-1270

34. Shashaj B, Luciano R, Contoli B, Morino GS, Spreghini MR, Rustico C. Reference ranges of HOMA-IR in normal-weight and obese young caucasians. *Acta Diabetol* (2016) 53(2):251–60. doi: 10.1007/s00592-015-0782-4

35. de Andrade MIS, Oliveira JS, Sá Leal V, da Silva Lima NM, Costa EC, de Aquino NB. Identification of cutoff points for homeostatic model assessment for insulin resistance index in adolescents: systematic review. *Rev Paul Pediatr* (2016) 34(2):234–42. doi: 10.1016/j.rpped.2015.08.006

36. *Obesity and overweight: Key facts*. World Health Organization (2018). Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

37. Sisson SB, Krampe M, Anundson K, Castle S. Obesity prevention and obesogenic behavior interventions in child care: A systematic review. *Prev Med* (2016) 87:57–69. doi: 10.1016/j.ypmed.2016.02.016

38. Bryant M, Farrin A, Christie D, Jebb SA, Cooper AR, Rudolf M. Results of a feasibility randomised controlled trial (RCT) for WATCH IT: a programme for obese children and adolescents. *Clin Trials* (2011) 8(6):755–64. doi: 10.1177/1740774511424766

39. Starbała A, Bawa S, Wojciechowska M, Weker H. The energy value of the diet and the consumption of macronutrients by obese children attending kindergarten. *Bromat Chem Toksyko* (2009) 3:747–53.

40. Ranucci C, Pippi R, Buratta L, Aiello C, Gianfredi V, Piana N. Effects of an intensive lifestyle intervention to treat overweight/obese children and adolescents [online]. *BioMed Res Int* (2017) 2017(5):1–11. doi: 10.1155/2017/8573725

41. Bathrellou E, Yannakoulia M, Papanikolaou K, Pehlivanidis A, Pervanidou P, Kanaka-Gantenbein C. Development of a multi-disciplinary intervention for the treatment of childhood obesity based on cognitive behavioral therapy. *Child Family Behav Ther* (2010) 32(1):34–50. doi: 10.1080/07317100903539873

42. Al-Khudairy. L, Loveman E, Colquitt JL, Mead E, Johnson RE, Fraser H. Diet, physical activity and behavioural interventions for the treatment of overweight or obese adolescents aged 12 to 17years (Review) [online]. *Cochrane Database Systematic Rev* (2017) 6:1–344. doi: 10.1002/14651858.CD012691

43. Skouterisa H, McCabea M, Ricciardellia LA, Milgromb J, Baurc LA. Aksand. Parent-child interactions and obesity prevention: A systematic review of the literature [online]. *Early Child Dev Care* (2012) 182(2):153–74. doi: 10.1080/03004430.2010.548606

44. Knöpfli BH, Radtke T, Lehmann M, Schätzle B, Eisenblättera J, Gachnanga A. Effects of a multidisciplinary inpatient intervention on body composition, aerobic fitness, and quality of life in severely obese girls and boys. *J Adolesc Health* (2008) 42(2):119–27. doi: 10.1016/j.jadohealth.2007.08.015

45. Seo YG, Lim H, Kim Y, Ju Y, Lee HJ, Jang HB. The effect of a multidisciplinary lifestyle intervention on obesity status, body composition, physical fitness, and cardiometabolic risk markers in children and adolescents with obesity. *Nutrients* (2019) 11(1):1–16. doi: 10.3390/nu11010137

46. Bradlee ML, Singer MR, Qureshi MM, Moore LL. Food group intake and central obesity among children and adolescents in the third national health and nutrition examination survey (NHANES III). *Public Health Nutr* (2009) 13(6):797–805. doi: 10.1017/S1368980009991546

47. Abreu S, Santos R, Moreira C, Santos PS, Mota J, Moreira P. Food consumption, physical activity and socio-economic status related to BMI, waist circumference and waist-to-height ratio in adolescents. *Public Health Nutr* (2013) 17(8):1834–49. doi: 10.1017/S1368980013001948

48. Hirschler V, Oestreicher K, Beccaria M, Hidalgo M, Maccallini G. Inverse association between insulin resistance and frequency of milk consumption in low-income argentinean school children. *J Pediatr* (2009) 154:101–5. doi: 10.1016/j.jpeds.2008.06.036

49. Dougkas A, Reynolds CK, Givens ID, Elwood PC, Minihane AM. Associations between dairy consumption and body weight: A review of the evidence and underlying mechanisms. *Nutr Res Rev* (2011) 24(1):72–95. doi: 10.1017/S095442241000034X

50. Layman DK. The role of leucine in weight loss diets and glucose homeostasis. *J Nutr* (2003) 133(1):261S–7S. doi: 10.1093/jn/133.1.261S

51. Reinehr T, Andler W. Changes in the atherogenic risk factor profile according to degree of weight loss. *Arch Dis Child* (2004) 89:419–22. doi: 10.1136/adc.2003.028803

52. Hunt LP, Ford A, Sabin MA, Crowne EC, Shield JPH. Clinical measures of adiposity and percentage fat loss: Which measure most accurately reflects fat loss and what should we aim for? *Arch Dis Child* (2007) 92:399–403. doi: 10.1136/adc.2006.103986

53. Kolotourou M, Radley D, Chadwick P, Smith L, Orfanos S, Kapetanakis V. Is BMI alone a sufficient outcome to evaluate interventions for child obesity? *Child Obes* (2013) 9(4):350–6. doi: 10.1089/chi.2013.0019

54. Reinehr T, Roth C, Menke T, Andler W. Adiponectin before and after weight loss in obese children. *J Clin Endocrinol Metab* (2004) 89(8):3790–4. doi: 10.1210/jc.2003-031925

55. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H. 2016 ESC / EAS guidelines for the treatment of lipid disorders. *Kardiol Pol* (2016) 74(11):1234–318. doi: 10.5603/KP.2016.0157

56. Wengle JG, Hamilton JK, Manlhiot C, Bradley TJ, Katzman DK, Sananes R. The 'Golden keys' to health – a healthy lifestyle intervention with randomized individual mentorship for overweight and obesity in adolescents. *Paediatr Child Health* (2011) 16(8):473–8. doi: 10.1093/pch/16.8.473

57. Gingras V, Rifas-Shiman SL, Taveras EM, Oken E, Hivert MF. Dietary behaviors throughout childhood are associated with adiposity and estimated insulin resistance in early adolescence: a longitudinal study [online]. *Int J Behav Nutr Phys Act* (2018) 15(1):129. doi: 10.1186/s12966-018-0759-0

58. Karatzi K, Moschonis G, Barouti AA, Lionis C, Chrousos GP, Manios Y. Dietary patterns and breakfast consumption in relation to insulin resistance in children. the healthy growth study. *Public Health Nutr* (2014) 17(12):2790–7. doi: 10.1017/S1368980013003327

59. Visuthranukul C, Sirimongkol P, Prachansuwan A, Pruksananonda C. Chomtho s., low-glycemic index diet may improve insulin sensitivity in obese children. *Pediatr Res* (2015) 78(5):567–73. doi: 10.1038/pr.2015.142

60. Buyken AE1, Mitchell P, Ceriello A, Brand-Miller J. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia* (2010) 53(3):406–18. doi: 10.1007/s00125-009-1629-8

61. Dalen JE, Devries S. Diets to prevent coronary heart disease 1957–2013: What have we learned? *Am J Med* (2014) 127(5):364–9. doi: 10.1016/j.amjmed.2013.12.014

62. de Jongh S, Ose L, Szamosi T, Gagné C, Lambert M, Scott R. Efficacy and safety of statin therapy in children with familial hypercholesterolemia: a randomized, double-blind, placebo-controlled trial with simvastatin. *Circulation* (2002) 106(17):2231–7. doi: 10.1161/01.CIR.0000035247.42888.82

63. Aldridge V, Dovey TM, Halford JCG. The role of familiarity in dietary development. *Dev Rev* (2009) 29(1):33–44. doi: 10.1016/j.dr.2008.11.001

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 09 December 2022
DOI 10.3389/fendo.2022.1033179

OPEN ACCESS

EDITED BY
Malgorzata Wojcik,
Jagiellonian University Medical College, Poland

REVIEWED BY
Domenico Corica,
University of Messina, Italy
Ilja Vietor,
Medical University of Innsbruck, Austria

*CORRESPONDENCE
Giuseppina Rosaria Umano
giuseppinarosaria.umano@unicampania.it

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 31 August 2022
ACCEPTED 28 November 2022
PUBLISHED 09 December 2022

CITATION
Palumbo S, Umano GR, Aiello F, Cirillo G, Miraglia del Giudice E and Grandone A (2022) Circulating levels of DLK1 and glucose homeostasis in girls with obesity: A pilot study. *Front. Endocrinol.* 13:1033179. doi: 10.3389/fendo.2022.1033179

Circulating levels of DLK1 and glucose homeostasis in girls with obesity: A pilot study

Stefania Palumbo, Giuseppina Rosaria Umano*, Francesca Aiello, Grazia Cirillo, Emanuele Miraglia del Giudice and Anna Grandone

Department of the Woman, of the Child, of General and Specialized Surgery, University of Campania "Luigi Vanvitelli", Naples, Italy

Introduction: DLK1 gene is considered a molecular gatekeeper of adipogenesis. DLK1 mutations have been reported as a cause of central precocious puberty associated with obesity and metabolic syndrome with undetectable DLK1 serum levels. We investigated the association between DLK1 circulating levels with clinical and biochemical parameters in obese adolescents and healthy controls.

Methods: Sixty-five obese adolescents and 40 controls were enrolled and underwent a complete clinical examination and biochemical assessment for glucose homeostasis and DLK1 plasma levels.

Results: We observed lower DLK1 levels in cases compared to controls. Moreover, we found a negative correlation between DLK1 and HOMA-IR and a direct correlation with insulin-sensitivity index.

Discussion: Our findings suggest that DLK1 might be involved in metabolic derangement in obese children.

KEYWORDS

Dlk1, insulin-resistance, children and adolescents, obesity, adipose tissue

Introduction

DLK1 (Delta-like 1 homolog) is a membrane-bound protein that plays an important role in inhibiting adipocyte differentiation (1, 2). It is part of the Notch signalling pathway controlling many developmental processes also having neuroendocrine function, suggested by its postnatal expression in hypothalamic nuclei (3, 4). It is located on chromosome 14 in the imprinted region 14q32, whose maternal uniparental disomy causes Temple's syndrome, a condition characterized by hypotonia, prenatal growth failure, short postnatal stature, early puberty, and truncal obesity (5). Moreover,

mutations in *DLK1* gene have been reported as a cause of central precocious puberty associated with obesity and metabolic syndrome with undetectable DLK1 serum levels (6). Animal model studies have shown that *DLK1* knockout mice exhibit growth retardation and obesity (7), while *DLK1* overexpression leads to decreased fat mass, diet-induced obesity resistance and reduced insulin signalling (8–10). In line with these data, deficiency of DLK1 in humans, both for imprinting defects such as in Temple syndrome and in cases of mutations in the gene, is associated with undetectable DLK1 levels and childhood and adolescent obesity.

The aim of the present preliminary study is to investigate circulating levels of DLK1 within the context of human paediatric obesity and its relationship with clinical and biochemical parameters.

Materials and methods

The study was conducted at paediatric endocrinology clinic of University of Campania Luigi Vanvitelli of Naples, Italy. Considering the relationship between DLK1 and puberty and the possible sex dependent nature of this relationship, we focused only on girls in this pilot study, also as the completion of pubertal development through registering age at menarche can be assessed with greater certainty in girls compared to boys. We enrolled 65 girls with obesity (mean age:12.3 ± 5.5; BMI z-score:2.9 ± 0.8) with normal puberty onset time (thelarche >8 years or age at menarche >10 years) and a body mass index (BMI) above the 95th percentile and 40 pubertal stage-matched control female patients (mean age:12.1 ± 1.2 BMI z-score: -0.6 ± 1.2). Clinical examination was performed in all girls, including weight and height measurement, and BMI z-score calculation according to the LMS (least mean squares) method. All blood samples were drawn at 8:00 a.m. from an antecubital vein, clotted, centrifuged, and serum was stored at −20°C until analyses were performed.

Fasting samples for glucose, insulin, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDLC), serum aspartate transaminase (AST) and alanine transaminase (ALT) were obtained. All subjects with obesity underwent a standard two-hour oral glucose tolerance test administrating 1,75g/kg glucose up to 75gr orally. Blood samples for plasma glucose and insulin were obtained every 30 minutes. Indexes of insulin-resistance (homeostasis model assessment of insulin resistance, HOMA-IR, and whole-body insulin sensitivity index, WBISI) were calculated as previously described (11).

Serum Dlk1 concentrations were determined using the commercially available Human DLK1 ELISA

(MyBioSource, San Diego, CA, USA) with a detection limit of 0.216 ng/mL. Intra-assay and inter-assay coefficients of variation (CVs) listed by the manufacturer were of <10 and <12%, respectively.

Continuous variables were checked for normality according to the Kolmogorov-Smirnov test. Differences for continuous variables were investigated with Student t-test for independent samples and Mann-Whitney U test as appropriate. Chi Square test was performed to test differences in categorical variables. Spearman correlation analyses were performed to test the correlation between DLK1 levels and insulin-resistance measures. Data are expressed as mean and standard deviation or median and interquartile range according to normal or not normal distribution.

Hepatic steatosis was defined as present or absent according to abdominal ultrasound. It was assessed according to abnormally intense echoes arising from the hepatic parenchyma, and liver-kidney differences in echo amplitude. Two experienced radiologists performed the ultrasound for hepatic steatosis detection.

Results

The anthropometric and biochemical characteristics of the cohort are reported in (Table 1). The two groups did not differ in age distribution (12.1 ± 1.2 in girls with obesity and 12.3 ± 2.5 in control group, p=0.76) and pubertal stage (21% prepubertal children in control group and 16% in girls with obesity, p=0.57). As expected, the group with obesity showed significantly higher z-score BMI compared to controls (p<0.0001). The results obtained from the serum assay of circulating protein revealed lower levels in the group of subjects with obesity with a median of 3.36 ng/ml (IQR 3.10) compared with the group of healthy patients with a median of 4.58 ng/ml (IQR 1.88, p=0.01). A significant negative correlation between DLK1 levels and HOMA-IR was also observed (r=-0.28; p= 0.03) (Figure 1A) while a direct correlation was found with insulin sensitivity index (WBISI) (r=0.28; p=0.03) (Figure 1B). Four out 65 (6%) obese girls showed prediabetes (1 IFG and 3 IGT). DLK1 levels were significantly lower in girls with prediabetes compared to normoglycemic girls (p=0.02). Abdominal ultrasound was available for 53 out 65 obese girls. Among them, NAFLD was present in 39.6% of cases. No difference for DLK1 levels were found between girls with NAFLD and girls without NAFLD.

Discussion

Our study reported lower serum levels of DLK1 in subjects with obesity suggesting its role in the regulation of adiposity even in the absence of syndromic condition or precocious puberty.

The role of DLK1 in obesity is not completely clear but studies in animal models have shown that DLK1 may play a role not only in adipogenesis but also in adaptive thermogenesis in adipose tissue, the phenomenon also called browning (12).

10.3389/fendo.2022.1033179

TABLE 1 Clinical and laboratory features of the study cohort.

Parameters	Ctrl	OB	p
N	40	65	
Age (y)	12.1 ± 1.2	12.3 ± 2.5	0.76
z-score BMI	-0.6 ± 1.2	2.9 ± 0.8	**<0.0001**
Age of Menarche (y)	11.5 ± 0.6	11.2 ± 1	0.50
LDL-cholesterol (mg/dl)	-	93.1 ± 29	
HDL-cholesterol (mg/dl)	-	44.4 ± 9.9	
Fasting glucose (mg/dl)	77.0 ± 6.1	77.7 ± 10.8	0.82
Fasting Insulin (mcU/ml)	–	23.7 ± 20.4	
HOMA-IR	–	4.4 ± 3.6	
WBISI	–	4.8 ± 4.6	
DLK1 (ng/ml)	4.58 (IQR 1.88)	3.36 (IQR 3.10)	**0.01**

All the values are expressed as mean ± SD. Significant differences are in bold. Ctrl, controls; HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range; N, number; OB, patients with obesity; WBISI, whole-body insulin sensitivity index.

Future studies focusing on the role of DLK1 in this process in humans would be warranted, as it could represent an interesting target for the treatment of obesity and its complications. Moreover, data about correlation between Dlk1 levels and both body fat percentage and insulin resistance are conflicting. In the present study has been observed a weak, albeit statistically significant, negative correlation between DLK1 serum levels and insulin-resistance degree in girls with obesity. This data is in line with those observed by Demir Çalteki et al. in a cohort of women with polycystic ovary syndrome (PCOS) (13) with low DLK1 levels and an inverse correlation between HOMA-IR and DLK1. In addition, a previous study revealed lower DLK1 serum concentrations in patients with obesity and Type 2 Diabetes (T2D) compared to non-T2D subjects (14). A longitudinal study on a large cohort of adult diabetic patients showed significantly lower levels of DLK1 in 4 individuals with increased fasting plasma glucose or whose homeostasis model assessment of β-cell function (HOMA-β) was decreased at the follow-up compared to the control group. Notably, these results were significant in women but not in men (15). Also, studies on animal models reported how DLK1 can influence fat and glucose metabolism in the liver. In particular, DLK1 administration in mice promotes hepatic fatty acid oxidation and inhibits gluconeogenesis (16). These findings supported the hypothesis that DLK1 plays a role in glucose/insulin homeostasis. In particular, scientific evidence indicates that DLK1 inhibits Notch1 function. Notch activation pathologically affects lipogenesis and gluconeogenesis, finally increasing insulin-resistance (16). Therefore, the DLK1 inhibitory activity on Notch1 signaling might mediate the modulatory effect of DLK1 on glucose metabolism.

Paternally transmitted foetal DLK1 genotype affected maternal DLK1 levels that were positively associated with insulin-resistance and inversely correlated with insulin secretion during the third trimester of pregnancy (17). In contrast, other studies have reported that DLK1 is negatively associated with insulin sensitivity during pregnancy and in non-

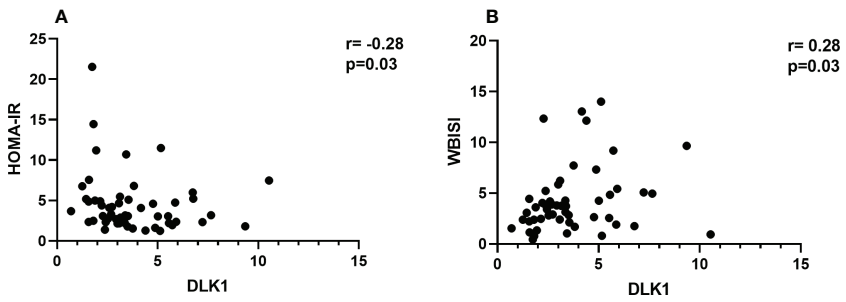


FIGURE 1
Spearman correlation analyses between DLK1 levels and insulin resistance measures in girls with obesity. **(A)** displays the correlation between DLK1 and HOMA-IR. **(B)** refers to the correlation between DLK1 and WBISI. Spearman correlation coefficient (rho, r) and significance level of the test statistic (p-value, p) are reported. Legend: HOMA-IR, homeostasis model assessment of insulin resistance; WBISI, whole-body insulin sensitivity index.

diabetic men (18). In diabetic men, DLK1 was shown to reduce skeletal muscle glucose uptake without affecting hepatic gluconeogenesis (18). Similarly, in a cohort of pre-pubertal Spanish children DLK1 serum levels were positively correlated with plasma insulin, HOMA-IR, and free fatty acids (19). This effect was dependent on changes in dehydroepiandrosterone-sulphate (DHEA-S) levels suggesting a reciprocal influence of adrenal hormones and DLK1 on adrenal gland function and metabolic control (19). These contradictory findings highlight the need to further investigate the role of DLK1 in glucose homeostasis. The dynamic expression of DLK1 and the controversies in literature findings can be explained by the heterogeneity of the individuals included in the several studies in terms of age, pubertal stage, gender, comorbidities (diabetes, obesity, cardiovascular diseases), or other variables. Some reports have reported that sexual hormones influence DLK1 levels. The hypothesis of a hormonal influence might partly explain the different results reported in literature (20, 21). Therefore, considering the imprinted nature of *DLK1* gene, a sexual dimorphism of DLK1 effects might be speculated.

This study presents several limitations that should be acknowledged, such as the small sample size and the lack of data on HOMA-IR and WBISI in the non-obese controls.

In conclusion, preliminary data obtained in the present study show lower DLK1 serum levels in subjects with obesity compared to lean controls. Although we report a weak correlation with insulin resistance, this finding would suggest a DLK1-mediated metabolic effect. Confirmation of this result on a larger population would allow to add circulating DLK1 level assessment as a new marker of metabolic alterations in adolescents with obesity. New studies will be needed to further investigate and confirm these data and to clarify in humans the role of DLK1 in insulin secretion, adipogenesis and in the browning process.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethic Commitee of the University of Campania Luigi Vanvitelli. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

SP: Writing - original draft, investigation; GU: Formal analysis, FA: Data curation; GC: Project administration, methodology; EG: Writing - review & editing; AG: Conceptualization, Resources, Supervision. All authors contributed to the article and approved the submitted version.

Acknowledgments

This work was supported by a grant (390) funded by "VALERE: VAnviteLli pEr la RicErca" program of University of Campania "L. Vanvitelli".

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.

References

1. Mitterberger MC, Lechner S, Mattesich M, Kaiser A, Probst D, Wenger N, et al. DLK1(PREF1) is a negative regulator of adipogenesis in $CD105^{+}/CD90^{+}/CD34^{+}/CD31^{-}/FABP4^{-}$ adipose derived stromal cells from subcutaneous abdominal fat pats of adult women. *Stem Cell Res* (2012) 9:35–48. doi: 10.1016/j.scr.2012.04.001

2. Hudak CS, Sul HS. Pref-1, a gatekeeper of adipogenesis. *Front Endocrinol* (2013) 4:79. doi: 10.3389/fendo.2013.00079

3. Traustadóttir GÁ, Lagoni LV, Ankerstjerne LBS, Bisgaard HC, Jensen CH, Andersen DC. The imprinted gene delta like non-canonical notch ligand 1 (Dlk1) is conserved in mammals, and serves a growth modulatory role during tissue development and regeneration through notch dependent and independent mechanisms. *Cytokine Growth Factor Rev* (2019) 46:17–27. doi: 10.1016/j.cytogfr.2019.03.006

4. Falix FA, Aronson DC, Lamers WH, Gaemers IC. Possible roles of DLK1 in the notch pathway during development and disease. *Biochim Biophys Acta* (2012) 1822(6):988–95. doi: 10.1016/j.bbadis.2012.02.003

5. Ioannides Y, Lokulo-Sodipe K, Mackay DJ, Davies JH, Temple IK. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. *J Med Genet* (2014) 51:495–501. doi: 10.1136/jmedgenet-2014-102396

6. Dauber A, Cunha-Silva M, Macedo DB, Brito VN, Abreu AP, Roberts SA, et al. Paternally inherited DLK1 deletion associated with familial central precocious puberty. *J Clin Endocrinol Metab* (2017) 102:1557–67. doi: 10.1210/jc.2016-3677

7. Carreras-Badosa G, Remesar X, Prats-Puig A, Xargay-Torrent S, Lizarraga-Mollinedo E, de Zegher F, et al. Dlk1 expression relates to visceral fat expansion

and insulin resistance in male and female rats with postnatal catch-up growth. *Pediatr Res* (2019) 86:195–201. doi: 10.1038/s41390-019-0428-2

8. Wang Y, Kim KA, Kim JH, Sul HS. Pref-1, a preadipocyte secreted factor that inhibits adipogenesis. *J Nutr* (2006) 136:2953–6. doi: 10.1093/jn/136.12.2953

9. Villena JA, Choi CS, Wang Y, Kim S, Hwang YJ, Kim YB, et al. Resistance to high-fat diet-induced obesity but exacerbated insulin resistance in mice overexpressing preadipocyte factor-1 (Pref-1): A new model of partial lipodystrophy. *Diabetes* (2008) 57:3258–66. doi: 10.2337/db07-1739

10. Moon YS, Smas CM, Lee K, Villena JA, Kim KH, Yun EJ, et al. Mice lacking paternally expressed pref-1/Dlk1 display growth retardation and accelerated adiposity. *Mol Cell Biol* (2002) 22:5585–92. doi: 10.1128/MCB.22.15.5585-5592.2002

11. Marzuillo P, Di Sessa A, Umano GR, Nunziata L, Cirillo G, Perrone L, et al. Novel association between the nonsynonymous A803G polymorphism of the n-acetyltransferase 2 gene and impaired glucose homeostasis in obese children and adolescents. *Pediatr Diabetes* (2017) 18:478–84. doi: 10.1111/pedi.12417

12. Rhee M, Kim JW, Lee MW, Yoon KH, Lee SH. Preadipocyte factor 1 regulates adipose tissue browning *via* TNF-α-converting enzyme-mediated cleavage. *Metabolism* (2019) 101:153977. doi: 10.1016/j.metabol.2019.153977

13. Demir Çaltekin M, Caniklioğlu A, Eris Yalçın S, Aydoğan Kırmızı D, Baser E, Yalvaç ES. DLK1 and nesfatin-1 levels and the relationship with metabolic parameters in polycystic ovary syndrome: Prospective, controlled study. *Turk J Obstet Gynecol* (2021) 18(2):124–30. doi: 10.4274/tjod.galenos.2021.39024

14. Flehmig G, Scholz M, Klöting N, Fasshauer M, Tönjes A, Stumvoll M, et al. Identification of adipokine clusters related to parameters of fat mass, insulin sensitivity and inflammation. *PloS One* (2014) 9:e99785. doi: 10.1371/journal.pone.0099785

15. Lee SH, Rhee M, Yang HK, Ha HS, Lee JH, Kwon HS, et al. Serum preadipocyte factor 1 concentrations and risk of developing diabetes: A nested case-control study. *Diabetes Med* (2016) 33:631–8. doi: 10.1111/dme.12871

16. Lee YH, Yun MR, Kim HM, Jeon BH, Park BC, Lee BW, et al. Exogenous administration of DLK1 ameliorates hepatic steatosis and regulates gluconeogenesis *via* activation of AMPK. *Int J Obes (Lond)* (2016) 40:356–65. doi: 10.1038/ijo.2015.173

17. Petry CJ, Burling KA, Barker P, Hughes IA, Ong KK, Dunger DB. Pregnancy serum DLK1 concentrations are associated with indices of insulin resistance and secretion. *J Clin Endocrinol Metab* (2021) 106:e2413–22. doi: 10.1210/clinem/dgab123

18. Jensen CH, Kosmina R, Rydén M, Baun C, Hvidsten S, Andersen MS, et al. The imprinted gene delta like noncanonical notch ligand 1 (Dlk1) associates with obesity and triggers insulin resistance through inhibition of skeletal muscle glucose uptake. *EBioMedicine* (2019) 46:368–80. doi: 10.1016/j.ebiom.2019.07.070

19. Hermida C, Garcés C, de Oya M, Cano B, Martínez-Costa OH, Rivero S, et al. The serum levels of the EGF-like homeotic protein dlk1 correlate with different metabolic parameters in two hormonally different children populations in Spain. *Clin Endocrinol (Oxf)* (2008) 69(2):216–24. doi: 10.1111/j.1365-2265.2008.03170.x

20. Faje AT, Fazeli PK, Katzman D, Miller KK, Breggia A, Rosen CJ, et al. Inhibition of pref-1 (preadipocyte factor 1) by oestradiol in adolescent girls with anorexia nervosa is associated with improvement in lumbar bone mineral density. *Clin Endocrinol (Oxf)* (2013) 79:326–32. doi: 10.1111/cen.12144

21. Aronis KN, Kilim H, Chamberland JP, Breggia A, Rosen C, Mantzoros CS. Preadipocyte factor-1 levels are higher in women with hypothalamic amenorrhea and are associated with bone mineral content and bone mineral density through a mechanism independent of leptin. *J Clin Endocrinol Metab* (2011) 96(10):E1634–9. doi: 10.1210/jc.2011-0600

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 12 December 2022
DOI 10.3389/fendo.2022.1036109

OPEN ACCESS

EDITED BY
Dénes Molnár,
University of Pécs, Hungary

REVIEWED BY
Michael Edwin Edmonds,
King's College Hospital NHS Foundation Trust, United Kingdom
Malgorzata Wojcik,
Jagiellonian University Medical College, Poland
Joanna Nazim,
Jagiellonian University Medical College, Poland

*CORRESPONDENCE
Anna Kącka
annakacka15@gmail.com
Barbara Głowińska-Olszewska
barbara.glowinska-olszewska@umb.edu.pl

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 03 September 2022
ACCEPTED 03 November 2022
PUBLISHED 12 December 2022

CITATION
Kącka A, Charemska A, Jarocka-Cyrta E and Głowińska-Olszewska B (2022) Comparison of novel markers of metabolic complications and cardiovascular risk factors between obese non-diabetic and obese type 1 diabetic children and young adults. *Front. Endocrinol.* 13:1036109. doi: 10.3389/fendo.2022.1036109

Comparison of novel markers of metabolic complications and cardiovascular risk factors between obese non-diabetic and obese type 1 diabetic children and young adults

Anna Kącka[1*], Anna Charemska[1], Elżbieta Jarocka-Cyrta[1] and Barbara Głowińska-Olszewska[2*]

[1]Department of Clinical Pediatrics, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Provincial Specialist Children's Hospital, Olsztyn, Poland, [2]Department of Pediatrics, Endocrinology, Diabetology with Cardiology Division, Medical University of Białystok, Białystok, Poland

Introduction: For the past years, the prevalence of obesity is growing in the general population of children, as well as among diabetic patients, resulting in increased risk of cardiovascular complications. Type 1 diabetes mellitus (T1DM) is one of the most common chronic sdiseases in children and young adults, leading to decreased life quality and lifespan, with obesity being recognized recently as a major contributing factor to these health problems. The objective of this study was to analyze and compare the selected novel markers for metabolic complications of obesity and vascular risk factors between obese non-diabetic and obese T1DM children and young adults.

Methods: One hundred four subjects, aged between 10 and 24 years (31 with T1DM and excessive body weight, 41 with obesity without diabetes, and 32 with T1DM and normal weight), and 32 matched lean controls were included in the study. Clinical characteristics, blood pressure measurements, daily requirement for insulin, HbA1c%, plasma lipids, fetuin-A, E-selectin, and osteoprotegerin levels were compared with respect to body mass index (BMI), body mass index standard deviation score (BMI-SDS), and carotid intima-media thickness (cIMT) of common carotid arteries.

Results: Patients with T1DM and excessive body weight compared to non-diabetic obese subjects had similar values of systolic blood pressure (125.6 ± 8.2 vs. 127.3 ± 12.9 mmHg, $p = 0.515$), diastolic blood pressure (78.19 ± 7.03 vs. 78.02 ± 8.01 mmHg, $p = 0.918$), cholesterol (175.26 ± 34.1 vs. 163.51 ± 26.08 mg/dl, $p = 0.102$), LDL (108.03 ± 32.55 vs. 112.22 ± 26.36 mg/dl, $p = 0.548$), and triglyceride levels (118.19 ± 71.20 vs. 117 ± 55.80 mg/dl, $p = 0.937$); all values were found to be higher compared to non-obese T1DM and healthy controls. HbA1c level and insulin resistance indices were significantly worse in T1DM

10.3389/fendo.2022.1036109

obese vs. T1DM non-obese patients. Fetuin-A levels were higher among obese non-diabetic patients (p = 0.01), and E-selectin and osteoprotegerin levels were similar in both groups with obesity, but higher than in the reference group. There were no statistical differences in cIMT with T1DM with normal weight, excessive weight, and non-diabetic obese children; however, the cIMT value was higher compared to the reference group.

Discussion: Novel markers of metabolic complications of obesity are similar between obese T1DM and non-diabetic subjects. Obesity in patients with T1DM results in worse metabolic control, insulin resistance, and increased risk for vascular complications.

KEYWORDS

type 1 diabetes mellitus, obesity, fetuin-A, E-selectin, osteoprotegerin

Introduction

In the last decades, the prevalence of obesity and diabetes mellitus has been rapidly growing (1). Once considered a problem in high-income countries, overweight and obesity are nowadays recognized as an epidemic worldwide, resulting in metabolic complications such as insulin resistance, dyslipidemia, hypertension, metabolic syndrome, and non-alcoholic fatty liver disease. It is also recognized as a risk factor for cardiovascular diseases.

According to WHO, over 39 million children under 5 years and 340 million children aged 5–19 are overweight or obese (2–4). Type 1 diabetes mellitus (T1DM) is one of the most common chronic diseases leading to decreased life quality and lifespan. The main cause of death among diabetic patients is cardiovascular complications. T1DM itself is recognized as a high-risk factor for cardiovascular disease (5–7). Others include hypertension, decreased HDL, and increased triglyceride levels. The prevalence of obesity in patients with T1DM is also rising and is associated with insulin pump therapy, early onset, puberty, female sex, and low levels of physical activity. Excessive body weight in children with T1DM has recently been recognized as a significant factor contributing to complications on various stages of the disease. Furthermore, the prevalence of insulin resistance, associated with being overweight and/obese, was previously linked to type 2 diabetes and is increased in children with T1DM (8). It is now recognized that insulin resistance results in worse glycemic control, higher HbA1c, and higher atherogenic lipid profile, and contributes to earlier development of microangiopathy as well as macroangiopathy. Measurement of insulin resistance in T1DM is difficult due to hypoinsulinemia, and methods such as HOMA-IR (homeostasis model assessment of insulin resistance) cannot be used. Therefore, the euglycemic–hyperinsulinemic clamp has been proposed, but not commonly used in practice, because it is labor-intensive and invasive. For clinical purposes, estimated glucose disposal rate (eGDR) has been developed, which is strongly correlated with clamp-measured insulin resistance. The formula is based on clinical measurements, such as hypertension status, waist-to-hip ratio, and HbA1c%, or especially in children—age, daily insulin requirement, and HbA1c% (9–12).

Additionally, to estimate the visceral adiposity dysfunction associated with cardiometabolic risk, a sex-specific index based on waist circumference, BMI, triglycerides, and HDL—visceral adiposity index (VAI)—was created (13).

The obesity epidemic caused increased interest in factors released by adipose tissue, such as inflammatory cytokines, fatty acids, and adipocytokines. In addition to well-described adipocytokines and markers of the inflammatory process, new prognostic indicators of an increased risk of developing cardiovascular diseases are still being sought.

While recent studies have shown a good correlation between risk of cardiovascular disease and some novel metabolic markers [osteoprotegerin (OPG), fetuin-A, and E-selectin] in adults, limited studies have been conducted in children (14).

Fetuin-A (Alpha-2 Heremans Schmid glycoprotein), which is a negative acute phase, also causes insulin resistance by enhancing insulin receptor tyrosine kinase activity and insulin receptor auto-phosphorylation. The fetuin-A production is increased by hyperlipidemia and hyperglycemia. The current studies provide evidence that higher levels of fetuin-A are also associated with higher risk of cardiovascular complications (15). In a case–cohort study, Weikert et al. showed that patients with high fetuin-A concentrations had a fourfold increased risk for myocardial infarction and ischemic stroke compared to subjects

with low fetuin-A levels (16). OPG is a cytokine member of the tumor necrosis factor (TNF) involved in bone metabolism and vascular calcification and atherogenesis (17). Recent studies showed that the RANK/RANKL/OPG pathway is important for the regulation of obesity, as well as associations between OPG levels and ischemic heart disease and insulin resistance (18). Alharbi et al. found that serum OPG level was significantly elevated in obese with insulin resistance patients compared to control subjects (19).

Moreover, Perez de Ciriza et al. in their study showed that patients with the metabolic syndrome had higher OPG than patients without. OPG correlated with carotid intima-media thickness (cIMT) and patients with atherosclerosis had higher OPG concentrations (20).

E-selectin is an endothelial adhesion molecule known to be integrally involved in the development of atherosclerotic plaque by promoting the adhesion of leukocytes to the endothelial wall. Levels of E-selectin are also increased in obesity (21). The MIAMI study that examined the relationship between various circulating markers of inflammation and CIMT found that E-selectin was strongly correlated to atherosclerotic burden and CIMT and inversely correlated to HDL-c (22).

Recent studies have shown that obesity and T1DM in youth are associated with greater cIMT. It is also influenced by hypertension, dyslipidemia, and poor glycemic control (23–25).

The objective of the study was to analyze and compare selected novel markers of metabolic complications of excessive body weight and classical cardiovascular risk factors between obese non-diabetic and obese T1DM children. The following variables were analyzed: daily insulin requirement, insulin resistance, measurement of the cIMT, and levels of fetuin-A, E-selectin, and OPG.

Therefore, the aim of this study was to investigate the link between obesity among type 1 diabetic patients, novel markers, and risk of cardiovascular complications.

We hypothesized that our results might help to identify the group of patients with higher risk of macroangiopathy as well as create the therapeutic goals for these patients, which might delay the development of chronic complications.

Methods

The study was performed in the Pediatric Endocrinology and Diabetology Division, Department of Clinical Pediatrics as well as Outpatient Clinics of Provincial Specialist Children's Hospital in Olsztyn between 2019 and 2022. The Ethics Committee of University of Warmia and Mazury approved this study (approval number KB/13/2019). Written informed consent forms were acquired from parents and patients older than 16 years.

Patients

One hundred four patients, aged between 10 and 24 years (31 with T1DM and obesity, 41 with obesity, and 32 with T1DM and normal weight), were enrolled in the study. The onset of T1DM must have been at least 2 years prior. The control group consisted of 32 age-matched healthy peers (BMI < 90 pc and BMI-SDS < 1).

The following inclusion criteria were used for the participants: excessive body weight was defined by BMI > 90 pc and BMI-SDS > 1 for children with T1DM and BMI > 97 pc and BMI-SDS > 2 for children with simple obesity based on BMI-for-age percentile charts of the nationally representative group. Clinical remission of diabetes, more than one autoimmune comorbidity, and microvascular complications were exclusion criteria for children with T1DM. Further exclusion criteria for all the participants included acute infection, previous surgery, or trauma 1 month prior.

Physical examination and clinical data

Weight, height, and waist circumference were measured. Body mass index (BMI kg/m^2) was calculated by the following formula: weight (kg)/height^2 (m^2). Standardized BMI (BMI-SDS) was calculated by the following formula: (BMI – BMI 50 pc)/0.5× (BMI 50 pc – BMI 3 pc). Waist circumference SDS was calculated by the following formula: (waist circumference – waist circumference 50 pc)/0.5× (waist circumference 50 pc – waist circumference 3 pc). Obtained data were referenced to polish percentile charts according to age and sex (26). The average of three measurements was taken to determine blood pressure.

In patients with T1D, data including diabetes duration and daily requirement of insulin were collected.

Laboratory methods

Venous blood samples were obtained after 8–12 h of fasting for laboratory tests. Eight milliliters of blood was collected and then centrifuged for 10 min at 2,000 turns per minute. Blood tests, including glycated hemoglobin (HbA1c), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), blood glucose (BG), insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and 25-hydroksyvitamin D, were performed using standard methods in the Diagnostic Laboratory of Provincial Specialist Children's Hospital in Olsztyn. The remaining material (serum) was stored at a temperature of −80°C until the determination.

To analyze novel markers of metabolic complications and cardiovascular risk factors including fetuin-A, E-selectin, and OPG, commercially accessible rapid sandwich immunoassay ELISA kits were used at the Institute of Animal Reproduction and Food Research of Polish Academy of Science.

In addition, in children with obesity, oral glucose toleration test (OGTT) was performed (1.75 g/kg, maximum 75 g of glucose). Insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) index using the following formula: fasting insulin × fasting blood glucose (mg/dl)/405. Interpretation of OGTT and diagnosis of prediabetes were established according to the criteria of Polish Society of Diabetes 2022 (27).

The following formulas were used for assessment of insulin resistance: for non-diabetic patients, HOMA-IR, while for type 1 diabetic patients, eGDR.

Additionally, visceral adipose function was expressed by the VAI.

eGDR was calculated using two indirect methods:

eGDR 1: 20.91 + [1.51 × (boy 1, girl 0)] − [0.1 × (age in years)] − [0.13 × (waist circumference in cm)] − [0.3 × HbA1c%] − [2.11 × daily insulin requirement], and eGDR2: 21.158 + (−0.09 × waist circumference in cm) + (−3.407 × 1 for HBP) + (−0.551 × HbA1c %). Lower values indicate greater insulin resistance (28, 29).

VAI was calculated according to sex using the following formula: girls: (waist circumference/36.58 + [1.89 × BMI]) × (TG/0.81) × (1.52/HDL) boys: (waist circumference/39.68 + [1.88 × BMI]) × (TG/1.03) × (1.31/HDL) (13).

Ultrasound

PHILIPS, Toshiba Apolio 500 ultrasound devices were used for measuring cIMT based on standard protocol. Covered end-diastolic (minimum diameter) IMT of the far walls (the distance between the leading edge of the first echogenic line and the leading edge of the second echogenic line) within a distance larger than 1 cm from the bifurcation was measured. The mean value of six measurements (three from the left and three from the right carotid artery) was included in the analyses (30, 31).

Statistical analysis

Statistical analysis was performed using STATISTICA v.13.3 software. Quantitative variables were expressed as mean and standard deviation (SD).

The values of categorized variables were presented in terms of cardinality (*N*).

To analyze the differences between the studied parameters in individual groups, the following parametric tests were used: Student's *t*-test for the comparison of two groups and the ANOVA test (with the *post-hoc* NIR test) in the case of a larger number of groups for the variables expressed on the quantitative scale.

The analysis of correlations was performed using the Pearson correlation coefficient.

Statistically significant results were found at the level of $p < 0.05$.

Results

Patients with T1DM and excessive body weight compared to non-diabetic obese subjects had similar values of systolic blood pressure (125.6 ± 8.2 vs. 127.3 ± 12.9 mmHg, $p = 0.515$), diastolic blood pressure (78.19 ± 7.03 vs. 78.02 ± 8.01 mmHg, $p = 0.918$), cholesterol (175.26 ± 34.1 vs. 163.51 ± 26.08 mg/dl, $p = 0.102$), LDL (108.03 ± 32.55 vs. 112.22 ± 26.36 mg/dl, $p = 0.548$), and triglyceride levels (118.19 ± 71.20 vs. 117 ± 55.80 mg/dl, $p = 0.937$); all values were higher compared to non-obese T1DM and healthy controls. The general characteristics of the study groups are shown in Table 1.

We noted a difference in glycemic control among diabetic patients. Mean HbA1c levels from the year prior to the study and obtained during the study were higher in obese T1DM patients, 7.99 ± 0.93% and 8.1 ± 1.21%, than in non-obese T1DM patients, 7.61 ± 0.89% and 7.780 ± 0.91% ($p = 0.099$, $p = 0.252$), although not significant. Daily dosage of the insulin was similar; however, insulin resistance indices eGDR1 and eGDR2 were significantly lower in obese T1DM patients than in non-obese T1DM patients: eGDR1: 5.16 ± 1.33 vs. 6.96 ± 1.32; eGDR2: 9.37 ± 1.21 vs. 10.66 ± 0.9 ($p = 0.0001$, $p = 0.0001$), meaning insulin resistance (Table 2). Daily dosage of the insulin was similar 0.83±0.16 in T1DM vs 0.85±0.17 [IU/kg/24hrs] (p=0.593).

Comparing novel markers of metabolic complications and cardiovascular risk factors revealed that fetuin-A levels were higher among obese non-diabetic children, 667.18 ± 363.12 vs. 388.87 ± 253.75 [μg/ml] ($p = 0.01$); E-selectin, 815.87 ± 751.92 vs. 582.01 ± 645.75 [ng/ml], and OPG levels, 0.10 ± 0.04 vs. 0.10 ± 0.03 [ng/ml], were similar in both groups with obesity. However, there was statistical difference in E-selectin and OPG levels between the obese non-diabetic group, the obese with T1DM group, and the control group: 238.78 ± 434.90 [ng/ml] ($p < 0.0002$; $p < 0.035$); 0.08 ± 0.03 [ng/ml] ($p < 0.012$; $p < 0.022$) (Table 3; Figures 1A–C).

There were no statistical differences in intima-media thickness between patients with T1DM with normal weight, 0.47 ± 0.12 [mm], excessive weight, 0.44 ± 0.04 [mm], and non-diabetic obese children, 0.44 ± 0.05 [mm]; however, the cIMT value was higher than the reference group, 0.38 ± 0.03 [mm] ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$) (Figure 2).

A significant positive correlation was found among the obese non-diabetic patients between BMI, BMI-SDS, and waist circumference with OPG: $r = 0.560$ ($p < 0.001$); $r = 0.618$ ($p < 0.001$); $r = 0.547$ ($p < 0.001$). Among obese T1D children, fetuin-A levels were significantly positively correlated with BMI,

10.3389/fendo.2022.1036109

TABLE 1 General characteristics of the study groups.

	Obese group (*N* = 41)	T1DM group (*N* = 32)	T1DM obese group (*N* = 31)	Control group (*N* = 32)	*p*
Gender (M/F) [*n* (%)]	16 (39.0%)/25 (61.0%)	13 (40.6%)/19 (59.4%)	10 (32%)/21 (68%)	9 (28.1%)/23 (71.9%)	
Age [years]	13.82 ± 2.87 [bc]	15.19 ± 3.29	15.63 ± 2.59 [a]	13.84 ± 2.70	0.017
Height [cm]	164.34 ± 11.59	163.94 ± 12.00	164.72 ± 11.42	160.15 ± 13.30	0.395
Body weight [kg]	92.29 ± 23.84 [abc]	54.44 ± 13.05 [b]	73.45 ± 12.98 [a]	48.88 ± 10.88	0.0001
BMI [kg/m^2]	33.82 ± 6.32 [abc]	20.00 ± 2.55 [b]	26.85 ± 2.53 [a]	18.70 ± 2.31	0.0001
BMI-SDS	5.24 ± 2.20 [abc]	0.21 ± 0.64 [b]	2.49 ± 0.87 [a]	−0.05 ± 0.59	0.0001
Waist circumference [cm]	97.87 ± 13.66 [abc]	69.00 ± 7.27 [ab]	80.42 ± 6.98 [a]	63.86 ± 6.27	0.0001
Waist circumference—SDS	4.62 ± 1.93 [abc]	0.25 ± 0.65 [b]	1.83 ± 0.80 [a]	−0.21 ± 0.66	0.0001
Systolic blood pressure [mm/Hg]	127.32 ± 12.93 [ac]	120.16 ± 10.02 [ab]	125.58 ± 8.18 [a]	110.44 ± 7.10	0.0001
Diastolic blood pressure [mm/Hg]	78.01 ± 8.01 [ac]	73.94 ± 7.95 [ab]	78.19 ± 7.03 [a]	69.88 ± 7.30	0.0001
Cholesterol [mg/dl]	163.51 ± 26.08 [a]	164.69 ± 32.42 [a]	175.26 ± 34.10 [a]	149.06 ± 26.25	0.007
TG [mg/dl]	117.00 ± 55.80 [a]	71.31 ± 18.76 [b]	118.19 ± 71.2 [a]	63.03 ± 27.42	0.0001
HDL [mg/dl]	48.22 ± 9.25 [abc]	62.09 ± 12.30	59.97 ± 15.76	58.56 ± 14.31	0.0001
LDL [mg/dl]	112.22 ± 26.36 [a]	99.75 ± 28.72 [a]	108.03 ± 32.55 [a]	85.91 ± 20.66	0.001

The data are presented as mean ± SD; ANOVA test.
[a]$p < 0.05$, compared to the control group.
[b]$p < 0.05$, compared to T1DM obese.
[c]$p < 0.05$, compared to T1DM in post-hoc tests.

$r = 0.572$ ($p < 0.001$), BMI-SDS, $r = 0.723$ ($p < 0.0001$), HDL [mg/dl], $r = 0.514$ ($p < 0.01$), and daily insulin requirement, $r = 0.577$ ($p < 0.001$), and negatively with eGDR2, $r = -0.521$ ($p < 0.01$).

In non-diabetic obese patients, cIMT was positively correlated with BMI, $r = 0.675$ ($p = 0.003$), BMI-SDS, $r = 0.679$ ($p = 0.003$), waist circumference SDS, $r = 0.638$ ($p = 0.006$), SBP, $r = 0.6523$ ($p = 0.036$), DBP, $r = 0.600$ ($p = 0.011$), TG, $r = 0.5069$ ($p = 0.017$),

TABLE 2 Glycemic control in type 1 diabetic patients .

	T1DM group	T1DM obese group	p
Gender M/F(%)	13 (40,6%)/ 19 (59.4%)	10 (32,3%)/ 21 (67,7%)	
Age [years]	15.19±3.29	15.63±2.59	0.56
Height [cm]	163.94±12.00	164.72±11.42	0.791
Body weight [kg]	54.44±13.05	73.45±12.98	0.0001*
T1D duration [years]	8.67±4.52	7.09±2.96	0.107
Mean HbA1c from last year	7.61±0.89	7.99±0.93	0.099
HbA1c last [%]	7.78±0.91	8.10±1.21	0.252
Daily insulin requirement [IU/kg/24hrs]	0.83±0.16	0.85±0.17	0.593
eGDR1	6.96±1.32	5.16±1.33	0.0001*
eGDR2	10.66±0.90	9.37±1.21	0.0001*

The data are presented as mean±SD *p<0.05 in t-student test.

10.3389/fendo.2022.1036109

TABLE 3 Comparison of the novel markers between non – diabetic and T1DM obese patients.

	Obese group		T1DM obese group		p
	Mean±SD	Med	Mean±SD	Med	
E-selectin [ng/ml]	815.87±751.92	296.78	582.01 ± 645.75	274.13	0.280
Osteoprotegerin [ng/ml]	0.10 ± 0.04	0.10	0.10 ± 0.03	0.09	0.914
Fetuin A [ug/ml]	667.18 ± 363.12	807.42	388.87 ±253.75	300.26	0.01*

The data are presented as mean±SD.
*$p < 0.05$ in t-student test.

fasting insulin level, $r = 0.767$ ($p = 0.000$), HOMA-IR, $r = 0.768$ ($p = 0.000$), and OPG level, $r = 0.528$ ($p = 0.029$).

In the group of obese children with T1D, cIMT was positively correlated with TG, $r = 0.611$ ($p = 0.027$), and VAI, $r = 0.611$ ($p = 0.027$). Negative correlation was reported with HDL, $r = -0.694$ ($p = 0.008$).

Discussion

The relationship of body weight and adiposity with cardiovascular risk factors and novel markers for metabolic complications in children and young adults with T1DM is not fully understood (32).

In the DCCT/EDIC study, excess weight gain among the patients with T1DM was reported to be associated with sustained increases in central obesity, insulin resistance, dyslipidemia, and hypertension, as well as more extensive atherosclerosis (33, 34).

It is important to remember that the American Heart Association classified T1DM as a high cardiovascular risk factor for pediatric patients who are at risk of obesity (35).

Because children and young adults with T1DM and excessive body weight have a higher likelihood of having coexisting hypertension, dyslipidemia, and elevated alanine aminotransferase, the problem of metabolic syndrome among them should also be considered.

Merger et al., in their cross-sectional study, suggest that T1DM with metabolic syndrome is an independent risk factor for T1DM patients in developing macrovascular and microvascular comorbidities (36). Moreover, atherogenic lipid profile is also associated with unsatisfactory diabetes control. The prospective SEARCH study showed that the frequency of dyslipidemia with inadequate glycemic control (HbA1c ≥ 9%), longer T1D duration, obesity, and hypertension correlated with higher cIMT (37). The results from our study showed the association of BMI and BMI-SDS with atherogenic lipid profile and higher blood pressure in obese patients with T1DM, which is consistent with previous studies. However, no significant differences in SBP, DBP, total cholesterol, LDL, and TG levels were found between obese diabetic and non-diabetic patients, suggesting that obesity and its complications, such as diabetes mellitus, might increase the risk of cardiovascular diseases. Relatively little is known about novel markers such as fetuin-A, E-selectin, and OPG among young patients with T1DM and their correlation with body weight, insulin resistance, and risk of cardiovascular diseases. Siraz et al., in their study, presented that patients with fetuin-A levels above the cutoff value had poorer glycemic control and higher TG levels (38). The association between insulin resistance and higher fetuin-A level among the male patients with T1DM was reported in a previous study (39). Moreover, the connection between serum fetuin-A concentration and the development of cardiovascular complications in diabetics has been reported (40).

One of the most intriguing novel findings of our study is that fetuin-A levels were higher among obese non-diabetic children than in obese T1DM. It could be explained by the hypothesis that in patients without diabetes, fetuin-A plays a potentially protective role against coronary artery disease and acute cardiovascular events and also prevents spontaneous mineral precipitation in the vasculature (41–43). This might suggest that in diabetic patients, the inflammatory process occurs faster compared to non-diabetic obese patients, regardless of obesity. Moreover, fetuin-A levels among obese diabetic patients correlated with obesity, higher daily insulin requirement, and insulin resistance. These findings indicate that fetuin-A can be used as a parameter for predicting cardiovascular complications of T1DM and for monitoring poor glycemic control.

However, further research should be performed to establish precise cutoff points.

It is now recognized that a higher E-selectin level is a marker for development of atherosclerosis. Several studies reported increased levels in patients with obesity, metabolic syndrome, and T1DM. It was also noted that among the diabetic patients, there is a positive correlation between HbA1c, diastolic blood pressure, cholesterol, TG, and E-selectin levels.

In our study, serum E-selectin levels were similar among obese patients with T1DM and non-diabetic patients, but higher than the other groups. In contrast to other groups, no obvious

10.3389/fendo.2022.1036109

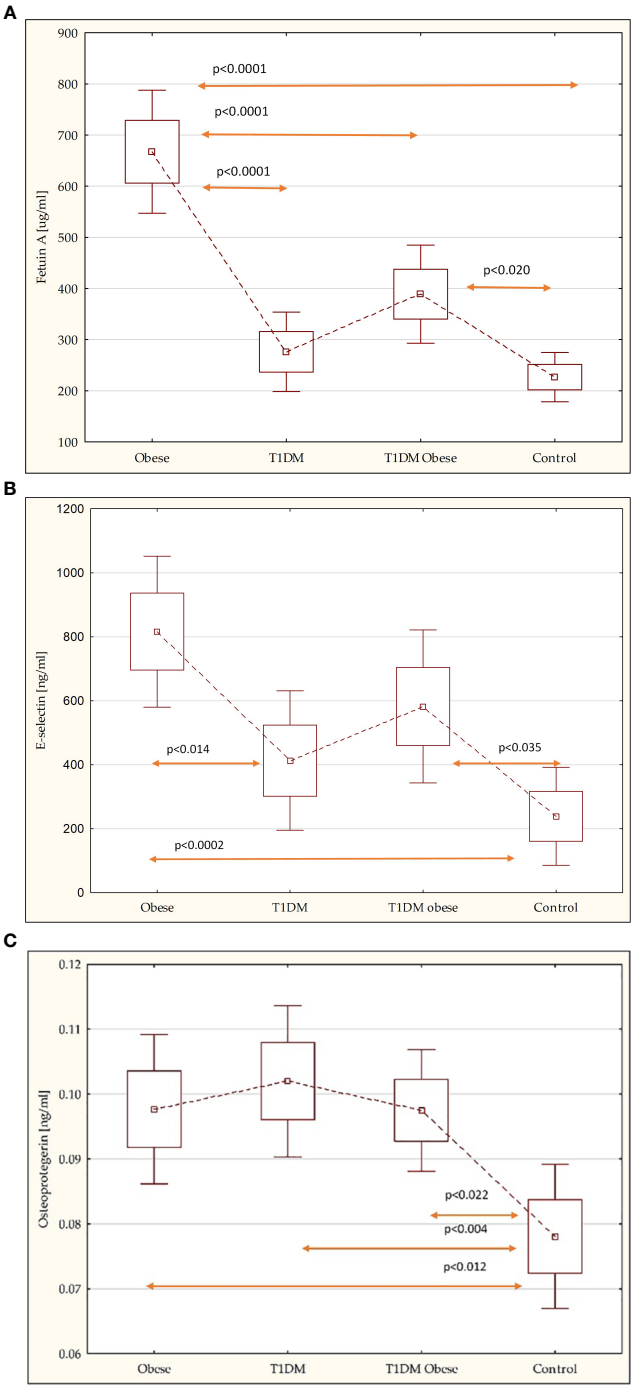


FIGURE 1
(A) Comparison of the fetuin-A in the study groups. **(B)** Comparison of the E-selectin in the study groups. **(C)** Comparison of the osteoprotegerin in the study groups.

correlation between classical risk factors and novel markers was noted (44, 45).

OPG is a bone-related peptide that can be found in different tissues, including bone, heart, and vascular endothelial cells. Current data suggest that increased plasma OPG concentrations are associated with atherosclerosis in the general population and are an independent predictor of cardiovascular complications in a large cohort of patients with T1DM 1 (46).

Our investigations show that OPG levels did not differ between obese T1DM patients and non-diabetic obese subjects, but were

10.3389/fendo.2022.1036109

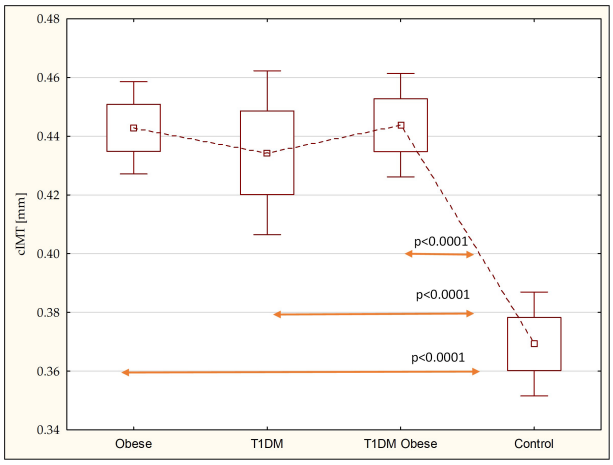


FIGURE 2
Comparison of the cIMT in the study groups.

higher in healthy individuals. Those findings are in line with those obtained by Gelluzi et al. and Ayina et al. (47). In the study, we also confirmed a positive correlation between OPG and BMI, BMI-SDS, and waist circumference in non-diabetic obese patients. The discrepancy in results may be a consequence of the study population; patients with T1DM and obesity had statistically lower BMI. Moreover, obesity is associated with elevated triglycerides and LDL and low HDL. However, in the present study, OPG levels did not correlate with TG, HDL, or LDL in both groups. Gannage-Yared et al. have detected a positive correlation between OPG and the HOMA index (48). Kim et al. have also determined an association between serum OPG levels and HOMA-IR in both normal and diabetic patients (49). In our study, we could not establish such a correlation between OPG levels and indexes of insulin sensitivity and insulin resistance. The role of OPG in the pathogenesis of atherosclerosis is still unclear. There is a discussion whether OPG synthesis is a compensatory mechanism to counteract the atherosclerotic process or whether OPG is an active compound in the atherosclerotic process (50, 51). Our results suggest that OPG might be involved in metabolic processes associated with atherosclerosis development. Further prospective studies are required to establish whether increased OPG levels in diabetic children in general as well as among those with obesity can predict later development of endothelial dysfunction and vascular complications.

In the study, we also reported that both of our obese groups —the non-diabetic group and the group with T1DM—had significantly higher cIMT compared to the control group. Even though there was no statistical difference between them, differences in cardiovascular risk factors were noticed.

In our non-diabetic obese patients, cIMT was positively correlated with BMI, BMI-SDS, and waist circumference-SDS. These findings are consistent with the data presented by the International Childhood Vascular Structure Evaluation Consortium. Abnormal cIMT was described in patients with cardiovascular complications including hypertension. Even though none of the study subjects was diagnosed with hypertension, there was a strong positive correlation between SBP, DBP, and cIMT among the non-diabetic group (23, 52). Moreover, obesity-related insulin resistance can induce atherothrombotic mechanisms, reduce fibrinolytic balance, and impair endothelial function. The possible relationship between cIMT and insulin resistance/hyperinsulinemia in children and adolescents remains inconsistent, with some studies reporting an adverse relation between insulin resistance and vascular measures while others observed no significant relation at all (53, 54). Our findings are in agreement with results of recent investigations where insulin resistance was associated with carotid wall thickness among non-diabetic children and adolescents with obesity.

Some publications report that T1DM patients have significantly increased cIMT levels compared to control subjects (55, 56). The SEARCH CVD Study clearly stated that increased BMI was a CV risk factor in young people with T1D and influenced cIMT (38).

Data obtained from our study were inconsistent. Similar to other studies, cIMT values were higher among diabetic patients, regardless of BMI, but strongly correlated with visceral adipose tissue, whose activity was expressed by VAI and triglyceride levels. Morisawa et al. indicate that OPG is also significantly associated with endothelial function and its concentration can be a useful predictor of early carotid atherosclerosis and higher cIMT (57). A similar correlation was noted in the group of obese non-diabetic patients.

Limitations of the study

We realize that our study has some limitations. First of all, the sample size was small, and the small number of patients were included. The age range included children and adults; however, only 10 subjects were older than 18 years (6 in the group with T1DM and normal weight and 4 in the group with T1DM and excessive body weight).

There were differences between the degree of obesity among diabetic and non-diabetic patients, and the stage of puberty was not considered. Further studies are necessary to report the degree of obesity among T1DM children and young adults and its influence on the levels of novel markers as well as cardiovascular complications.

Conclusion

Obesity in children and young adults with T1DM results in worse metabolic control, insulin resistance, and increased risk for vascular complications. However, novel markers of metabolic complications of obesity are similar between obese diabetic and non-diabetic patients.

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.

Ethics statement

The studies involving human participants were reviewed and approved by The Bioethics Committee of University of Warmia and Mazury (approval number KB/13/2019). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AK conceptualized and designed the study, collected data, performed statistical analysis, prepared tables and figures, and wrote and edited the manuscript. BG-O conceptualized and designed the study, interpreted the results, and designed and revised the manuscript. AC was involved in the design, conception, and revision of the manuscript. EJ-C read and approved the final version of the manuscript. All authors contributed to the article and approved the version submitted.

Funding

This research was supported by grants from Medical School of University of Warmia and Mazury in Olsztyn, Poland.

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.

References

1. Norris JM, Johnson RK, Stene LC. Type 1 diabetes-early life origins and changing epidemiology. *Lancet Diabetes Endocrinol* (2020) 8(3):226–38. doi: 10.1016/S2213-8587(19)30412-7

2. World Health Organization (WHO). *Obesity and overweight* . Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (Accessed 9 June 2021).

3. Majcher A, Czerwonogrodzka-Senczyna A, Kądziela K, Rumińska M, Pyrżak B. Development of obesity from childhood to adolescents. *Pediatr Endocrinol Diabetes Metab* (2021) 27(2):70–5. doi: 10.5114/pedm.2021.105297

4. Sahoo K, Sahoo B, Choudhury AK, Sofi NY, Kumar R, Bhadoria AS. Childhood obesity: causes and consequences. *J Family Med Prim Care* (2015) 4 (2):187–92. doi: 10.4103/2249-4863.154628

5. Magliano DJ, Boyko EJ. IDF Diabetes Atlas 10th edition scientific committee.. In: *IDF DIABETES ATLAS [Internet]. 10th ed.*. Brussels: International Diabetes Federation (2021). Available at: https://diabetesatlas.org.

6. Ogle GD, James S, Dabelea D, Pihoker C, Svennson J, Maniam J, et al. Global estimates of incidence of type 1 diabetes in children and adolescents: Results from the international diabetes federation atlas, 10. *Diabetes Res Clin Pract* (2021) 183:109083. doi: 10.1016/j.diabres.2021.109083

7. Manyanga T, Sellers EA, Wicklow BA, Doupe M, Fransoo R. Not as skinny as we used to think: Body mass index in children and adolescents at diagnosis of type 1 diabetes mellitus. *J Diabetes Complications* (2016) 30(2):292–4. doi: 10.1016/j.jdiacomp.2015.11.022

8. Jarosz-Chobot P, Polanska J, Szadkowska A, Kretowski A, Bandurska-Stankiewicz E, Ciechanowska M, et al. Rapid increase in the incidence of type 1 diabetes in polish children from 1989 to 2004, and predictions for 2010 to 2025. *Diabetologia* (2011) 54(3):508–15. doi: 10.1007/s00125-010-1993-4

9. Wolosowicz M, Lukaszuk B, Chabowski A. The causes of insulin resistance in type 1 diabetes mellitus: Is there a place for quaternary prevention? *Int J Environ Res Public Health* (2020) 17(22):8651. doi: 10.3390/ijerph17228651

10. Danielson KK, Drum ML, . Estrada CL, Lipton RB. Racial and ethnic differences in an estimated measure of insulin resistance among individuals with type 1 diabetes. *Diabetes Care* (2010) 33(3):614–9. doi: 10.2337/dc09-1220

11. Corbin KD, Driscoll KA, Pratley RE, Smith SR, Maahs DM, Mayer-Davis EJ. Advancing care for type 1 diabetes and obesity network (ACT1ON). obesity in type 1 diabetes: Pathophysiology, clinical impact, and mechanisms. *Endocr Rev* (2018) 39(5):629–63. doi: 10.1210/er.2017-00191

12. Vilarrasa N, San Jose P, Rubio MÁ, Lecube A. Obesity in patients with type 1 diabetes: Links, risks and management challenges. *Diabetes Metab Syndr Obes* (2021) 14:2807–27. doi: 10.2147/DMSO.S223618

13. Amato MC, Giordano C, Pitrone M, Galluzzo A. Cut-off points of the visceral adiposity index (VAI) identifying a visceral adipose dysfunction associated with cardiometabolic risk in a Caucasian Sicilian population. *Lipids Health Dis* (2011) 10:183. doi: 10.1186/1476-511X-10-183

14. Pagan LU, Gatto M, Martinez PF, Okoshi K, Okoshi MP. Biomarkers in cardiovascular disease: The role of fetuin-a. *Arq Bras Cardiol* (2022) 118(1):22–3. doi: 10.36660/abc.20210980

15. Shim YS, Kang MJ, Oh YJ, Baek JW, Yang S, Hwang IT. Fetuin-a as an alternative marker for insulin resistance and cardiovascular risk in prepubertal children. *J Atheroscler Thromb* (2017) 24(10):1031–8. doi: 10.5551/jat.38323

16. Weikert C, Stefan N, Schulze MB, Pischon T, Berger K, Joost HG, et al. Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. *Circulation* (2008) 118(24):2555–62. doi: 10.1161/CIRCULATIONAHA.108.814418

17. Tschiderer L, Klingenschmid G, Nagrani R, Willeit J, Laukkanen JA, Schett G, et al. Osteoprotegerin and cardiovascular events in high-risk populations: Meta-analysis of 19 prospective studies involving 27 450 participants. *J Am Heart Assoc* (2018) 7(16):e009012. doi: 10.1161/JAHA.118.009012

18. Rashad NM, El-Shal AS, Shalaby SM, Abdel-Nour HM, Sarhan WM. Osteoprotegerin expression and serum values in obese women with type 2 diabetes mellitus. *Mol Biol Rep* (2021) 48(11):7095–104. doi: 10.1007/s11033-021-06699-x

19. Kalkan R, Becer E. RANK/RANKL/OPG pathway is an important for the epigenetic regulation of obesity. *Mol Biol Rep* (2019) 46(5):5425–32. doi: 10.1007/s11033-019-04997-z

20. Pérez de Ciriza C, Moreno M, Restituto P, Bastarrika G, Simón I, Colina I, et al. Circulating osteoprotegerin is increased in the metabolic syndrome and associates with subclinical atherosclerosis and coronary arterial calcification. *Clin Biochem* (2014) 47(18):272–8. doi: 10.1016/j.clinbiochem.2014.09.004

21. Al-Shorman A, Al-Domi H, Faqih A. Markers of subclinical atherosclerosis in schoolchildren with obesity and metabolic syndrome. *Swiss Med Wkly* (2020) 147:w14446. doi: 10.4414/smw.2020.14446

22. Porta B, Baldassarre D, Camera M, Amato M, Arquati M, Brusoni B, et al. E-selectin and TFPI are associated with carotid intima-media thickness in stable IHD patients: the baseline findings of the MIAMI study. *Nutr Metab Cardiovasc Dis* (2008) 18(4):320–8. doi: 10.1016/j.numecd.2007.01.008

23. Zhao M, López-Bermejo A, Caserta CA, Medeiros CCM, Kollias A, Bassols J, et al. International childhood vascular structure evaluation consortium. metabolically healthy obesity and high carotid intima-media thickness in children and adolescents: International childhood vascular structure evaluation consortium. *Diabetes Care* (2019) 42(1):119–25. doi: 10.2337/dc18-1536

24. Klonowska B, Charemska D, Jabłońska J, Banach A, Kącka A, Szynkarczuk E, et al. Carotid artery intima-media thickness (cIMT) in young type 1 diabetic patients in relation to comorbid additional autoimmune diseases and microvascular complications. *Pediatr Endocrinol Diabetes Metab* (2016) 22(3). doi: 10.18544/PEDM-22.03.0057

25. Sun YP, Cai YY, Li HM, Deng SM, Leng RX, Pan HF. Increased carotid intima-media thickness (CIMT) levels in patients with type 1 diabetes mellitus (T1DM): A meta-analysis. *J Diabetes Complications* (2015) 29(5):724–30. doi: 10.1016/j.jdiacomp.2015.03.018

26. . Available at: https://www.mp.pl/pediatria/praktyka-kliniczna/procedury/13874,rys-11-siatki-centylowe-wskaznika-wzglednej-masy-ciala-bmi-chlopcow-a-i-dziewczat-b-warszawskich.

27. Majid H, Masood Q, Khan AH. Homeostatic model assessment for insulin resistance (HOMA-IR): A better marker for evaluating insulin resistance than fasting insulin in women with polycystic ovarian syndrome. *J Coll Phys Surg Pak* (2017) 27(3):123–6.

28. *Guidelines on the management of patients with diabetes 2021* . Available at: https://ptdiab.pl/images/docs/zalecenia/2021-Guidelines-on-the-management-of-patients-with-diabetes.pdf.

29. Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ. Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* (2000) 49(4):626–32. doi: 10.2337/diabetes.49.4.626

30. Szadkowska A, Pietrzak I, Szlawska J, Kozera A, Gadzicka A, Młynarski W. Abdominal obesity, metabolic syndrome in type 1 diabetic children and adolescents. *Pediatr Endocrinol Diabetes Metab* (2009) 15(4):233–9. Available at: https://www.researchgate.net/publication/44585398_Abdominal_obesity_metabolic_syndrome_in_type_1_diabetic_children_and_adolescents.

31. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* (1986) 74(6):1399–406. doi: 10.1161/01.cir.74.6.1399

32. Salgado AL, Carvalho LD, Oliveira AC, Santos VN, Vieira JG, Parise ER. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. *Arq Gastroenterol* (2010) 47(2):165–9. doi: 10.1590/s0004-28032010000200009

33. Jamiołkowska-Sztabkowska M, Głowińska-Olszewska B, Bossowski A. C-peptide and residual β-cell function in pediatric diabetes – state of the art. *Pediatr Endocrinol Diabetes Metab* (2021) 27(2):123–33. doi: 10.5114/pedm.2021.107165

34. Lipsky LM, Gee B, Liu A, Nansel TR. Body mass index and adiposity indicators associated with cardiovascular biomarkers in youth with type 1 diabetes followed prospectively. *Pediatr Obes* (2017) 12(6):468–76. doi: 10.1111/ijpo.12167

35. Purnell JQ, Zinman B, Brunzell JDDCCT/EDIC Research Group. The effect of excess weight gain with intensive diabetes mellitus treatment on cardiovascular disease risk factors and atherosclerosis in type 1 diabetes mellitus: results from the diabetes control and complications Trial/Epidemiology of diabetes interventions and complications study (DCCT/EDIC) study. *Circulation* (2013) 127(2):180–7. doi: 10.1161/CIRCULATIONAHA.111.077487

36. de Ferranti SD, Steinberger J, Ameduri R, Baker A, Gooding H, Kelly AS, et al. Cardiovascular risk reduction in high-risk pediatric patients: A scientific statement from the American heart association. *Circulation* (2019) 139(13):e603–34. doi: 10.1161/CIR.0000000000000618

37. Merger SR, Kerner W, Stadler M, Zeyfang A, Jehle P, Müller-Korbsch M, et al. DPV initiative; German BMBF competence network diabetes mellitus. prevalence and comorbidities of double diabetes. . *Diabetes Res Clin Pract* (2016) 119:48–56. doi: 10.1016/j.diabres.2016.06.003

38. Shah AS, Dabelea D, Fino NF, Dolan LM, Wadwa RP, D'Agostino RJr, et al. Predictors of increased carotid intima-media thickness in youth with type 1 diabetes: The SEARCH CVD study. *Diabetes Care* (2016) 39(3):418–25. doi: 10.2337/dc15-1963

39. Şiraz ÜG, Doğan M, Hatipoğlu N, Muhtaroğlu S, Kurtoğlu S. Can fetuin-a be a marker for insulin resistance and poor glycemic control in children with type 1 diabetes mellitus? *J Clin Res Pediatr Endocrinol* (2017) 9(4):293–9. doi: 10.4274/jcrpe.4532

40. Jung CH, Kim BY, Kim CH, Kang SK, Jung SH, Mok JO. Associations of serum fetuin-a levels with insulin resistance and vascular complications in patients with type 2 diabetes. *Diabetes Vasc Dis Res* (2013) 10(5):459–67. doi: 10.1177/1479164113490766

41. Dabrowska AM, Tarach JS, Wojtysiak-Duma B, Duma D. Fetuin-a (AHSG) and its usefulness in clinical practice. review of the literature. *BioMed Pap Med Fac Univ Palacky Olomouc Czech Repub* (2015) 159(3):352–9. doi: 10.5507/bp.2015.018

42. Vörös K, Gráf LJr, Prohászka Z, Gráf L, Szenthe P, Kaszás E, et al. Serum fetuin-a in metabolic and inflammatory pathways in patients with myocardial infarction. *Eur J Clin Invest* (2011) 41(7):703–9. doi: 10.1111/j.1365-2362.2010.02456.x

43. Zhao ZW, Lin CG, Wu LZ, Luo YK, Fan L, Dong XF, et al. Serum fetuin-a levels are associated with the presence and severity of coronary artery disease in patients with type 2 diabetes. *Biomarkers* (2013) 18(2):160–4. doi: 10.3109/1354750X.2012.762806

44. Carrizo Tdel R, Prado MM, Velarde MS, Díaz EI, Bazán MC, Abregú AV. E-selectina soluble en una población infanto-juvenil con diabetes tipo 1 [Soluble e-selectin in children and adolescents with type 1 diabetes. *Med (B Aires)* (2008) 68(3):193–7. Available at: https://www.researchgate.net/publication/23157909_Soluble_E-selectin_in_children_and_adolescents_with_type_1_diabetes.

45. El Wakeel MA, El-Kassas GM, Amer AF, Elbatal WH, Sabry RN, EL-Ghaffar M, et al. E-selectin and vascular complications in children with type 1 diabetes mellitus. *Med Res J* (2014) 13(1):27–32. doi: 10.1097/01.MJX.0000446937.40653.3d

46. Galluzzi F, Stagi S, Salti R, Toni S, Piscitelli E, Simonini G, et al. Osteoprotegerin serum levels in children with type 1 diabetes: a potential modulating role in bone status. *Eur J Endocrinol* (2005) 153(6):879–85. doi: 10.1530/eje.1.02052

47. Ayina Ayina CN, Sobngwi E, Essouma M, Noubiap JJN, Boudou PH, Ngoa LSE, et al. Osteoprotegerin in relation to insulin resistance and blood lipids in sub-Saharan African women with and without abdominal obesity. *Diabetol Metab Syndr* (2015) 7:47. doi: 10.1186/s13098-015-0042-3

48. Gannagé-Yared MH, Yaghi C, Habre B, Khalife S, Noun R, Germanos-Haddad M, et al. Osteoprotegerin in relation to body weight, lipid parameters insulin sensitivity, adipocytokines, and c-reactive protein in obese and non-obese young individuals: results from both cross-sectional and interventional study. *Eur J Endocrinol* (2008) 158(3):353–9. doi: 10.1530/EJE-07-0797

49. Yeşilkaya E, Bideci A, Çamurdan O, Boyraz M, Vurucu S, Cinaz P. Association of osteoprotegerin and rankl levels with insulin resistance in

pubertal obese children. *Open Med* (2009) 5:261–7. doi: 10.2478/s11536-009-0065-y

50. Gordin D, Soro-Paavonen A, Thomas MC, et al. Osteoprotegerin is an independent predictor of vascular events in Finnish adults with type 1 diabetes. *Diabetes Care* (2013) 36:1827–33. doi: 10.2337/dc12-2170

51. Lambrinoudaki I, Tsouvalas E, Vakaki M, Kaparos G, Stamatelopoulos K, Augoulea A, et al. Osteoprotegerin, soluble receptor activator of nuclear factor- κ b ligand, and subclinical atherosclerosis in children and adolescents with type 1 diabetes mellitus. *Int J Endocrinol* (2013) 2013:102120. doi: 10.1155/2013/102120

52. Widjaja NA, Irawan R, Prihaningtyas RA, Ardiana M, Hanindita MH. Carotid intima-media thickness, hypertension, and dyslipidemia in obese adolescents. *Pan Afr Med J* (2019) 34:134. doi: 10.11604/pamj.2019.34.134.18309

53. Santos IS, Bittencourt MS, Goulart AC, Schmidt MI, Diniz MFHS, Lotufo PA, et al. Insulin resistance is associated with carotid intima-media thickness in non-diabetic subjects. a cross-sectional analysis of the ELSA-brasil cohort baseline. *Atherosclerosis* (2017) 260:34–40. doi: 10.1016/j.atherosclerosis.2017.03.011

54. Asghari G, Dehghan P, Mirmiran P, Yuzbashian E, Mahdavi M, Tohidi M, et al. Insulin metabolism markers are predictors of subclinical atherosclerosis among overweight and obese children and adolescents. *BMC Pediatr* (2018) 18 (1):368. doi: 10.1186/s12887-018-1347-9

55. Cerit MN, Sendur HN, Bolayır B, Cerit ET, Cindil E, Yaşım Aktürk M, et al. Evaluation of common carotid artery in type 1 diabetes mellitus patients through speckle tracking carotid strain ultrasonography. *Diagn Interv Radiol* (2021) 27 (2):195–205. doi: 10.5152/dir.2021.20025

56. Stanković SM, Zivić SR, Šaranac L, Cvetković V, Pešić M, Vasić K, et al. Determinants of atherosclerosis in children and adolescents with diabetes type 1. *Endokrynol Pol* (2012) 63(6):414–9. Available at: https://journals.viamedica.pl/endokrynologia_polska/article/view/25127/19956.

57. Morisawa T, Nakagomi A, Kohashi K, Kosugi M, Kusama Y, Atarashi H, et al. Osteoprotegerin is associated with endothelial function and predicts early carotid atherosclerosis in patients with coronary artery disease. *Int Heart J* (2015) 56(6):605–12. doi: 10.1536/ihj.15-150

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 14 December 2022
DOI 10.3389/fendo.2022.1061671

OPEN ACCESS

EDITED BY
Malgorzata Wojcik,
Jagiellonian University Medical College, Poland

REVIEWED BY
Ewa Barg,
Wroclaw Medical University, Poland
Srividya Vasu,
Scientific Advisor/Helio Genomics, United States

*CORRESPONDENCE
Iwona Pietrzak
iwona.pietrzak@vp.pl

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 06 October 2022
ACCEPTED 21 November 2022
PUBLISHED 14 December 2022

CITATION
Chylińska-Frątczak A, Pietrzak I, Michalak A, Wyka K and Szadkowska A (2022) Autoimmune reaction against pancreatic beta cells in children and adolescents with simple obesity. *Front. Endocrinol.* 13:1061671. doi: 10.3389/fendo.2022.1061671

Autoimmune reaction against pancreatic beta cells in children and adolescents with simple obesity

Aneta Chylińska-Frątczak[1†], Iwona Pietrzak[2*†], Arkadiusz Michalak[2,3], Krystyna Wyka[4] and Agnieszka Szadkowska[2]

[1]Department of Pediatrics, Endocrinology, Diabetology and Nephrology, Maria Konopnicka University Pediatrics Center, Lodz, Poland, [2]Department of Pediatrics, Diabetology, Endocrinology and Nephrology, Medical University of Lodz, Lodz, Poland, [3]Department of Biostatistics and Translational Medicine, Medical University of Lodz, Lodz, Poland, [4]Department of Pediatrics, Oncology and Hematology, Medical University of Lodz, Lodz, Poland

Introduction: One of the most important complications of obesity is insulin resistance, which leads to carbohydrate metabolism disorders such as type 2 diabetes. However, obesity is also associated with development of an autoimmune response against various organs, including pancreatic beta cells. The prevalence of such autoimmune processes in children and their possible contribution to the increased incidence of type 1 diabetes is currently unclear. Therefore, the present study assessed the prevalence of autoantibodies against pancreatic islet beta cell's antigens in children and adolescents with simple obesity.

Material and methods: This prospective observational study included pediatric patients (up to 18 years of age) with simple obesity hospitalized between 2011 and 2016 at the Department of Pediatrics, Diabetology, Endocrinology and Nephrology of the Medical University of Lodz. Children with acute or chronic conditions that might additionally affect insulin resistance or glucose metabolism were excluded. Collected clinical data included sex, age, sexual maturity ratings (Tanner`s scale), body height and weight, waist and hip circumference, amount of body fat and lean body mass. Each participant underwent a 2-hour oral glucose tolerance test with simultaneous measurements of glycaemia and insulinemia at 0`, 60` and 120`. In addition, glycated hemoglobin HbA1c, fasting and stimulated c-peptide, total cholesterol, as well as high- and low-density cholesterol and triglycerides were measured. Insulin resistance was assessed by calculating HOMA-IR index. The following autoantibodies against pancreatic islet beta cells were determined in each child: ICA - antibodies against cytoplasmic antigens of pancreatic islets, GAD - antibodies against glutamic acid decarboxylase, ZnT8 - antibodies against zinc transporter, IA2 - antibodies against tyrosine phosphatase, IAA – antibodies against insulin.

Results: The study group included 161 children (57.4% boys, mean age 13.1 ± 2.9 years) with simple obesity (mean BMI z-score +2.2 ± 1.6). Among them, 28 (17.4%) were diagnosed with impaired glucose metabolism during OGTT [23 (82.2%) – isolated impaired glucose tolerance (IGT), 3 (10.7%) – isolated impaired fasting glucose (IFG), 2 (7.1%) – IFG and IGT]. Of the children tested, 28 (17.4%) were tested positive for at least one islet-specific autoantibody [with similar percentages in boys (15, 17.4%) and girls (13, 17.3%), p=0.9855], with ICA being the most common (positive in 18, 11.2%), followed by IAA (7, 4.3%), ZnT8 (5, 3.1%), GADA (3, 1.9%) and IA2 (1, 0.6%). There was no association between the presence of the tested antibodies and age, sex, stage of puberty, parameters assessing the degree of obesity, HbA1c, lipid levels and basal metabolic rate. However, autoantibody-positive subjects were more likely to present IFG or IGT in OGTT compared to those who tested completely negative (9, 32.1% vs 19, 14.3%, p=0.0280). Their HOMA-IR was also significantly higher (HOMA-IR: 4.3 ± 1.9 vs 3.4 ± 1.9, p=0.0203) and this difference remained statistically significant after adjusting for sex and age (p=0.0340).

Conclusions: Children and adolescents with simple obesity presented a higher prevalence of markers of autoimmune response against pancreatic beta cells than the general population. Most often, they had only one type of antibody - ICA. The presence of autoimmune response indicators against pancreatic islet antigens is more common in obese patients with impaired carbohydrate metabolism and is associated with lower insulin sensitivity.

KEYWORDS

obesity, diabetes, anti-islet autoantibodies, children, adolescents

Introduction

Excess body weight has become one of the most crucial challenges for pediatric healthcare. According to the World Health Organization report, the prevalence of overweight and obesity among children aged 5-19 has increased 4.5 times since 1975, meaning that in 2016, 340 million children and adolescents worldwide were overweight or obese and 124 million were obese (1). Excess body weight is associated with an increased risk of a number of disorders, the most prominent of which are disorders of carbohydrate metabolism, including diabetes (2–6). In principle, obesity is known to cause insulin resistance and secondary insulin secretion abnormalities that lead to the development of type 2 diabetes (T2D) (7). However, in children and adolescents, the impact of excess body weight might be more nuanced.

Primarily, it has been shown that the clinical course of T2D in children and adolescents differs from that in adults and exhibit a faster rate of decline in endogenous insulin secretion (8–10). Moreover, up to 1/3 of patients with T2D can be detected with the presence of at least one type of antibodies against islet antigens (11, 12). Moreover, excess body weight has been also hypothesized to contribute to the development of type 1 diabetes (T1D), the most common type of diabetes in childhood. According to the accelerator hypothesis, in individuals with HLA-dependent genetic predisposition, excess weight gain reduces insulin sensitivity, which in turn leads to pancreatic beta cells apoptosis and activation of an autoimmune response (13, 14). In addition, adipose tissue generates chronic low-grade inflammation and adversely affects the immunotolerance mechanisms (15). However, attempts to clinically verify this hypothesis have met with mixed success – the observed associations between excess body weight (birth weight, weight gain in infants and older children) and the incidence of T1D (5, 16–19) turned out to be inconclusive and largely based on selected groups of children with an underlying genetic risk for T1D.

Therefore, it is worth taking a closer look at the group of obese children who might be at increased risk not only for T2D, but also for T1D. The aim of this study was to investigate the presence of T1D-related autoimmune markers in children and adolescents with simple obesity and test whether they are associated with the development of carbohydrate metabolism disorders in those patients.

Subjects and methods

Ethics statement

This was a prospective observational study approved by the local Bioethics Committee of the Medical University of Lodz (No. RNN/224/15/KE) and conducted in accordance with the principles set forth in the Declaration of Helsinki

Subjects

Between 2011-2016, we recruited children and adolescents up to 18 years of age who were routinely admitted to the Department due to obesity (defined as BMI percentile ≥95th percentile) and underwent screening for glucose metabolism abnormalities. They were invited to take part in an extended panel of metabolic tests, including screening for T1D. Individuals with acute or chronic conditions that might predispose to carbohydrate metabolism disorders were excluded. We also collected data on family history of T1D and other autoimmune disorders to minimize potential bias in autoantibody prevalence due to family burden. This step was done during a follow-up period, so not all participants were available.

Methods

Each participant underwent a comprehensive medical examination with nutritional assessment. Anthropometric parameters were measured by Harpenden stadiometer (accuracy of 0.1 cm), TANITA MC-980MA (accuracy of 0.1 kg) and non-stretchable tape (accuracy of 0, 1 cm). Body composition was analyzed by bioelectrical impedance analysis (BIA-TANITA MC-980MA), and basal metabolic rate (BMR) was calculated. Sexual maturity ratings were assessed in each child using the Tanner scale. Glycated hemoglobin (HbA1c) was measured with high-performance liquid chromatography (Bio-Rad Variant, Bio-Rad Laboratories, Hercules, USA). Lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) was performed in each child according to commonly accepted methods in the hospital laboratory.

Each participant underwent a 2-hour oral glucose tolerance test (OGTT), during which blood glucose and insulin levels were measured at 0`, 60` and 120` - then the presence of glucose metabolism disorders was determined in accordance to the ISPAD 2018 Recommendations (20). In addition, c-peptide levels (ELISA) were measured for each patient in the fasting state and 6` after stimulation with 1mg of intravenous glucagon.

Each child also underwent a comprehensive screening for pancreatic islet autoantibodies performed by the Laboratory of Immunopathology and Genetics, which is the reference laboratory for Poland, certified during the Islet AutoAntibody Standardization Program - IASP 2012-2019 (LAB604). The following autoantibodies were measured in serum (method, IASP-certified sensitivity/specificity and cut-off for positivity reported respectively):

- ICA - antibodies against cytoplasmic antigens of pancreatic islets (indirect immunofluorescence method using human pancreatic sections, 72.0% and 94.4%, cut-off: 5-10 μ. JDF depending on the substrate used),
- GAD - antibodies against glutamic acid decarboxylase (RSR ELISA method, USA, 82% and 98.9%, cut-off ≥10),
- ZnT8 - antibodies against zinc transporter (RSR ELISA method, USA, 76% and 97.8%, cut-off ≥15),
- IA2 - antibodies against tyrosine phosphatase (RSR ELISA method, USA, 70% and 95.6%, cut-off≥20),
- IA/IAA – antibodies against insulin (RIA method, UK, 42% and 100%, cut-off≥10).

Statistical analysis

Body mass index (BMI) was calculated according to standard equation [weigh/(height in m) ^2], z-scores and percentiles were calculated based on national growth charts (21). Waist-to-height ratio (WHtR) and waist-to-hip ratio (WHT) were calculated in the standard way. Insulin resistance was assessed using HOMA-IR index (Homeostatic Model Assessment – Insulin Resistance), calculated according to the standard equation (fasting insulinemia (mU/ml) x fasting blood glucose (mmol/l)/22.5).

Distributions of continuous variables were assessed using Shapiro-Wilk test. Afterwards, tests between the groups were performed using t-test [results reported as mean± standard deviation (SD)] or Mann-Whitney`s U test (results as medians and 25-75% ranges). Qualitative variables were analyzed using the chi^2 test or fisher`s exact test for small groups.

Due to the lack of a control group, the prevalence of antibodies to pancreatic islet antigens in the study group was compared with data from the literature (22–24).

Results

Demographic, anthropometric, and metabolic parameters

The study group included 161 children (57.4% boys, mean age 13.1 ± 2.9 years) with simple obesity (mean BMI z-score +2.2+/0.4) (Table 1). Among them, 28 (17.4%) were diagnosed with impaired glucose metabolism during OGTT [23 (82.2%) – isolated impaired

glucose tolerance (IGT), 3 (10.7%) – isolated impaired fasting glucose (IFG), 2 (7.1%) – IFG and IGT].

The prevalence of anti-islet antibodies

Of the children tested, 28 (17.4%) were positive for at least one islet-specific autoantibody [with similar percentages in boys (15, 17.4%) and girls (13, 17.3%), p=0.9855], with ICA being the most common (positive in 18, 11.2%), followed by IAA (7, 4.3%), ZnT8 (5, 3.1%), GAD (3, 1.9%) and IA2 (1, 0.6%). The prevalence of positive results for most autoantibodies in the study group was similar to general population (historic reference for IAA – 4%, anti-GAD – 2%, IA2 – 0.8%, ZnT8 -2%, all p>0.05). However, we noted that obese children were more often positive for at least one autoantibody (17.3% vs 4.9%, p<0.0001) and particularly for ICA autoantibodies (11.2% vs historical reference of 3%, p<0.0001).

Association of the autoimmune response against pancreatic islets with participants` clinical characteristics

Children positive for at least one autoantibody were non-significantly older (13.8 ± 2.8 vs 13.0 ± 2.9, p=0.1840) than those who tested negative and presented similar sex maturity ratings (median 3 (25-75%: 2 to 5) vs 3 (25-75%: 2 to 5), p= 0.4135), HbA1c (5.4 ± 0.3 vs 5.3 ± 0.3, p=0.3111), c-peptide (fasting: 3.4 ± 1.2 vs 3.0 ± 1.2, p=0.1420; stimulated: 8,7 ± 3,7 vs 7,6 ± 2,8, p=0.0925) and lipid profile. They were also comparable in terms of BMI z-scores, body fat percentage and estimated Basal Metabolic Rate. However, autoantibody-positive subjects had a higher waist to height ratio (WHtR: 0,63 ± 0,03 vs 0,59 ± 0,1, p=0.047 - adj. for sex and age).

The relationship between the occurrence of an autoimmune reaction against pancreatic islets and the carbohydrate metabolism disorders

Children with at least one positive autoantibody were more likely to present IFG or IGT in OGTT compared with those who tested completely negative (9, 32.1% vs 19, 14.3%, p=0.0280). The autoantibody-positive children showed significantly higher glycemia at 60 min. of OGTT and higher insulinemia at all three time points of OGTT (Figure 1). Their HOMA-IR index was significantly higher (HOMA-IR: 4.3 ± 1.9 vs 3.4 ± 1.9, p=0.0203) and the difference remained statistically significant after adjusting for sex and age (p=0.0340). A detailed comparison between the groups is presented in Table 1.

Association of autoimmune response against pancreatic islets with family history of type 1 diabetes and other autoimmune diseases

In 54 (33.5%) children, a family history of type 1 diabetes or other autoimmune diseases were unavailable. Among the 107 children with a complete family history available, none of the 16 autoantibody-positive children (0%) had any known relatives with type 1 diabetes, compared with 7 autoantibody-negative children (7.7%), which was likely due to chance (p=0.5910). Similarly, both autoantibody-positive and autoantibody-negative children had a similar prevalence of type 1 diabetes or other autoimmune diseases in first degree relatives (autoantibody-positive: 2 (12.5%) vs autoantibody-negative: 15 (16/5%), p=1.0000)

Patients with multiple anti-islet antibodies

Out of autoantibody-positive children, four (2.5%) were positive for at least two autoantibodies, marking pre-clinical stages of type 1 diabetes – their exact titers and patients` clinical features are included in Table 2. Over the following seven years, only one subject developed clinically evident type 1 diabetes as an adult (more data unavailable).

Discussion

Our most important observation is that the prevalence of at least one islet-specific autoantibody in a sample of children and adolescents with simple obesity was as much as 3.5 times higher than in general population. The prevalence of islet autoantibodies is well-established in patients with T1D or at increased risk of developing the disease (first degree relationship to the patient, genetic screening) (24, 25).

In contrast, reports on the frequency of autoimmune anti-islet responses in people with excess body weight or with T2D are less numerous and primarily relate to adults. About 2-12% of adults with clinically diagnosed T2D were found to have detectable levels of antibodies to pancreatic islet antigens in peripheral blood (26–28). It was also suggested that the degree of obesity might be associated with the risk of developing islet autoantibodies in adults with T2D - among 204 patients with T2D and excess body weight, the presence of at least one of the three antibodies (GADA, IA2, and ZnT8A) was found in 6.4% of all subjects and in as many as 12.8% when considering those with severe obesity (BMI > = 40 kg/m2) (29). On the other hand, studies on pediatric patients with T2D reported the presence of at least one anti-islet antibody in approximately 30% of them (11, 30–32). The presence of at least

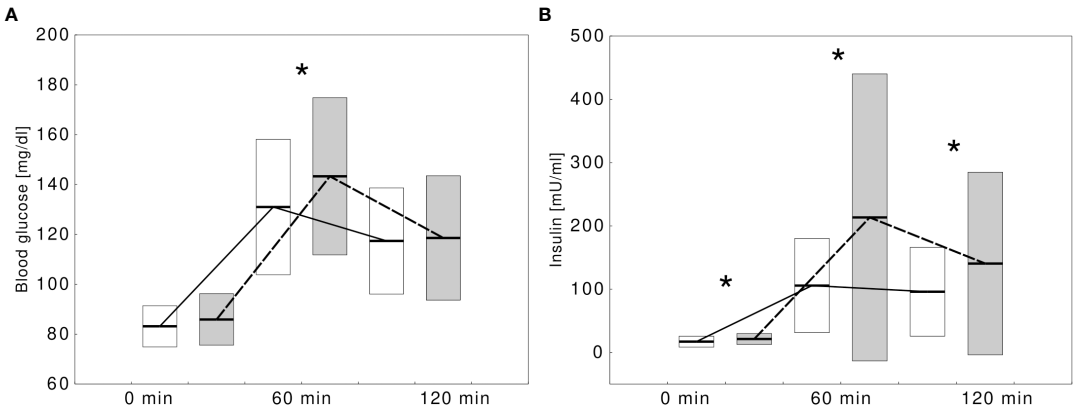


FIGURE 1
Comparison of blood glucose **(A)** and insulin **(B)** concentrations during oral glucose tolerance test in obese children without type 1 diabetes-specific autoantibodies (white boxes, solid line) and with at least one autoantibody (gray boxes, dashed lines). Significant differences ($p<0.05$) were marked with an asterix (*).

one anti-islet antibody (GADA or IA2) in obese children with T2D was associated with younger age, lower insulin secretion, higher HbA1c, and a higher incidence of diabetic ketoacidosis at diagnosis (33). Our estimated prevalence of positive autoantibodies was, as expected, lower than that observed in children and adolescents with T2D.

Relatively few papers have examined the anti-islet autoimmune response in obese children and adolescents without previously diagnosed diabetes. Cambuli et al. assessing the presence of anti-islet antibodies (GADA, IA2, IAA) in 686 overweight/obese children and adolescents (mean age 10.3 ± 3.2 years) found the presence of at least one anti-islet antibody in 2.18% of the subjects (2 antibodies in 0.7% and 1 antibody in 0.15%) and this value did not differ significantly from the result obtained in the control group with normal body weight (1.8%) (34). GADA-1.89% was the most frequently detected, followed by IA2-0.87% and IAA-0.43%. The apparent discrepancy with our observed prevalence results from including ICA in our study (and to a lesser extent, ZnT8). In our study of obese children and adolescents, ICA was the most common anti-islet antibody, its prevalence significantly increased compared to literature reports for general population (22–24). ICA antibodies were the first identified antibodies against pancreatic islet antigens and were identified using indirect immunofluorescence on frozen sections of human pancreas with blood group 0. The demonstration of ICA by indirect immunofluorescence is an illustration of general islet autoimmunization and identifies several beta-cell antigens simultaneously (24). The finding of isolated positive ICA despite negative GADA, IA-2A, IAA and ZnT8A results in children with newly diagnosed T1D indicates that ICA testing detects a wide range of islet-specific antibodies and sometimes cross-reacting antibodies in serum samples (35). Pollannen et al. in the Finnish population-based Diabetes Prediction and Prevention (DIPP) type 1 study, which aims to monitor the emergence of anti-islet autoantibodies in children with increased HLA-dependent susceptibility to T1D, showed high sensitivity of ICA in identifying clinical disease progression, but unlike previously published 5-year follow-up data, lower specificity than other autoantibodies tested by biochemical methods (IAA, GADA, IA2) (36). It should also be kept in mind that the inconclusive results of the work on the predictive value of ICA for the development of T1D may be due to the fact that ICA determination is the most operator-dependent test of all the tests measuring the level of anti-islet autoantibodies (23, 37). In our study, the determination of all types of antibodies against pancreatic islet antigens was performed in a reference laboratory for Poland, certified during the Islet AutoAntibody Standardization Program - IASP 2012-2019.

We also need to comment on the patients in whom we observed multiple positive islet autoantibodies, although the numbers was too low for statistical analysis. It is estimated that 70% of patients who have at least 2 types of anti-islet antibodies will develop clinical type 1 diabetes mellitus within 10 years (38). Our study identified 4 individuals with at least 2 anti-islet antibodies, one of them was also diagnosed with dysglycemia (IGT). According to the ADA criteria, they were individuals with type 1 diabetes, 3 individuals in the first stage (autoimmunity), and one individual in the second stage (autoimmunity and dysglycemia) (39). Only for the latter individual, follow-up data were available - after 7 years since testing, he was clinically diagnosed with T1D (stage III) and insulin therapy had to be implemented.

In the classical view, decreased insulin sensitivity caused by excess adipose tissue heralds the progression of metabolic disorders towards pre-diabetes (IFG, IGT) and then diabetes (40, 41). In the present study, impaired glucose metabolism in

TABLE 1 Clinical and biochemical characteristics of obese children and adolescents according to the presence of beta cell autoantibodies.

	All patients (N=161)		Ab- (N=133)		Ab+ (N=28)		p-value (adj. for sex and age)
	N with available data	Mean ± SD	N with available data	Mean ± SD	N with available data	Mean ± SD	
Age [years]	161	13.1 ± 5.8	133	13.0 ± 2.9	28	13.8 ± 2.8	0.1840
BMI z-score	161	2.2 ± 1.6	133	2.2 ± 0.4	28	2.3 ± 0.4	0.7382
Waist (cm)	109	95.9 ± 72.0	89	95.3 ± 10.5	20	98.6 ± 11.5	0.2271 (0.6896)
Hips (cm)	107	102.8 ± 78.0	89	102.1 ± 11.6	18	106.5 ± 13.8	0.1590 (0.6929)
WHR	106	0.9 ± 0.7	88	0.9 ± 0.1	18	0.9 ± 0.1	0.7959 (0.2889)
WHtR	96	0.6 ± 0.5	78	0.59 ± 0.1	18	0.63 ± 0.03	0.0739 (0.0471)
Body composition, fat percentage [%]	131	38.1 ± 23.9	112	38.2 ± 6.7	19	37.4 ± 6.3	0.6193 (0.6449)
BMR [kJ]	131	7 563.5 ± 4 996.0	112	7513.6 ± 1464.6	19	7857.4 ± 1345.7	0.3406 (0.9558)
OGTT glycemia 0’	161	83.6 ± 59.0	133	83.2 ± 8.2	28	85.9 ± 10.3	0.1297
OGTT glycemia 60’	161	133.1 ± 78.6	133	131.0 ± 27.2	28	143.3 ± 31.5	0.0360
OGTT glycemia 120’	161	117.6 ± 63.0	133	117.4 ± 21.3	28	118.6 ± 24.9	0.7926
OGTT insulin 0’	108	18.0 ± 1.0	87	17.1 ± 8.7	21	21.3 ± 8.6	0.0496
OGTT insulin 60’	106	127.0 ± 3.0	85	105.7 ± 74.4	21	213.4 ± 226.8	0.0004
OGTT insulin 120’	107	104.3 ± 9.9	87	96.0 ± 70.2	20	140.5 ± 144.2	0.0447
C-peptide – fasting [ng/ml]	150	3.1 ± 1.1	122	3.0 ± 1.2	28	3.4 ± 1.2	0.1420
C-peptide – stimulated [ng/ml]	145	7.8 ± 2.3	120	7.6 ± 2.8	25	8.7 ± 3.7	0.0925
C-peptide – ratio after stimulation	143	2.7 ± 0.6	118	2.7 ± 0.9	25	2.7 ± 0.9	0.9709
HOMA-IR	158	3.5 ± 0.2	132	3.4 ± 1.9	26	4.3 ± 1.9	0.0203 (0.0340)
HbA1c [%]	159	5.4 ± 4.4	131	5.4 ± 0.3	28	5.5 ± 0.3	0.3111
cholesterol [mg/dl]	109	169.5 ± 99.0	87	171.4 ± 34.2	22	162.2 ± 35.7	0.2644
TG [mg/dl]	109	126.4 ± 32.7	87	130.3 ± 70.6	22	110.8 ± 62.9	0.2409
HDL [mg/dl]	110	45.0 ± 26.6	88	45.3 ± 9.9	22	43.8 ± 10.8	0.5540
LDL [mg/dl]	108	105.6 ± 8.9	86	106.1 ± 33.5	22	103.5 ± 28.5	0.7410

(BMI, body mass index; WHR, waist to hip ratio; WHtR, waist to height ratio; BMR, basal metabolic rate; OGTT, oral glucose tolerance test; HOMA, IR, Homeostatic Model Assessment – Insulin Resistance; BMR, basal metabolic rate HDL, cholesterol, high, density lipoprotein, cholesterol; LDL, cholesterol, low, density lipoprotein, cholesterol).

the form of IFG and/or IGT was found in 28 (17.4%) obese children and adolescents (none of the subjects were diagnosed with diabetes). In comparison, studies from European countries estimate the incidence of IFG/IGT in obese children and adolescents at 5.7-17.1%, and type 2 diabetes at 1.4% (42, 43). The observed differences may be related to the methodology of studies (age of subjects, definition of obesity) and the influence of such recognized risk factors as e.g., eating habits, physical activity, genetic conditions, or socioeconomic factors (40). A prospective study by Galderisi et al. involving a multi-ethnic group of 526 obese adolescents (10.6-14.2 years of age) showed the transient nature of IGT - after 2 years, 65% of respondents returned from IGT to normal glucose tolerance (NGT), 27% maintained IGT and 8% developed T2D (44). Unfortunately, long-term follow-up data were not collected in our study to ascertain the future clinical, metabolic and immune status of the patients (except for the one already-mentioned case).

In the study by Cambuli et al. in children with excess body weight, impaired glucose metabolism were identified in 11.37% of subjects (IFG-8.16%, IGT-3.2%, diabetes-0.6%), and individuals with positive antibodies characterized by a higher incidence of pre-diabetes or diabetes mellitus and higher glycaemia at 120 min. OGTT than those without antibodies (27% vs 11% and 133 mg/dL vs 105.4 mg/dL, respectively) (34). This was consistent with our observation. HbA1c, however, was comparable in both subgroups.

We also investigated the possible relationship between the presence of autoimmune markers and the degree of obesity. The

TABLE 2 Characteristics of obese children and adolescents with multiple beta cell autoantibodies (≥ 2Ab).

Patient ID		1	2	3	4
Autoantibodies and cut-offs	ICA (>0)	0	**20**	**40**	**40**
	anti-GAD (>=10)	0.73	**17**	**131.14**	**131.1**
	anti-IA2 (>=20)	5.24	1.33	10.26	0
	ZnT8 (>=15)	**22.06**	3.29	**16.17**	0.8
	IAA (>=10)	**23.14**	3.41	**41.96**	1.58
Clinical characteristics	Age	13.05	12.47	16.68	17.23
	Sex	F	F	M	M
	Family history	N/A	N/A	Negative	Negative
	Fasting c-peptide	3.16	3.07	5.57	2.3
	HbA1c	5.2	5.3	5.2	5.5
	HOMA-IR	4.3	4.5	3.9	6.34
	BMR	7510	8117	9912	8118
	Fasting blood glucose	79	96	82	82
	Blood glucose – 120` of OGTT	130	81	**169**	118

(ICA, antibodies against cytoplasmic antigens of pancreatic islets; GAD, antibodies against glutamic acid decarboxylase; ZnT8, antibodies against zinc transporter; IA2, antibodies against tyrosine phosphatase; IAA, anti-insulin antibodies; HOMA-IR- Homeostatic Model Assessment – Insulin Resistance; BMR-basal metabolic rate).

effect of type of obesity was not assessed, as all study participants were diagnosed with abdominal obesity. In our group, neither BMI z-score nor total body fat was related to the presence of islet autoantibodies. While BMI and body fat are good indicators of obesity, it was mainly the increase in visceral adipose tissue that was associated with insulin resistance and low-grade chronic inflammation, contributing not only to impaired insulin function and impaired insulin secretion, but also to the promotion of autoimmune responses against various organs (40, 45–49). We could not reliably measure or estimate that characteristics in this study setting. However, we noted that seropositive patients were characterized by higher WHtR, which corresponds to the severity of abdominal obesity, as well as higher insulin resistance, consistent with reports that abdominal obesity and insulin resistance have a negative effect on the development of an autoimmune response against pancreatic islet antigens (47, 48). According to a study in overweight/obese adults with T2D by Philla et al., the presence of antibodies was associated with lower resting C-peptide levels (29). We did not find a similar relationship in obese children and adolescents, either in terms of fasting or stimulated C-peptide secretion. Similarly, we found no significant differences in the lipid profiles reported in adults (islet autoantibodies associated with higher serum HDL-cholesterol concentration) (29).

The pubertal pediatric population has the physiologically lowest insulin sensitivity and the highest incidence of type 2 diabetes (50, 51). Girls are more likely to develop type 2 diabetes than boys, which may be due to overstimulation of the insulin receptor in pancreatic beta cells by endogenous estrogen (52). The pubertal period is also associated with a higher incidence of T1D than the early school age (53). In the present study, there was no correlation between age, gender, and pubertal stage in obese children and adolescents and the occurrence of anti-islet autoimmunity.

Genetic factors are crucial in the development of both T1D and T2D, and a burdensome family history and/or the presence of a genetically determined susceptibility to develop diabetes may be the determining factors tin bringing children and adolescents under close clinical observation and laboratory screening (36, 51). In the present study, data on the presence of T1D and other autoimmune diseases in 1st and 2nd degree relatives were obtained in 2/3 of patients. Obese children with anti-islet autoimmunity did not differ significantly in terms of family burden of autoimmune diseases, including T1D, from children without autoantibodies. It should be added that one patient diagnosed with T1D stage II according to ADA criteria had no family history of T1D or other autoimmune diseases.

There are several limitations of this work that should be disclosed. First, this was a single-center study, and patients were assessed at a single visit, without subsequent follow-up for autoimmunity markers or progression towards diabetes (except in one case). There was also no healthy control group available, which forced us to use literature-based data on the islet-autoantibody prevalence as reference. Finally, the analysis did not consider the effect of genetic HLA-dependent and HLA-independent susceptibility on the development of diabetes, and in some cases, it was not possible to verify information on the family burden of diabetes in the subjects.

On the other hand, we provided data from a fairly large sample of children and adolescents with simple obesity. The patients were of the same ethnic origin and were not pre-selected and matched in terms of genetic predisposition to develop diabetes. Anthropometric parameters were interpreted using national percentile grids, and both the effect of subjects' age and pubertal stage were included in

the data analysis. Five types of antibodies to pancreatic islet antigens were tested, and all tests were performed at a single reference center. Not only the frequency of carbohydrate metabolism disorders was analyzed, but also parameters determining pathogenetic phenomena related to glucose homeostasis - insulin sensitivity (HOMA-IR) and insulin secretion (OGTT, C-peptide - fasting and glucagon stimulated) were examined.

Conclusions

Children and adolescents with simple obesity in this study presented significantly higher rates of being positive for T1D-related islet autoantibodies, however, most of the difference was due to an increased prevalence of positive ICA. The presence of autoimmune markers was associated with insulin resistance and risk of prediabetes. Our results support that hypothesis that obesity might play a role in the development of T1D, but further research is needed. It might be worthwhile to extend diabetes screening in obese children and adolescents to include monitoring of the autoimmune response against pancreatic beta cells.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Bioethics Committee of the Medical University of Lodz (No. RNN/224/15/KE). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AC-F conceived the study, contributed to the study design, data collection, analysis, and drafting of the manuscript, IP contributed to the study design, data collection, analysis and wrote the first draft of the manuscript. AM contributed to the study design, data collection, analysis, and drafting of the manuscript, KW contributed to the analysis and drafting of the manuscript, AS conceived the study, contributed to the study design, analysis and drafting of the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors thank Mrs. Karolina Piątek (English interpreter; k.piatek@eukc.pl) who verified linguistically this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial and 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.

References

1. *Obesity and overweight*. Available at: https://www.who.int/news-room/fact-sheet/detail/obesity-and-overweight (Accessed 15 September 2022).

2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. (The GBD 2013 obesity collaboration) global, regional and national prevalence of overweight and obesity in children and adults 1980-2013: A systematic analysis lancet. *Lancet* (2014) 384(9945):766–81. doi: 10.1016/S0140-6736(14)60460-8

3. Faienza MF, Wang DQ, Frühbeck G, Garruti G, Portincasa P. The dangerous link between childhood and adulthood predictors of obesity and metabolic syndrome. *Intern Emerg Med* (2016) 11:175–82. doi: 10.1007/s11739-015-1382-6

4. Lindberg L, Danielsson P, Persson M, Marcus C, Hagman E. Association of childhood obesity with risk of early all-cause and cause-specific mortality: A Swedish prospective cohort study. *PloS Med* (2020) 17:e1003078. doi: 10.1371/journal.pmed.1003078

5. Nucci AM, Virtanen SM, Cuthbertson D, Ludvigsson J, Einberg U, Huot C, et al. TRIGR investigators growth and development of islet autoimmunity and type 1 diabetes in children genetically at risk. *Diabetologia* (2021) 64:826–35. doi: 10.1007/s00125-020-05358-3

6. Obita G, Alkhatib A. Disparities in the prevalence of childhood obesity-related comorbidities: A systematic review front public health. *Front Public Health* (2022) 10:923744. doi: 10.3389/fpubh.2022.923744

7. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L. The many faces of diabetes: a disease with increasing heterogeneity. *Lancet* (2014) 383:1084–94. doi: 10.1016/S0140-6736(13)62219-9

8. Wilmot E, Idris I. Early onset type 2 diabetes: risk factors, clinical impact and management. *Ther Adv Chronic Dis* (2014) 5:234–44. doi: 10.1177/2040622314548679

9. Dabelea D, Mayer-Davis EJ, Andrews JS, Dolan LM, Pihoker C, Hamman RF, et al. Clinical evolution of beta cell function in youth with diabetes: the SEARCH for diabetes in youth study. *Diabetologia* (2012) 55:3359–68. doi: 10.1007/s00125-012-2719-6

10. Hannon TS, Arslanian SA. The changing face of diabetes in youth: lessons learned from studies of type 2 diabetes. *Ann N Y Acad Sci* (2015) 1353:113–37. doi: 10.1111/nyas.12939

11. Umpaichitra V, Banerji MA, Castells S. Autoantibodies in children with type 2 diabetes mellitus. *J Pediatr Endocrinol Metab* (2002) 15 Suppl 1:525–30.

12. Reinehr T, Schober E, Wiegand S, Thon A, Holl RDPV-Wiss Study Group. Beta-cell autoantibodies in children with type 2 diabetes mellitus: subgroup or misclassification? *Arch Dis Child* (2006) 91:473–7. doi: 10.1136/adc.2005.088229

13. Wilkin TJ. The accelerator hypothesis: weight gain as the missing link between type I and type II diabetes. *Diabetologia* (2001) 44:914–22. doi: 10.1007/s001250100548

14. Knerr I, Wolf J, Reinehr T, Stachow R, Grabert M, Schober E, et al. The a'ccelerator hypothesis': relationship between weight, height, body mass index and age at diagnosis in a large cohort of 9,248 German and Austrian children with type 1 diabetes mellitus. *Diabetologia* (2005) 48:2501–2504. doi: 10.1007/s00125-005-0033-2

15. Tsigalou C, Vallianou N, Dalamaga M. Autoantibody production in obesity: Is there evidence for a link between obesity and autoimmunity? *Curr Obes Rep* (2020) 9:245–54. doi: 10.1007/s13679-020-00397-8

16. Lamb MM, Yin X, Zerbe GO, Klingensmith GJ, Dabelea D, Fingerlin TE, et al. Height growth velocity, islet autoimmunity and type 1 diabetes development: the diabetes autoimmunity study in the young diabetologia. *Diabetologia* (2009) 52:2064–71. doi: 10.1007/s00125-009-1428-2

17. Verbeeten KC, Elks CE, Daneman D, Ong KK. Association between childhood obesity and subsequent type 1 diabetes: A systematic review and meta-analysis. *Diabetes Med* (2011) 28:10–8. doi: 10.1111/j.1464-5491.2010.03160.x

18. Larsson HE, Vehik K, Haller MJ, Liu X, Akolkar B, Hagopian W, et al. Growth and risk for islet autoimmunity and progression to type 1 diabetes in early childhood: The environmental determinants of diabetes in the young study. *Diabetes* (2016) 65:1988–95. doi: 10.2337/db15-1180

19. Ferrara-Cook C, Geyer SM, Evans-Molina C, Libman IM, Becker DJ, Gitelman SE, et al. Type 1 diabetes TrialNet study group excess BMI accelerates islet autoimmunity in older children and adolescents. *Diabetes Care* (2020) 43:580–7. doi: 10.2337/dc19-1167

20. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, et al. ISPAD clinical practice consensus guidelines 2018: Definition, epidemiology, diagnosis and classification of diabetes in children and adolescents. *Pediatr Diabetes* (2018) Suppl 27:7–19. doi: 10.1111/pedi.12773

21. Kułaga Z, Różdżyńska-Świątkowska A, Grajda A, Gurzkowska B, Wojtyło M, Góźdź M, et al. Percentile charts for growth and nutritional status assessment in polish children and adolescents from birth to 18 year of age standardy Medyczne/Pediatria. *Standardy Medyczne/Pediatria* (2015) 12:119–35.

22. Smeets S, Diedert Luc De Paep DL, Stangé G, Verhaeghen K, van der Auwera B, Keymeulen B, et al. Insulitis in the pancreas of non-diabetic organ donors under age 25 years with multiple circulating autoantibodies against islet cell antigens. *Virchows Arch* (2021) 479:295–304. doi: 10.1007/s00428-021-03055-z

23. Velluzzi F, Secci G, Sepe V, Klersy C, Shattock M, Foxon R, et al. Prediction of type 1 diabetes in sardinian schoolchildren using islet cell autoantibodies: 10-year follow-up of the sardinian schoolchildren type 1 diabetes prediction study. *Acta Diabetol* (2016) 53:73–9. doi: 10.1007/s00592-015-0751-y

24. Franke B, Galloway TS, Wilkin TJ. Developments in the prediction of type 1 diabetes mellitus, with special reference to insulin autoantibodies. *Diabetes Metab Res Rev* (2005) 2:395–415. doi: 10.1002/dmrr.554

25. Ross C, Ward ZJ, Gomber A, Owais M, Yeh YM, Reddy CL, et al. The prevalence of islet autoantibodies in children and adolescents with type 1 diabetes mellitus: A global scoping review. *Front Endocrinol (Lausanne)* (2022) 13:815703. doi: 10.3389/fendo.2022.815703

26. Davis TME, Wright AD, Mehta ZM, Cull CA, Stratton IM, Bottazzo GF, et al. Islet autoantibodies in clinically diagnosed type 2 diabetes: prevalence and relationship with metabolic control (UKPDS 70). *Diabetologia* (2005) 48:695–702. doi: 10.1007/s00125-005-1690-x

27. Zinman B, Kahn SE, Haffner SM, O'Neill MC, Heise MA, Freed MI, et al. Phenotypic characteristics of GAD antibody-positive recently diagnosed patients with type 2 diabetes in north America and Europe. *Diabetes* (2004) 53:3193–200. doi: 10.2337/diabetes.53.12.3193

28. Trabucchi A, Faccinetti NI, Guerra LL, Puchulu FM, Frechtel GD, Poskus E, et al. Detection and characterization of ZnT8 autoantibodies could help to screen latent autoimmune diabetes in adult-onset patients with type 2 phenotype. *Autoimmunity* (2012) 45:137–42. doi: 10.3109/08916934.2011.604658

29. Pilla SJ, Balasubramanyam A, Knowler WC, Lazo M, Nathan DM, Pi-Sunyer X, et al. Islet autoantibody positivity in overweight and obese adults with type 2. *Diabetes Autoimmun* (2018) 51:408–16. doi: 10.1080/08916934.2018.1547711

30. Reinehr T, Schober E, Wiegand S, Thon A, Holl Ron behalf of the DPV-Wiss Study Group. β-cell autoantibodies in children with type 2 diabetes mellitus: subgroup or misclassification? *Arch Dis Child* (2006) 91:473–7. doi: 10.1136/adc.2005.088229

31. Alyafei F, Soliman A, Alkhalaf F, Sabt A, De Sanctis V, Elsayed N, et al. Prevalence of β-cell antibodies and associated autoimmune diseases in children and adolescents with type 1 diabetes (T1DM) versus type 2 diabetes (T2DM) in Qatar. *Acta BioMed* (2018) 89(S5):32–9. doi: 10.23750/abm.v89iS4.7359

32. Tfayli H, Bacha F, Gungor N, Arslanian S. Phenotypic type 2 diabetes in obese youth: secretion in islet cell antibody-negative versus -positive patients. *Diabetes* (2009) 58:738–44. doi: 10.2337/db08-1372

33. Rivera-Vega MY, Flint A, Winger DG, Libman I, Silva Arslanian S. Obesity and youth diabetes: Distinguishing characteristics between islet cell antibody positive vs. negative patients over time. *Pediatr Diabetes* (2015) 16:375–81. doi: 10.1111/pedi.12249

34. Cambuli VM, Incani M, Cossu E, Congiu T, Scano F, Pilia S, et al. Prevalence of type 1 diabetes autoantibodies (GADA, IA2, and IAA) in overweight and obese children. *Diabetes Care* (2010) 33:820–2. doi: 10.2337/dc09-1573

35. Andersson C, Kolmodin M, Ivarsson S-A, Carlsson A, Forsander G, Lindblad B, et al. Islet cell antibodies (ICA) identify autoimmunity in children with new onset diabetes mellitus negative for other islet cell antibodies. *Pediatr Diabetes* (2014) 15:336–44. doi: 10.1111/pedi.12093

36. Pöllänen PM, Ryhänen SJ, Toppari J, Ilonen J, Vähäsalo P, Veijola R, et al. Dynamics of islet autoantibodies during prospective follow-up from birth to age 15 years. *J Clin Endocrinol Metab* (2020) 105:e4638–51. doi: 10.1210/clinem/dgaa624

37. Bonifacio E, Achenbach P. Birth and coming of age of islet autoantibodies. *Clin Exp Immunol* (2019) 198:294–305. doi: 10.1111/cel.13360

38. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA* (2013) 309:2473–9. doi: 10.1001/jama.2013.6285

39. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the endocrine society, and the American diabetes association. *Diabetes Care* (2015) 38:1964–74. doi: 10.2337/dc15-1419

40. Valaiyapathi B, Gower B, Ashraf AP. Pathophysiology of type 2 diabetes in children and adolescents. *Curr Diabetes Rev* (2020) 16:220–9. doi: 10.2174/1573399814666180608074510

41. Weiss R, Magge SN, Santoro N, Giannini C, Boston R, Holder T, et al. Glucose effectiveness in obese children: Relation to degree of obesity and dysglycemia. *Diabetes Care* (2015) 38:689–95. doi: 10.2337/dc14-2183

42. Hagman E, Reinehr T, Kowalski J, Ekbom A, Marcus C, Holl RW. Impaired fasting glucose prevalence in two nationwide cohorts of obese children and adolescents. *Int J Obes (Lond)* (2014) 38:40–5. doi: 10.1038/ijo.2013.124

43. Koutny F, Weghuber D, Bollow E, Greber-Platzer S, Hartmann K, Körner A, et al. Prevalence of prediabetes and type 2 diabetes in children with obesity and increased transaminases in European German-speaking countries. *Anal APV initiative Pediatr Obes* (2020) 15:e12601. doi: 10.1111/ijpo.12601

44. Galderisi A, Giannini C, Weiss R, Kim G, Shabanova V, Santoro N, et al. Trajectories of changes in glucose tolerance in a multiethnic cohort of obese youths: an observational prospective analysis. *Lancet Child Adolesc Health* (2018) 2:726–35. doi: 10.1016/S2352-4642(18)30235-9

45. González N, Moreno-Villegas Z, González-Bris A, Egido J, Lorenzo O. Regulation of visceral and epicardial adipose tissue for preventing cardiovascular injuries associated to obesity and diabetes. *Cardiovasc Diabetol* (2017) 16:44. doi: 10.1186/s12933-017-0528-4

46. Hershkop K, Besor O, Santoro N, Pierpont B, Caprio S, Weiss R. Adipose insulin resistance in obese adolescents across the spectrum of glucose tolerance. *J Clin Endocrinol Metab* (2016) 101:2423–31. doi: 10.1210/jc.2016-1376

47. Versini M, Jeandel PY, Rosenthal E, Shoenfeld Y. Obesity in autoimmune diseases: not a passive bystander. *Autoimmun Rev* (2014) 13:981–1000. doi: 10.1016/j.autrev.2014.07.001

48. Tiberti C, Zampetti S, Capoccia D, Campagna G, Lucantoni F, Anastasi E, et al. Evidence of diabetes-specific autoimmunity in obese subjects with normal glucose tolerance. *Diabetes Metab Res Rev* (2018) 8 20:e3055. doi: 10.1002/dmrr.3055

49. Brooks-Worrell BM, Palmer JP. Setting the stage for islet autoimmunity in type 2 diabetes: Obesity-associated chronic systemic inflammation and endoplasmic reticulum (ER) stress. *Diabetes Care* (2019) 42:2338–46. doi: 10.2337/dc19-0475

50. Zeitler P, Arslanian S, Fu J, Pinhas-Hamiel O, Reinehr T, Tandon N, et al. ISPAD clinical practice consensus guidelines 2018 type 2 diabetes mellitus in youth. *Pediatr Diabetes* (2018) Suppl 27:28–46. doi: 10.1111/pedi.12719

51. Copeland KC, Zeitler P, Geffner M, Guandalini C, Higgins J, Hirst K, et al. Characteristics of adolescents and youth with recent-onset type 2 diabetes: the TODAY cohort at baseline. *J Clin Endocrinol Metab* (2011) 96:159–67. doi: 10.1210/jc.2010-1642

52. Nadal A, Alonso-Magdalena P, Soriano S, Quesada I, Ropero AB. The pancreatic beta-cell as a target of estrogens and xenoestrogens: Implications for blood glucose homeostasis and diabetes. *Mol Cell Endocrinol* (2009) 304:63–8. doi: 10.1016/j.mce.2009.02.016

53. Norris JM, Johnson RK, Stene LC. Type 1 diabetes–early life origins and changing epidemiology. *Lancet Diabetes Endocrinol* (2020) 8:226–38. doi: 10.1016/S2213-8587(19)30412-7

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 19 December 2022
DOI 10.3389/fendo.2022.1082684

OPEN ACCESS

EDITED BY
Artur Mazur,
University of Rzeszow, Poland

REVIEWED BY
Tetyana Chaychenko,
Kharkiv National Medical University, Ukraine
Vera M Zdravkovic,
University Children's Hospital, Belgrade, Serbia

*CORRESPONDENCE
Concepción M. Aguilera
caguiler@ugr.es

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 28 October 2022
ACCEPTED 23 November 2022
PUBLISHED 19 December 2022

CITATION
de Lamas C, Kalén A, Anguita-Ruiz A, Pérez-Ferreirós A, Picáns-Leis R, Flores K, Moreno LA, Bueno G, Gil Á, Gil-Campos M, Aguilera CM and Leis R (2022) Progression of metabolic syndrome and associated cardiometabolic risk factors from prepuberty to puberty in children: The PUBMEP study.
Front. Endocrinol. 13:1082684.
doi: 10.3389/fendo.2022.1082684

Progression of metabolic syndrome and associated cardiometabolic risk factors from prepuberty to puberty in children: The PUBMEP study

Carmela de Lamas[1,2†], Anton Kalén[1,2†], Augusto Anguita-Ruiz[3,4,5,6†], Alexandra Pérez-Ferreirós[1,2], Rosaura Picáns-Leis[1,2,7], Katherine Flores[5,8], Luis A. Moreno[5,9,10], Gloria Bueno[5,9,10,11], Ángel Gil[3,4,5], Mercedes Gil-Campos[5,8], Concepción M. Aguilera[3,4,5*] and Rosaura Leis[1,2,5,7]

[1]Unit of Investigation in Human Nutrition, Growth and Development of Galicia (GALINUT), University of Santiago de Compostela (USC), Santiago de Compostela, Spain, [2]Pediatric Nutrition Research Group, Institute of Sanitary Research of Santiago de Compostela (IDIS), University Clinical Hospital of Santiago - University of Santiago de Compostela (CHUS–USC), Santiago de Compostela, Spain, [3]Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix", Center of Biomedical Research, University of Granada, Armilla, Granada, Spain, [4]Instituto de Investigación Biosanitaria ibs, Granada, Spain, [5]The Center for Biomedical Research Network Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III (ISCIII), Madrid, Spain, [6]Institute for Global Health (ISGlobal), Barcelona, Spain, [7]Unit of Pediatric Gastroenterology, Hepatology and Nutrition, Pediatric Service, University Clinical Hospital of Santiago (CHUS), Santiago de Compostela, Spain, [8]Metabolism and Investigation Unit, Reina Sofia University Hospital, Maimónides Institute for Biomedical Research of Córdoba (IMIBIC), University of Córdoba, Córdoba, Spain, [9]GENUD (Growth, Exercise, NUtrition and Development) Research group, University of Zaragoza, Institute of Sanitary Research of Aragón (IIS Aragón), Zaragoza, Spain, [10]Agri-food Institute of Aragon (IA2), Zaragoza, Spain, [11]Unit of Pediatric Endocrinology, University Clinical Hospital Lozano Blesa, Zaragoza, Spain

Introduction: Metabolic syndrome (MetS) is a cluster of clinical and metabolic alterations related to the risk of cardiovascular diseases (CVD). Metabolic changes occurring during puberty, especially in children with overweight and obesity, can influence the risk of developing chronic diseases, especially CVD.

Methods: Longitudinal study based on the follow-up until puberty of a cohort of 191 prepubertal Spanish boys and girls without congenital, chronic, or inflammatory diseases: undernutrition: or intake of any drug that could alter blood glucose, blood pressure, or lipid metabolism. The following parameters were used to determine the presence of MetS: obesity, hypertension, hyperglycemia, hypertriglyceridemia, and low HDL-c.

Results: A total of 75·5% of participants stayed in the same BMI category from prepuberty to puberty, whereas 6·3% increased by at least one category. The prevalence of MetS was 9·1% (prepubertal stage) and 11·9% (pubertal stage). The risk of presenting alterations in puberty for systolic blood pressure (SBP), plasma triacylglycerols, HDL cholesterol (HDL-c), and HOMA-IR was

10.3389/fendo.2022.1082684

significantly higher in those participants who had the same alterations in prepuberty. MetS prevalence in puberty was predicted by sex and levels of HOMA-IR, BMI-z, and waist circumference in the prepubertal stage, in the whole sample: in puberty, the predictors were levels of HOMA-IR, BMI-z, and diastolic blood pressure in participants with obesity. Two fast-and-frugal decision trees were built to predict the risk of MetS in puberty based on prepuberty HOMA-IR (cutoff 2·5), SBP (cutoff 106 mm of Hg), and TAG (cutoff 53 mg/dl).

Discussion: Controlling obesity and cardiometabolic risk factors, especially HOMA-IR and blood pressure, in children during the prepubertal stage appears critical to preventing pubertal MetS effectively.

KEYWORDS

adolescent, cardiometabolic risk factors, child, metabolic syndrome, obesity, overweight, puberty

Introduction

Obesity has grown steadily over the past decades in all ages (1). In 2016, the prevalence of obesity was estimated at 50 million girls and 74 million boys worldwide (1). The prevalence of excess weight (overweight and obesity) in Europe, in children aged 2 to 10 years, ranges from less than 10% in the northern regions to more than 40% in the southern countries (2). Obesity in children is associated with comorbidities during this stage of life and increases the risk of diseases during adulthood (3, 4).

The metabolic syndrome (MetS) in adults is well-defined as a cluster of cardiovascular and diabetes risk factors including abdominal obesity, dyslipidemia (alterations in plasma levels of triacylglycerols due to excess or HDL-c due to deficiency), carbohydrate metabolism dysfunction, and hypertension (5). In children otherwise, there is lack of consensus on how to define MetS and which specific cut-off points should be used for each of the altered components. In a previous work from our group by Olza et al. (2011) (6), all internationally-accepted MetS definitions were compared according to their prevalence in a cohort of Spanish children with obesity, concluding that single cut-off points cannot be used to define abnormalities in children. Instead, values above the 90th, 95th, or 97th age- and sex-adjusted percentiles extracted from big representative epidemiological cohorts are recommended as cut-offs. Among suggested altered components participating in the definition of MetS, insulin resistance (IR), has been proposed to be critical, since it is one of the main signs of glucose intolerance (7). This disorder is related to organ, cellular, and biochemical alterations like increased endoplasmic reticulum oxidative stress, modified production of several hormones, augmented secretion of pro-inflammatory cytokines in adipose tissue, liver, and muscle, and elevated levels of biomarkers of endothelial vessel damage and blood coagulation homeostasis (8, 9). Nevertheless, this alteration is not considered by the majority of the currently accepted definitions, despite several claims for its inclusion have been recently raised (9).

The first adaptations of the MetS diagnostic criteria in children were carried out in the nineties of the last century (10, 11). Since then, more than 40 different definitions, including different sets of features and cut-offs, have been reported (12). The incidence of MetS in children under 18 years of age ranges from 0.2% to incidences greater than 12% (13–15). In 2015, a study determined the prevalence of MetS in the general population in Spain with the most widely used pediatric definitions (of which 5.7% employs NCEP-ATPIII criteria and 3.8% the International Diabetes Federation (IDF) criteria in adolescents between 12 and 17 years old) (16). In the group of children with obesity, the agreement between the two definitions was strong but lower in normal weight adolescents (16). In our previous from 2011, we demonstrated that an incidence that ranged between 7.6 and 30.8% during the prepubertal stage and between 9.7 and 41.2% in the pubertal stage depending on the used criteria (6). Likewise, various cross-sectional studies have reported a parallel increase in the incidence of obesity and MetS in all stages of childhood, regardless of the criteria used to define it (14, 15). Besides, MetS is directly related to obesity, and its incidence is greater as its severity increases (17, 18).

The body composition, with an increase in fat mass, and hormonal and metabolic changes occurring during puberty, such as increased IR, can influence the risk of developing chronic diseases, especially CVD (19, 20). These changes appear to affect individuals with or without overweight

10.3389/fendo.2022.1082684

differentially (21). Although a clear positive correlation has been established between age and MetS in adults, contradictory results have been reported in children (14). Some studies have shown significant differences in MetS prevalence between the prepubertal and pubertal stages (14, 15, 21). However, there is scarce information about how puberty affects the progression of MetS and associated cardiometabolic risk factors in longitudinal studies.

Hence, the PUBMEP study aimed to evaluate the prevalence of MetS and the progression of cardiometabolic risk factors related to it from prepuberty to puberty in a cohort of Spanish children. In this work, we also evaluate the utility of prepubertal risk factors, such as IR, not included in many of the current accepted MetS definitions for the prediction of pubertal MetS.

Materials and methods

Study design

The PUBMEP is a longitudinal study based on the follow-up of a cohort of children who had previously participated in the GENOBOX study (22–29). This project studied the relationship between genetic variants, markers of oxidative stress and inflammation, lifestyle, and cardiovascular risk in 1699 children and adolescents. Inclusion criteria: Prepubertal boys and girls at the time of the GENOBOX study who have already reached puberty at the time of the PUBMEP study start, were invited to participate. The following characteristics were considered as exclusion criteria: the presence of congenital, chronic, or inflammatory diseases or undernutrition: intake of any drug that could alter blood glucose, blood pressure or lipid metabolism: not being able to comply with the study procedures and being participating or having participated in the last three months in an investigation project.

A total of 374 subjects were contacted in the PUBMEP study, of which 49 were not located, 36 could not participate because they have changed their place of residence or meet any of the exclusion criteria and 98 declined the invitation. One hundred ninety-one answered affirmatively, and their parents or legal guardians accepted an appointment to receive all the information related to the PUBMEP study. Figure S1 depicts the flow chart of selected subjects.

Ethical considerations

The multicentric PUBMEP study was designed following the ethical principles of human research according to the seventh revision of the declaration of Helsinki (30) and current Spanish legislation on clinical research in humans. Moreover, the study was approved by the corresponding ethic committees on each of the participating centers (Code IDs GENOBOX: Córdoba01/2017, Santiago 2011/198, Zaragoza 12/2010 and PUBMEP: Córdoba 260/3408, Santiago 2016/522, Zaragoza 22/2016, Granada 01/2017). Parents and legal guardians and children over 12 years signed an informed consent before starting their participation.

Anthropometric parameters and pubertal stage assessment

The anthropometric evaluation was carried out with the participants in underwear or light sportswear and barefoot. Weight (kg) with an electronic medical scale (SECA® 701 – model class III digital display, Germany): height (cm) with a Harpenden wall-mounted stadiometer with a high-speed counter (Holtain® Ltd, United Kingdom): bicipital, tricipital, subscapular and suprailiac skin folds (SF) were determined with a skinfold Caliper (Holtain®, Wales, UK): and waist and hip perimeter (cm) with an inextensible tape measure (SECA®, Germany). All parameters were determined with standardized methods in which researchers from all participating centers were previously trained. The body mass index (BMI) [(weight (kg)/height (m)^2] was calculated and children were classified as having normal weight, overweight or obesity, according to BMI by using the Cole et al. (31) sex and age cut-offs for children. The waist circumference (WC) was related to the Spanish percentile tables of Ferrández et al. (32). The waist-to-hip ratio (WHR) was obtained. The assessment of the pubertal stage was carried out following the Tanner classification (33) and confirmed the prepubertal stage with a hormonal study. Systolic and diastolic blood pressure (SBP and DBP, respectively) were determined twice, after five minutes of rest, on the left arm of each participant while he was sitting. The measurement was performed with digital blood pressure monitor with a suitable cuff an (mofel M3, OMRON®, Japan). The mean values, expressed in millimeters of mercury adjusted for sex, age and height, were classified according to international references (34).

Biochemical analyses

The lipid profile and carbohydrate metabolism were studied in a blood sample collected after 12 h of fasting and without having performed more than 2 h of physical activity during the previous 24 h. The following parameters were determined to assess the lipid profile: total cholesterol and triacylglycerols (TAG) (Advia 2400 Chemistry system: Siemens healthcare diagnostics, Erlangen, Germany) and HDL-c and low-density lipoprotein cholesterol (LDL-c) (SAS-3 cholesterol profile kit – Helena Biosciences Europe: Tyne and Wear, UK). Plasma fasting glucose (Advia 2400 Chemistry system: Siemens healthcare diagnostics, Erlangen, Germany) and insulin (Advia centaur XP analyzer, Siemens healthcare diagnostics, Erlangen,

10.3389/fendo.2022.1082684

Germany) determinations were used to calculate the homeostasis model assessment of insulin resistance (HOMA-IR) index, which allowed us to assess carbohydrate metabolism.

Metabolic syndrome definition

To determine the presence of MetS we followed the recommendations by Olza et al. (2011) (6), which states the use of sex- and age-specific international criteria and cutoff points: obesity (BMI>95p) according to Cole et al. (2000) (31), and, at least, two altered components among the following: hypertension (BP z-score>95p) (34), carbohydrates metabolism dysfunction (hyperglycemia >100mg/dL) (35), hypertriglyceridemia (TAG z-score>95p) (36–39) and low HDL-c (HDL-c<5p) (40). When selecting the variable associated with the determination of fat mass, different options e.g., BMI, WC or WHR and cut-off points were evaluated. There was a very good correlation between all of them and BMI according to Cole et al. (2000) criteria (31) was finally selected because of its higher frequency of use. This definition therefore employs the latest available reference charts for Caucasians populations covering both prepubertal and pubertal stages.

Statistical analysis

For the final analysis, 10 participants belonging to the normal weight group who were outliers (had extreme values > p99) in any of the variables analyzed and 38 participants in whom some data were missing were excluded. Finally, 143 participants were included in the analysis.

A complete picture of the full analytical procedure is presented in Figure S2. The prepubertal-to-pubertal progression of body composition and cardiometabolic risk markers were analyzed using three approaches: 1) evaluation of the overall progression and differences between girls and boys, 2) prospective analysis of participants grouped based on their BMI status and MetS prevalence in prepuberty, and 3) retrospective analysis of participants grouped by MetS prevalence in puberty. For all three approaches, mixed-effects models were fitted for each of the analyzed phenotypes to investigate between- and within-group differences. The models included fixed effects for puberty stage, group, and their interactions, to allow within and between-group comparisons: and Tanner stage and sex to correct for any differences in pubertal status and sex between groups. A random effect for participants was included to account for repeated measures. All variables, except age, Tanner stage and glucose, were log-transformed. The models were used to test for progression in analyzed variables from prepubertal to pubertal stage within each group, pairwise differences at the prepubertal stage between groups, and pairwise differences in the prepubertal-pubertal evolution between groups. All tests were calculated using the Kenward-Roger method for degrees of freedom and corrected for false discovery rate using Benjamini–Yekutieli procedure. The models did not show a problem of non-normality or heteroscedasticity of residuals.

Further, to assess the within-participant association of cardiometabolic risk in prepuberty and puberty, logistic regressions were used to test if the prevalence of MetS in prepuberty affected the risk of MetS in puberty. Likewise, logistic regressions were used to test if altered levels of DBP, SBP, TAG, HDL-c, HOMA-IR and glucose in prepuberty affected the risk of having altered levels of these variables in puberty. The purpose of including HOMA-IR in these analyses was to demonstrate the relevance of prepubertal IR for the development of future MetS (even not participating in its definition). The cut-off point of HOMA-IR was considered 2.5 in the prepubertal stage: 3.38 in pubertal boys and 3.905 in pubertal girls. These IR cutoff values were extracted according to the 95th HOMA-IR percentile in a subset of 677 prepubertal and 778 pubertal Spanish children (41, 42). Although the ideal situation would involve to extract particular percentile cut-offs for each tanner stage, this was not possible in our study given the lack of enough individuals in each category for extracting reliable percentiles. Therefore, we claim to responsible use if extending these cutoffs to other populations, especially if individuals correspond to late tanner stages.

Two statistical approaches were used to test which cardiometabolic risk markers in prepuberty can predict MetS prevalence in puberty. First, a backward stepwise logistic regression tested which prepubertal levels of BMI z-score, WC, SBP, DBP, TAG, HDL-c, LDL-c, glucose, HOMA-IR and age, together with Tanner stage in puberty, would influence the prediction of MetS in puberty. The predictors did not suffer from multicollinearity (max VIF = 1.81). This procedure was repeated for the subsample of participants that presented obesity in prepuberty.

Secondly, two fast-and-frugal decision trees were constructed so that they potentially identify prepubertal children at risk of developing MetS in puberty (43). Fast-and-frugal decision trees provide easy to interpret decision trees, which are robust to overfitting (43). They have been successfully used to describe decision processes and provide prescriptive guides for effective real-world decision-making in medicine (44, 45), amongst other areas. To identify children at risk of developing MetS, a high sensitivity (i.e., correctly classifying individuals with MetS in puberty) over a high specificity (i.e., correctly classifying individuals without MetS in puberty) was considered more important for the purpose in this study. Therefore, the trees were evaluated using a weighted accuracy

10.3389/fendo.2022.1082684

metric. The trees with the best performance, when sensitivity was weighted 1.5 and 1.2 times higher than the specificity, were chosen. We used leave-one-out cross-validation (LOO-CV) to estimate how the trees were expected to perform in general when used to make predictions on data not used during the training of the model (46). This approach involves elaborating (training) the trees using all except one observation and then test the classification performance on the last observation. This procedure is repeated for each observation. The data were stratified on MetS prevalence in puberty to ensure cases of MetS in all folds. Due to the low number of MetS cases, bootstrapping was used within each fold to increase the sample size 25 times. The full CV procedure was repeated five times, giving a total of 20 estimates. We compared the predictive value of the two trees against those obtained by receiver operating characteristic (ROC) curves using prepubertal BMI-z and HOMA-IR, individually, to classify the true positive rate (TPR) against the false positive rate (FPR) of MetS in puberty. All statistical analyses were performed using the R statistical package (3.6.0 version).

Results

Participant characteristics, anthropometry, and plasma levels of cardiometabolic risk factors associated with the MetS in prepuberty and puberty are presented in Table 1. There were no significant differences between boys and girls in the

TABLE 1 Participant characteristics, anthropometry and cardiometabolic risk markers grouped by pre and pubertal stage and by sex.

Variable	Total (n = 143)			Girls (n = 71)			Boys (n = 72)		
	Pre	Pub	p-value	Pre	Pub	p-value	Pre	Pub	p-value
Age (yr)	7.8 ± 1.8	14.3 ± 1.9	<0.001	7.5 ± 1.7	13.9 ± 1.9	<0.001	8.2 ± 1.8	14.7 ± 1.7	<0.001
Tanner stage	0.0 ± 0.0	4.3 ± 1.1	<0.001	0.0 ± 0.0	4.5 ± 0.9	<0.001	0.0 ± 0.0	4.0 ± 1.1	<0.001
Anthropometry									
BMI (kg/m^2)	21.6 ± 4.6	26.3 ± 6.3	<0.001	21.2 ± 3.8	26.4 ± 6.3	<0.001	22.1 ± 5.2	26.2 ± 6.4	<0.001
BMI-z	1.92 ± 2.06	1.63 ± 1.74	0.183	1.59 ± 1.65	1.77 ± 1.86	0.999	2.24 ± 2.36	1.48 ± 1.62	0.027
BMI status									
NW	43 (30.1)	49 (34.3)	0.062	20 (28.2)	25 (35.2)	0.326	23 (31.9)	24 (33.3)	0.999
OW	31 (21.7)	39 (27.3)	0.169	17 (23.9)	19 (26.8)	0.999	14 (19.4)	20 (27.8)	0.619
OB	69 (48.3)	55 (38.5)	0.012	4 (47.9)	27 (38.0)	0.350	35 (48.6)	28 (38.9)	0.333
WC (cm)	72 ± 13	85 ± 16	<0.001	70 ± 10	84 ± 15	<0.001	73 ± 14	87 ± 17	<0.001
WHR	0.92 ± 0.07	0.86 ± 0.10	<0.001	0.91 ± 0.07	0.84 ± 0.11	<0.001	0.92 ± 0.07	0.89 ± 0.08	0.093
Sum SF (mm)	72 ± 33	84 ± 26	0.002	73 ± 28	88 ± 26	0.336	70 ± 38	80 ± 24	0.022
Cardiometabolic risk markers									
SBP (mm Hg)	104 ± 12	113 ± 14	<0.001	105 ± 10	111 ± 11	0.605	102 ± 13	116 ± 17	<0.001
DBP mm Hg)	62 ± 9	69 ± 9	<0.001	63 ± 9	69 ± 10	0.114	61 ± 9	68 ± 8	<0.001
Cholesterol (mg/dL)	169 ± 33	157 ± 29	0.002	170 ± 32	159 ± 26	0.605	167 ± 33	156 ± 31	0.011
LDL-c (mg/dL)	99 ± 28	91 ± 23	0.003	100 ± 27	91 ± 23	0.231	98 ± 29	91 ± 24	0.090
HDL-c (mg/dL)	56 ± 15	50 ± 12	<0.001	55 ± 15	50 ± 11	0.561	56 ± 14	49 ± 13	<0.001
TAG (mg/dL)	58 ± 28	75 ± 33	<0.001	63 ± 31	79 ± 37	<0.001	54 ± 24	70 ± 28	<0.001
Glucose (mg/dL)	84 ± 7	85 ± 9	0.007	83 ± 7	86 ± 8	0.002	84 ± 7	84 ± 9	0.999
Insulin (U/L)	8.0 ± 5.9	14.8 ± 9.5	<0.001	8.7 ± 6.0	16.2 ± 9.6	<0.001	7.3 ± 5.8	13.4 ± 9.2	<0.001
HOMA-IR	1.67 ± 1.32	3.15 ± 2.13	<0.001	1.79 ± 1.30	3.49 ± 2.25	<0.001	1.56 ± 1.35	2.82 ± 1.95	<0.001
Metabolic syndrome									
Prevalence (%)	13 (9.1)	17 (11.9)	0.239	9 (12.7)	10 (14.1)	0.999	4 (5.6)	7 (9.7)	0.702

Statistics presented; Mean ± SD; n (%). Pre-pub difference in total are controlled for sex and Tanner stage. Pre-pub differences and differences between sexes (in text) are controlled for Tanner stage. P-values are adjusted for false discovery rate using Benjamini–Yekutieli procedure.
Pre, Prebubertal stage; Pub, Pubertal stage; NW, Normal Weight; OW, Overweight; OB, Obesity; BMI-z, BMI z-score; Sum SF, Sum of skinfolds; WC, Waist circumference, WHR, Waist-hip ratio; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; LDL-c, Low-density lipoprotein cholesterol; HDL-c, High-density lipoprotein cholesterol; TAG, triacylglycerols; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

10.3389/fendo.2022.1082684

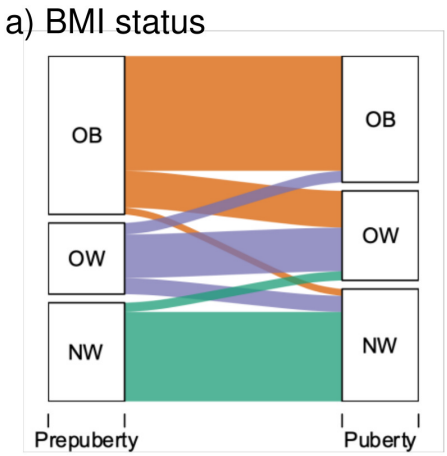
prepubertal stage for any variables. At puberty, girls were significantly younger ($p<0.001$), and had smaller WHR than boys.

Concerning the changes between prepuberty and puberty, a significant decrease in the prevalence of obesity was observed, with no significant differences in girls or boys. A significant increase in WC in the total sample and in girls and boys, and in the sum of skinfolds (SumSF) in the total sample and boys were also found. The WHR decreased significantly in the total sample and in girls.

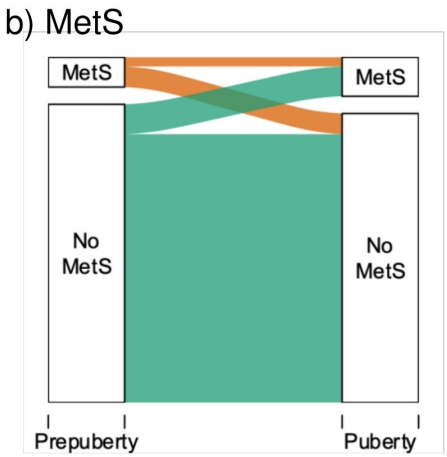
SBP and DBP increased significantly with puberty, this change being significant in both the total sample and in boys. However, TC, LDL-c and HDL-c decreased with puberty, changes being significant in the full sample and in boys. TAG increased significantly in all population, girls and in boys. About carbohydrate metabolism markers, glucose increased significantly in the total population and girls, while insulin and HOMA-IR increased significantly in both boys and girls. The prevalence of MetS was higher in pubertal boys and girls compared to prepuberty, but without significant differences between them.

Progression of categories of BMI, MetS and presence of altered cardiometabolic risk markers

The progression of BMI from prepuberty to puberty can be seen in Figure 1A. The majority of children (75.5%) stayed in the same category of BMI from prepuberty to puberty. For example, being obese in the prepubertal stage and continue as such in the pubertal stage. On the other hand, 6.3% increased at least one category (e.g., passed from overweight to obese), and 18.2% went down at least one (e.g., from obese to overweight). Among the



Prepuberty \ Puberty	OB	OW	NW
OB	50 (72.5)	16 (23.2)	3 (4.3)
OW	5 (16.1)	19 (61.3)	7 (22.6)
NW	0 (0)	4 (9.3)	39 (90.7)



Prepuberty \ Puberty	MetS	No MetS
MetS	4 (30.8)	9 (69.2)
No MetS	13 (10.0)	117 (90.0)

FIGURE 1
Progression from prepuberty to puberty of **(A)** BMI status and **(B)** prevalence of metabolic syndrome. Statistics presented: n (%). OB, Obesity: OW, Overweight: NW, Normal weight: MetS, Metabolic syndrome.

10.3389/fendo.2022.1082684

children who were overweight in the prepuberty 16.1% developed obesity. 72.5% of children with prepubertal obesity continued at puberty.

The progression of MetS prevalence can be seen in Figure 1B. Participants with MetS in prepuberty had 3.08 times higher risk of having MetS in puberty (95% CI: 0.98–7.27). The progression of the individual risk factors for MetS (13): hypertension, hypertriglyceridemia, low HDL-c, hyperglycemia (fasting plasma glucose > 100 mg/dL) (47), and HOMA-IR from prepuberty to puberty are presented in Figure 2. High prepubertal SBP increased the risk of high pubertal SBP by 3.17 times (95% CI: 1.60–6.55). Prepubertal hypertriglyceridemia increased the risk of pubertal hypertriglyceridemia by 3.61 times (95% CI: 1.28–8.65). Low prepubertal HDL-c increased the risk of low pubertal HDL-c by 8.56 times (95% CI: 2.35–22.77). High prepubertal HOMA-IR increased the risk of high pubertal HOMA-IR by 2.15 times (95% CI: 1.27–3.44). Prepubertal high DBP and hyperglycemia were not associated with pubertal high DBP and hyperglycemia respectively.

Prospective analysis by category of BMI and MetS prevalence in prepuberty

Participants were grouped by their prepubertal category of BMI and prevalence of MetS to analyze how obesity and MetS prevalence in prepuberty were related to the modification of anthropometry and cardiometabolic risk markers in puberty. There were no significant differences between the groups in how

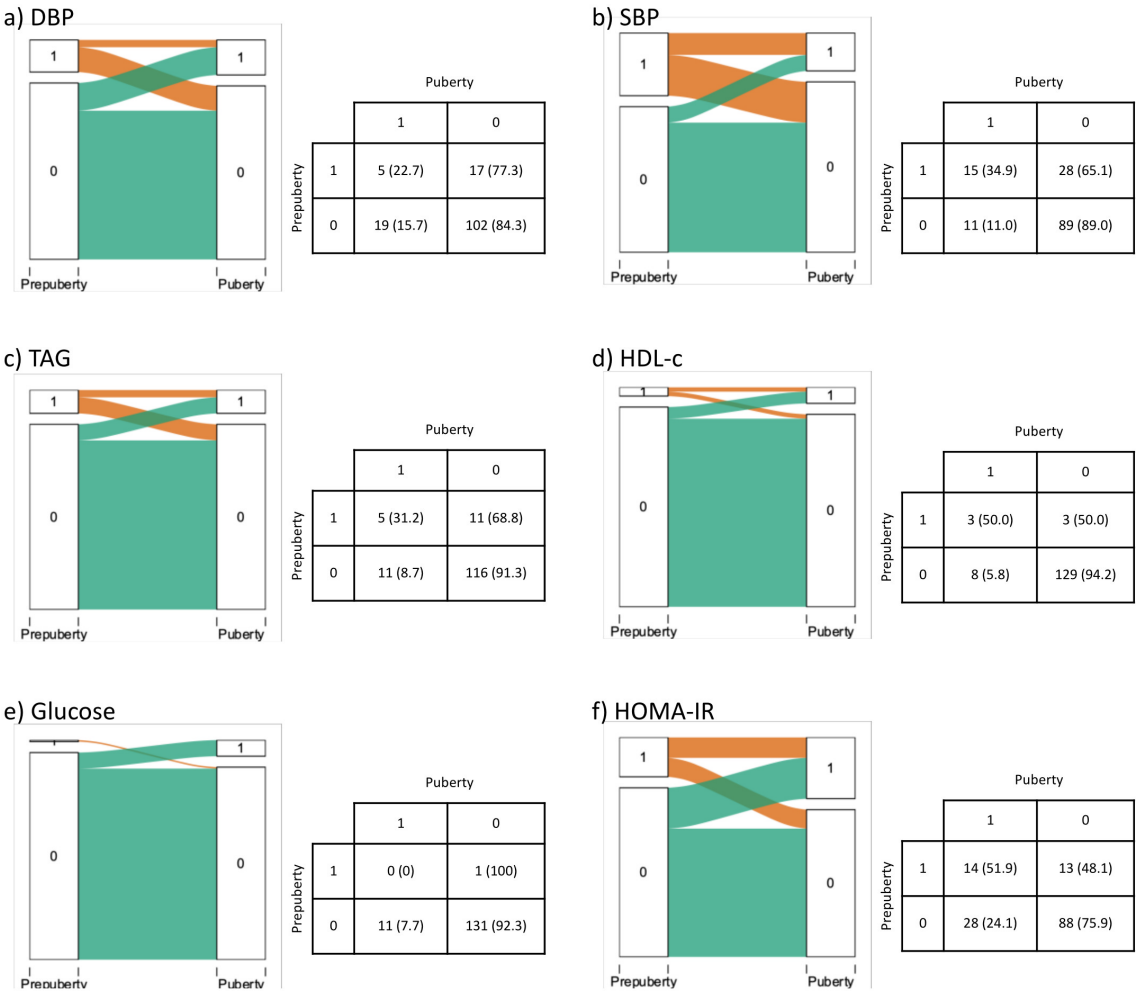


FIGURE 2
Progression of **(A)** DBP, **(B)** SBP, **(C)** TAG, **(D)** HDL-c, **(E)** Glucose and **(F)** HOMA-IR from prepuberty to puberty. 1 Indicates above the threshold, 0 below the threshold according to Olza et al. (6). Statistics presented: n (%). DBP, Diastolic blood pressure: HDL-c, High-Density Lipoprotein cholesterol: HOMA-IR, homeostasis model assessment of insulin resistance: SBP, Systolic blood pressure: TAG, Triacylglycerols.

10.3389/fendo.2022.1082684

they progressed from prepuberty to puberty in age nor in the rest of anthropometric parameters nor in cardiometabolic risk markers. BMI-z was fairly maintained from prepuberty to puberty: however, Obesity No-MetS prepubertal children exhibited a significant decrease, being the only one to show a reduction (-0.91). In contrast, a substantial increase in WC was observed in the four considered groups. Prepubertal children with obesity, both No-MetS and MetS, had significantly higher WC than normal weight and overweight children.Moreover, a significant increase was found for the Sum SF in the normal weight group (Table 2).

In relation to cardiometabolic risk markers, SBP and DBP increased significantly at puberty in Obesity No-MetS-children ($p<0.001$), while in the overweight group, only a significant increase in DBP was observed ($p=0.033$). Obesity MetS group in prepuberty had significantly higher SBP and DBP than the rest of groups. About the lipid profile, children with Obesity MetS in prepuberty had significantly lower levels of HDL-c and higher TAG than children of all other groups. Insulin and HOMA-IR levels increased significantly with puberty in all groups: they were significantly higher in overweight and obesity, both No-MetS and MetS, groups than normal weight group (Table 2).

Retrospective analysis by MetS prevalence in puberty

Participants were stratified in two groups, based on presence of MetS or no in puberty, to analyze the progression of anthropometric parameters and cardiometabolic risk markers from prepuberty to puberty (Table 3). The progression from prepuberty to puberty was significantly different between No MetS and MetS group for WHR, Sum SF and DBP.

A significant increase in BMI, WC and sum SF and a decrease in WHR with respect to prepubertal age were observed in the No-MetS group at puberty, while in the MetS

TABLE 2 Pre and pubertal levels and change of anthropometry and cardiometabolic risk markers, grouped by BMI status in prepuberty.

Variable	Normal weight in prepuberty (n = 43)			Overweight in prepuberty (n = 31)			Obesity No-MetS in prepuberty (n = 56)			Obesity MetS in prepuberty (n = 13)		
	Pre	Pub	Δ	Pre	Pub	Δ	Pre	Pub	Δ	Pre	Pub	Δ
Age (yr)	7.9 ± 1.8	14.8 ± 1.7	6.8 ± 2.6*	8.5 ± 1.7^{c}	14.6 ± 1.9	6.1 ± 2.4*	7.4 ± 1.9^{b}	13.7 ± 1.9	6.3 ± 2.3*	7.7 ± 1.4	14.6 ± 1.3	6.9 ± 2.2*
Tanner stage	0.0 ± 0.0	4.3 ± 1.1	4.3 ± 1.1	0.0 ± 0.0	4.4 ± 1.0	4.4 ± 1.0	0.0 ± 0.0	4.1 ± 1.1	4.1 ± 1.1	0.0 ± 0.0	4.7 ± 0.8	4.7 ± 0.8
Anthropometry												
BMI (kg/m^2)	16.2 ± 1.5	20.3 ± 2.9	4.1 ± 2.5	20.8 ± 1.9	25.3 ± 3.5	4.6 ± 3.5	25.1 ± 2.2	29.7 ± 5.3	4.6 ± 4.9	26.6 ± 4.1	34.2 ± 5.9	7.6 ± 3.7
BMI-z	-0.27 ± 0.55^{bcd}	-0.10 ± 0.70	0.17 ± 0.58	1.25 ± 0.44^{acd}	1.29 ± 0.98	0.04 ± 0.98	3.55 ± 1.50^{ab}	2.64 ± 1.40	-0.91 ± 1.80*	3.73 ± 1.96^{ab}	3.76 ± 1.43	0.03 ± 1.95
WC (cm)	58 ± 5^{bcd}	71 ± 9	13 ± 8*	70 ± 8^{acd}	82 ± 9	12 ± 11*	80 ± 9^{ab}	94 ± 14	15 ± 13*	83 ± 12^{ab}	104 ± 14	21 ± 8*
WHR	0.88 ± 0.06^{c}	0.83 ± 0.12	-0.07 ± 0.09*	0.91 ± 0.08	0.84 ± 0.07	-0.07 ± 0.07*	0.95 ± 0.06^{a}	0.90 ± 0.08	-0.05 ± 0.08*	0.94 ± 0.08	0.90 ± 0.07	-0.03 ± 0.04
Sum SF (mm)	35 ± 17^{bcd}	83 ± 27	51 ± 33*	77 ± 22^{ac}	86 ± 24	11 ± 27	94 ± 21^{ab}	82 ± 26	-10 ± 37	104 ± 24^{a}	93 ± 21	-7 ± 43
Cardiometabolic risk markers												
SBP (mm Hg)	101 ± 11^{d}	107 ± 11	7 ± 15	105 ± 9^{d}	112 ± 12	7 ± 11	102 ± 12^{d}	117 ± 13	15 ± 15*	116 ± 6^{abc}	121 ± 23	6 ± 22
DBP mm Hg)	62 ± 7^{d}	67 ± 7	5 ± 9	61 ± 8^{d}	68 ± 6	7 ± 8*	60 ± 7^{d}	68 ± 9	8 ± 11*	76 ± 12^{abc}	76 ± 17	0 ± 23
Cholesterol (mg/dL)	173 ± 31	162 ± 31	-10 ± 24	175 ± 37	154 ± 33	-21 ± 26*	162 ± 32	156 ± 24	-7 ± 26	170 ± 29	159 ± 30	-11 ± 25
LDL-c (mg/dL)	97 ± 27	90 ± 25	-8 ± 18	104 ± 33	89 ± 26	-14 ± 20*	96 ± 27	91 ± 21	-6 ± 21	108 ± 27	97 ± 25	-11 ± 20
HDL-c (mg/dL)	64 ± 15^{bcd}	58 ± 14	-6 ± 12	57 ± 16^{ad}	47 ± 11	-10 ± 14*	52 ± 10^{ad}	47 ± 10	-5 ± 10*	42 ± 10^{abc}	41 ± 9	-1 ± 8
TAG (mg/dL)	46 ± 14^{bd}	67 ± 29	21 ± 28*	67 ± 35^{ad}	73 ± 31	7 ± 30	55 ± 23^{d}	75 ± 30	19 ± 28*	92 ± 36^{abc}	101 ± 47	9 ± 46
Glucose (mg/dL)	84 ± 7	86 ± 9	2 ± 10	86 ± 6	85 ± 7	-1 ± 10	81 ± 7	84 ± 9	3 ± 11	86 ± 8	85 ± 9	-1 ± 12
Insulin (U/L)	4.6 ± 2.5^{bcd}	10.2 ± 4.8	5.6 ± 5.4*	8.7 ± 5.8^{a}	12.6 ± 5.8	4.0 ± 7.5*	9.3 ± 6.5^{a}	18.1 ± 11.4	8.8 ± 10.5*	12.2 ± 6.9^{a}	21.0 ± 10.7	8.8 ± 13.8*
HOMA-IR	0.95 ± 0.52^{bcd}	2.20 ± 1.15	1.25 ± 1.26*	1.87 ± 1.36^{a}	2.69 ± 1.34	0.82 ± 1.70*	1.89 ± 1.39^{a}	3.84 ± 2.59	1.95 ± 2.33*	2.68 ± 1.71^{a}	4.46 ± 2.40	1.78 ± 3.24*

Statistics presented: Mean ± SD: n (%). Pre-pub evolution, group differences in prepuberty and in evolution (in text) were controlled for sex and Tanner stage. P-values are adjusted for false discovery rate using Benjamini–Yekutieli procedure.

* Significant change from pre to pubertal stage within group: [a]significant difference from Normal Weight: [b]significant difference from Overweight: [c]significant difference from Obesity No-MetS: [d]significant difference from Obesity MetS.

MetS, Metabolic Syndrome; BMI-z, BMI Z-score; Sum SF, Sum of skinfolds; WC, Waist circumference, WHR, Waist-hip ratio; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; LDL-c, Low-density lipoprotein; HDL-c, High-density lipoprotein; TAG, triacylglycerol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

10.3389/fendo.2022.1082684

TABLE 3 Pre and pubertal levels and change of anthropometry and cardiometabolic risk markers grouped by the presence or absence of metabolic syndrome in puberty.

Variable	No MetS in Puberty (n = 126)			MetS in Puberty (n = 17)			Pre diff
	Pre	Pub	Δ	Pre	Pub	Δ	p-value
Age (yr)	7.8 ± 1.8	14.4 ± 1.9	6.6 ± 2.4*	7.9 ± 1.7	13.8 ± 1.7	5.9 ± 2.3*	0.999
Tanner stage	0.0 ± 0.0	4.2 ± 1.1	4.2 ± 1.1*	0.0 ± 0.0	4.5 ± 0.9	4.5 ± 0.9*	0.999
Anthropometry							
BMI (kg/m^2)	21.0 ± 4.4	25.1 ± 5.4	4.2 ± 3.6*	26.5 ± 3.2	35.0 ± 6.3	8.5 ± 4.5*	<0.001
BMI-z	1.68 ± 1.96	1.29 ± 1.47	-0.39 ± 1.41	3.71 ± 1.88	4.11 ± 1.64	0.40 ± 1.67	<0.001
WC (cm)	70 ± 12	82 ± 14	12 ± 11*	83 ± 10	108 ± 14	25 ± 10*	<0.001
WHR	0.91 ± 0.07	0.86 ± 0.10	-0.07 ± 0.08*	0.93 ± 0.07	0.93 ± 0.08	0.00 ± 0.07	0.999
Sum SF (mm)	66 ± 31	83 ± 24	20 ± 39*	108 ± 21	91 ± 35	-17 ± 51	<0.001
Cardiometabolic risk markers							
SBP (mm Hg)	103 ± 12	111 ± 11	9 ± 14*	109 ± 9	128 ± 23	20 ± 23*	0.314
DBP mm Hg)	62 ± 8	67 ± 7	5 ± 10*	64 ± 12	82 ± 11	18 ± 13*	0.999
Cholesterol (mg/dL)	169 ± 34	157 ± 30	-12 ± 26*	166 ± 28	159 ± 21	-7 ± 20	0.999
LDL-c (mg/dL)	99 ± 28	90 ± 24	-9 ± 20*	100 ± 30	94 ± 21	-6 ± 18	0.999
HDL-c (mg/dL)	57 ± 14	51 ± 12	-6 ± 12*	48 ± 12	40 ± 9	-8 ± 9	0.036
TAG (mg/dL)	55 ± 26	70 ± 28	15 ± 30*	80 ± 32	109 ± 47	29 ± 33*	0.004
Glucose (mg/dL)	83 ± 7	84 ± 8	1 ± 10	86 ± 7	89 ± 10	3 ± 15	0.908
Insulin (U/L)	7.1 ± 5.0	13.1 ± 7.6	6.0 ± 8.2*	14.5 ± 8.2	27.5 ± 12.4	13.0 ± 12.7*	<0.001
HOMA-IR	1.47 ± 1.09	2.76 ± 1.68	1.29 ± 1.83*	3.14 ± 1.91	6.05 ± 2.80	2.91 ± 3.04*	<0.001

Statistics presented: Mean ± SD: n (%). Pre-pub evolution, group differences in prepuberty and in evolution (in text) were controlled for sex and Tanner stage. P-values are adjusted for false discovery rate using Benjamini–Yekutieli procedure.

* Significant change from pre to pubertal stage within group.

MetS, Metabolic Syndrome; Pre diff, differences in prepuberty between the groups; BMI-z, BMI Z-score; Sum SF, Sum of skinfolds; WC, Waist circumference, WHR, Waist-hip ratio; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; LDL-c, Low-density lipoprotein; HDL-c, High-density lipoprotein; TAG, triacylglycerol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

group, there was a significant increase of BMI and WC. MetS group presented significantly higher BMI, BMI-z, WC, and sum SF than No-MetS group in prepuberty.

Concerning cardiometabolic risk factors, in the No-MetS group, a significant increase in SBP and DBP, insulin and HOMA-IR were observed. On the contrary, we found a significant decrease in the lipid profile parameters (cholesterol, LDL-c, and HDL-c), except for TAG, which increased significantly. In the MetS group, a significant increase in SBP and DBP, and TAG, insulin and HOMA-IR was noticed. In prepuberty, the MetS group at puberty presented significantly higher levels of TAG, insulin, and HOMA-IR and lower levels of HDL-c. The progression of BMI-z, WC, blood pressure, TAG, HDL-c, glucose, and HOMA-IR for participants with and without MetS in puberty are presented graphically in Figure S3.

Prediction of MetS prevalence in puberty

A backward stepwise logistic regression was used to ascertain which of BMI z-score, WC, SBP, DBP, TAG, HDL-c, LDL-c, glucose, HOMA-IR, and age in prepuberty was useful for the prediction of MetS in puberty. Prepubertal levels of HOMA-IR (OR=1.92: CI:1.29-2.98), BMI-z (OR=1.53: CI:1.06-2.30), WC (OR=1.09: CI:1.01-1.19), and sex (OR=5.69: CI:1.28-32.32) significantly predicted MetS prevalence in puberty (Table S1).

As all participants who presented MetS in puberty were obese at prepuberty, we used another stepwise logistic regression to test which cardiometabolic risk factors help to predict MetS within the group of 69 children with obesity in prepuberty. Within participants with obesity at prepuberty, HOMA-IR (OR=1.88: CI:1.19-3.28), BMI-z (OR=1.03: CI:1.00-1.05) and DBP (OR=0.92: CI:0.83-0.99) significantly predicted MetS prevalence in puberty (Table S2).

Fast-and-frugal decision trees were built to predict the risk of MetS in puberty based on body composition and cardiometabolic risk factors in prepuberty (Figure 3). Two trees are proposed with HOMA-IR cut-off 2,5, SBP cut-off 106 mm of Hg and TAG cut-off 53 mg/dl. One with the best accuracy is given when sensitivity is 1.5 times higher than the specificity (Figure 3A). The second one represents an alternate model when sensitivity is 1.2 times higher than the specificity (Figure 3B). The first decision tree correctly classified 82.4% of the total children and correctly classified 100% of the children with MetS in puberty. Moreover, out of the 25 false positives, 20 had at least one altered component of MetS or obesity, and five had neither altered features nor obesity. The second decision tree correctly classified 89.4% of the total children and correctly classified 94.1% of the children with MetS in puberty. Of

10.3389/fendo.2022.1082684

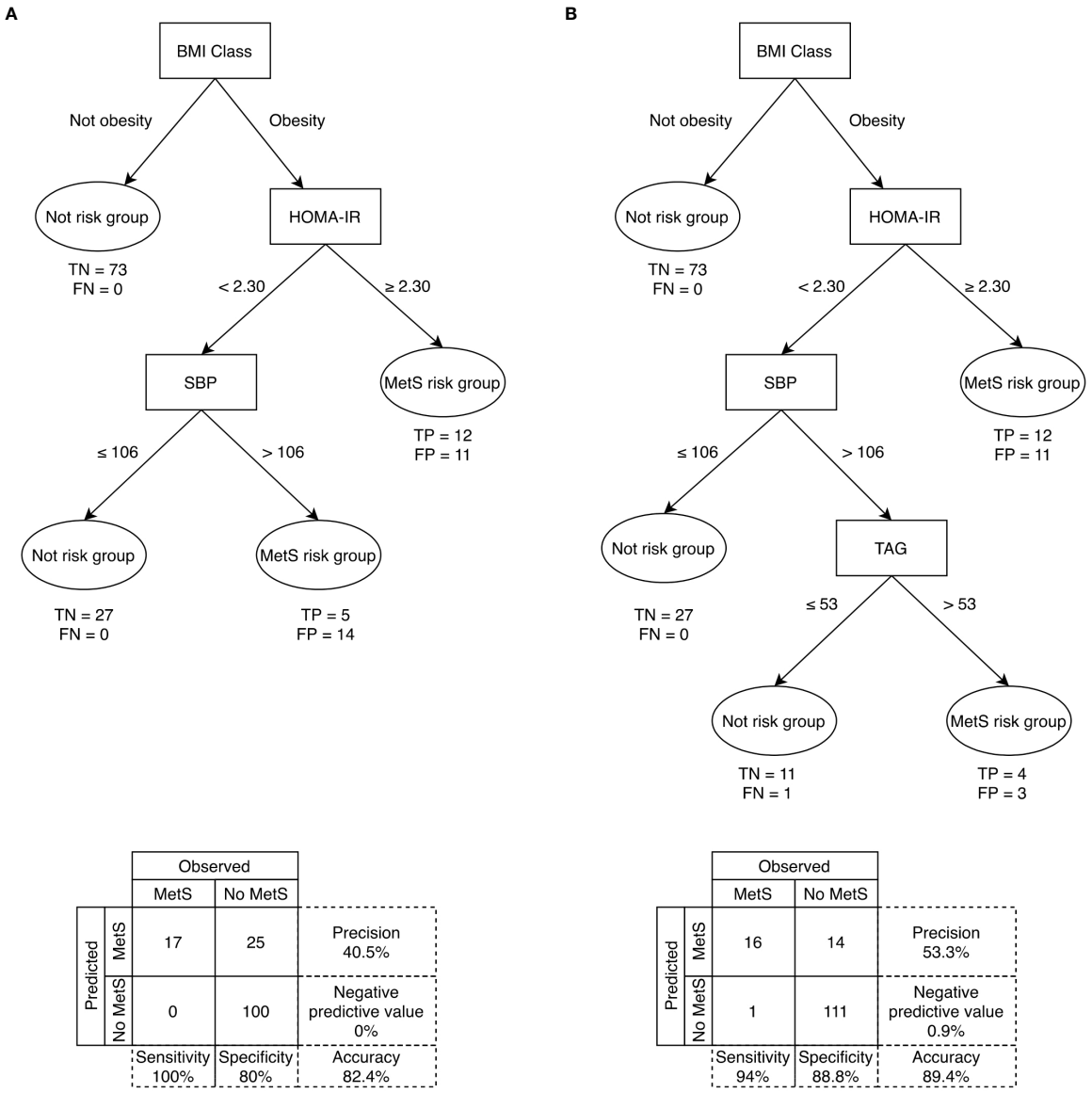


FIGURE 3
Fast-and-frugal decision trees for identifying prepubertal children in risk of developing MetS in puberty with sensitivity **(A)** 1.5 times and **(B)** 1.2 times more important than specificity. DBP= Diastolic blood pressure; FN, False negative; FP, False positive; HDL-c, High-Density Lipoprotein cholesterol; HOMA-IR= homeostasis model assessment of insulin resistance; MetS= Metabolic syndrome; SBP, Systolic blood pressure; TAG, Triacylglycerols; TN, True negative; TP, True positive. Sensitivity (i.e., correctly classifying individuals with MetS in puberty); specificity (i.e., correctly classifying individuals without MetS in puberty).

the 14 false positives, 11 had at least one altered component of MetS or obesity, and three had neither altered features nor obesity. LOO-CV showed that the first tree had an out-of-sample sensitivity of 100%, specificity of 85.6%, and accuracy of 87.3%. The second tree showed an out-of-sample sensitivity of 94.1%, specificity of 88.8%, and accuracy of 89.4%. The classification performance of the decision trees compared with the predictive values given by ROC curves when using only prepubertal BMI-z (area under curve =0.792) or HOMA-IR (area under curve =0.786) to predict pubertal MetS, is shown in Figure S4. Both prediction trees exhibited higher performance than using single predictors i.e., BMI-z or HOMA-IR.

10.3389/fendo.2022.1082684

Discussion

The hypothesis that the metabolic changes occurring during puberty is usually associated with anthropometric changes and IR leading to greater cardiovascular risk has been evidenced in the present study. Changes in anthropometric parameters related to increased cardiovascular risk were observed from prepuberty to puberty, especially WC, and in the MetS associated risk factors. A high percentage of prepubertal children maintained their BMI-z at puberty. In prepubertal children with obesity, a HOMA index >2.3 and a SBP >106 mm of Hg would identify 100% of prepubertal children with risk of MetS at pubertal age, with an accuracy of 82.4%. Indeed, the built-up decision- tree models for the prediction of MetS allowed us to determine, using only the BMI z-score, the HOMA-IR and the SBP in prepuberty, the individuals at high risk of presenting MetS at the pubertal stage. The possibility of early prediction of MetS in adolescents using simple cut-offs for cardiometabolic risk parameters at prepuberty, constitutes the major singularity and uniqueness of the present study, which may have important clinical implications in the early diagnosis and treatment of children at risk of developing premature CVD.

In our study, when evaluating the progression of BMI category, most of the participants remained in the same category (90.7% in normal weight, 61.3% in overweight and 72.5% in obesity). These results coincide with those of a narrative review published by Caprio et al. in 2020, with data collected from several studies, who estimated that approximately 80% of children with obesity maintained until adults (48).

The risk of MetS in puberty was significantly higher in participants with prepubertal MetS, what agrees with other publications (5). These results also support the data obtained from 285 participants in the Bogalusa Heart study followed for 15 years, where a positive correlation between prepubertal MetS and MetS in early adulthood was observed (49).

In the present work, within the participants who did not have prepubertal MetS, only 10% developed it in puberty. It is important to note that this 10% corresponded to individuals who, even without MetS, presented obesity in prepuberty. These results would reinforce those of other studies that conclude that individuals with obesity have a higher prevalence of MetS (14, 49). Furthermore, the incidence of MetS increases simultaneously as the BMI rises, as demonstrated by Kuschnir et al. (50).

In our study, the risk of presenting in puberty altered levels of SBP, TAG, HDL-c and HOMA-IR was significantly higher in those participants who had altered levels in prepuberty. However, this was not the case for DBP and glucose. These results are similar to those of the Québec family study, published in 2001, in which the progression of Sum SF of the trunk, mean arterial BP, HDL-c and TAG in 147 boys and girls from childhood to young adult were evaluated. Similarly, other authors shown the highest correlations for the Sum SF and HDL-c and the lowest for glucose (51).

When considering the progression of cardiometabolic risk factors by groups based on BMI and the presence of MetS in prepuberty, we observed that insulin, HOMA-IR and WC increased significantly within the four considered groups, as also reported by other authors (52). Our results indicate that prepubertal HOMA-IR, BMI-z, WC and female sex influence the prevalence of MetS. In prepubertal participants with obesity, HOMA-IR, BMI-z and SBP allowed predicting the prevalence of MetS in puberty. Many articles, both in children and adults, have pointed to BMI or WC as one of the most important predictive factors for the development of MetS (53–55). Likewise, some studies have also indicated alterations in IR and SBP as important risk factors, especially in populations at risk, such as relatives of individuals with diabetes mellitus (52, 56). Concerning the female sex, these data seem to contradict other publications that describe a greater risk of presenting MetS in boys (57) or do not describe differences between the sexes (58). The reason could be that the girls in our study had a significantly higher Tanner stage than boys, and this could be associated with a higher risk of MetS (14).

In our cohort, the HOMA-IR in the entire sample and in the prepubertal group with obesity, both in prospective and retrospective analysis, appeared the most important predictor of MetS in puberty. Most definitions of MetS in childhood do not include insulin or resistance as a feature (12), perhaps due to the difficulty of its determination and standardization of the analytical methodology among different clinical centers. However, as IR is an apparent prognostic factor of MetS in childhood, it could be interesting to use it already in prepuberty, as others have also suggested (51). Our own research group proposed it in a publication in 2015 (35). In this regard, in a recent survey, it has been shown that high BMI is not associated consistently with dyslipidemia and disturbed glucose metabolism in children and adolescents with classes III and IV obesity: therefore, measurements of cardiovascular risk factors instead of BMI seem preferable to counsel different treatment approaches (52).

Some studies (52) have shown that the measurement of adiposity but not its distribution, such as BMI, can leave out some people at risk, including children. However, when studying

the predictive factors of MetS, BMI z-score or WC z-score is normally used, obtaining similar results with high concordance. After carrying out the cross-validation of the tree predictive models, it is important to emphasize that the use of these models seems to be more convenient than using only HOMA-IR or DBP individually.

Limitations and strengths of the study

The main limitations of our study are, on the one hand, the low number of participants and the different grades of pubertal stages included. On the other hand, there is a certain risk of selection bias, since part of the participating individuals -those with the worst progression-, were followed up in the consultations of the participating hospitals, so it could have been easier to contact them or encourage them to participate in the study as they maintained contact with the research team. Another possible source of bias would be the difference in time elapsed between the two measurements (prepubertal and pubertal times) between the different participants.

The main strength of our study is the high significance obtained in the regression curves, although the number of participants was not high, as well as the high sensitivity, specificity, and accuracy of the MetS predictive models. Likewise, the fact that it was a prospective study allowed us to establish a correct temporal sequence and control how to measure the effects. Besides, standardized methodology was applied in all participated centers.

Conclusions

A large number of children with obesity in the Spanish population already presents MetS from a very early age (prepubertal period). The majority of the children presenting MetS in early-childhood continue as such when they enter into puberty, which drastically increase their probabilities of being adults with obesity, diabetes and CVDs. Prepubertal HOMA-IR and blood pressure are again evidenced as key risk factors for the prediction of pubertal MetS, as evidenced in our machine learning-based models. Therefore, the MetS criteria not considering IR should be revisited. This paper reinforces the importance of earl-life monitoring of metabolic health in children with obesity and the initiation of preventive programs from the prepubertal stages to avoid future comorbidities in adult populations.

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.

Ethics statement

The studies involving human participants were reviewed and approved by Ethic committees on each of the participating centers (Code IDs GENOBOX: Córdoba01/2017, Santiago 2011/198, Zaragoza 12/2010 and PUBMEP: Córdoba 260/3408, Santiago 2016/522, Zaragoza 22/2016, Granada 01/2017). Parents and legal guardians and children over 12 years signed an informed consent before starting their participation. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Design research: CMA, RL, MG-C, GB, LM and AG. Data analysis: AK, AA-R, AP-F, CL, AG, and RL. Writing of the original draft: CL, AK, AA-R, RP-L, KF and RL. Supervision: RL, CMA and AG. Paper Editing and revision: All authors have read and agreed to the final version of the manuscript.

Funding

This research was supported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I), Instituto de Salud Carlos III-Health Research Funding (FONDOS FEDER) (PI05/1968, PI11/01425, PI11/02042, PI11/02059, PI16/01301, PI16/01205, PI16/00871, PI20/00988, PI20/00924 and PI20/00563): CIBEROBN Network (CB15/00131, CB15/00043). The authors also acknowledge Instituto de Salud Carlos III for personal funding of AA-R: Contratos i-PFIS: doctorados IIS-empresa en ciencias y tecnologías de la salud de la convocatoria 2017 de la Acción Estratégica en Salud 2013–2016 (IFI17/00048). ÁG was funded by the Research Plan of the

Vice-Rectorate of Research and Transfer of the University of Granada, Spain. This paper will be included in CL doctorate, at the University of Santiago de Compostela, Spain.

Acknowledgments

The authors would like to thank the children and their parents for their participation in the study.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1082684/full#supplementary-material

References

1. Bentham J, Di Cesare M, Bilano V, Bixby H, Zhou B, Stevens GA, 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(10113):2627–42. doi: 10.1016/S0140-6736(17)32129-3

2. Ahrens W, Siani A, Adan R, De Henauw S, Eiben G, Gwozdz W, et al. Cohort profile: The transition from childhood to adolescence in European children-how I.Family extends the IDEFICS cohort. *Int J Epidemiol* (2017) 46(5):1394–5. doi: 10.1093/ije/dyw317

3. Lloyd LJ, Langley-Evans SC, McMullen S. Childhood obesity and risk of the adult metabolic syndrome: A systematic review. *Int J Obes* (2012) 36(1):1–11. doi: 10.1038/ijo.2011.186

4. Gepstein V, Weiss R. Obesity as the main risk factor for metabolic syndrome in children. *Front Endocrinol (Lausanne)* (2019) 10:568(August). doi: 10.3389/fendo.2019.00568

5. Morrison JA, Friedman LA, Gray-McGuire C. Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: The Princeton lipid research clinics follow-up study. *Pediatrics* (2007) 120(2):340–5. doi: 10.1542/peds.2006-1699

6. Olza J, Gil-Campos M, Leis R, Bueno G, Aguilera CM, Valle M, et al. Presence of the metabolic syndrome in obese children at prepubertal age. *Ann Nutr Metab* (2011) 58(4):343–50. doi: 10.1159/000331996

7. Mameli C, Zuccotti GV, Carnovale C, Galli E, Nannini P, Cervia D, et al. An update on the assessment and management of metabolic syndrome, a growing medical emergency in paediatric populations. *Pharmacol Res* (2017) 119:99. doi: 10.1016/j.phrs.2017.01.017

8. Orlando A, Nava E, Giussani M, Genovesi S. Adiponectin and cardiovascular risk. from pathophysiology to clinic: Focus on children and adolescents. *Int J Mol Sci* (2019) 20(13):3228. doi: 10.3390/ijms20133228

9. Ahrens W, Moreno LA, Mårild S, Molnár D, Siani A, De Henauw S, et al. IDEFICS consortium. metabolic syndrome in young children: definitions and results of the IDEFICS study. *Int J Obes (Lond)* (2014) 38 Suppl 2:S4–14. doi: 10.1038/ijo.2014.130

10. Bavdekar A, Yajnik CS, Fall CH, Bapat S, Pandit AN, Deshpande V, et al. Insulin resistance syndrome in 8-year-old Indian children: small at birth, big at 8 years, or both? *Diabetes* (1999) 48(12):2422–9. doi: 10.2337/diabetes.48.12.2422

11. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third national health and nutrition examination survey, 1988- 1994. *Arch Pediatr Adolesc Med* (2003) 157:821–7. doi: 10.1001/archpedi.157.8.821

12. Ford ES, Li C. Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? *J Pediatr* (2008) 152(2):160–4. doi: 10.1016/j.jpeds.2007.07.056

13. Pierlot R, Cuevas-Romero E, Rodríguez-Antolín J, Méndez-Hernández P, Martínez-Gómez M. Prevalencia de síndrome metabólico en niños y adolescentes de américa. *Tip* (2017) 20(1):40–9. doi: 10.1016/j.recqb.2016.11.004

14. Reisinger C, Nkeh-Chungag BN, Fredriksen PM, Goswami N. The prevalence of pediatric metabolic syndrome - a critical look on the discrepancies between definitions and its clinical importance. *Int J Obes* (2021) 45(1):12–24. doi: 10.1038/s41366-020-00713-113

15. Ekelund U, Anderssen S, Andersen LB, Riddoch CJ, Sardinha LB, Luan J, et al. Prevalence and correlates of the metabolic syndrome in a population-based sample of European youth. *Am J Clin Nutr* (2009) 89(1):90–6. doi: 10.3945/ajcn.2008.26649

16. Galera-Martínez R, García-García E, Vázquez-López MÁ, Ortiz-Pérez M, Ruiz-Sánchez AM, Martín-González M, et al. Prevalence of metabolic syndrome among adolescents in a city in the Mediterranean area: comparison of two definitions. *Nutr Hosp* (2015) 32(2):627–33. doi: 10.3305/nh.2015.32.2.92782015

17. Nam HK, Kim HR, Rhie YJ, Lee KH. Trends in the prevalence of extreme obesity among Korean children and adolescents from 2001 to 2014. *J Pediatr Endocrinol Metab* (2017) 30(5):517–23. doi: 10.1515/jpem-2016-0456

18. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* (2004) 350:2362–74. doi: 10.1056/NEJMoa031049

19. Rogol AD, Roemmich JN, Clark PA. Growth at puberty. *J Adolesc Heal* (2002) 31(6 SUPPL.):192–200. doi: 10.1016/S1054-139X(02)00485-8

20. Kelsey MM, Zeitler PS. Insulin resistance of puberty. *Curr Diabetes Rep* (2016) 16(7):64. doi: 10.1007/s11892-016-0751-5

21. Reinehr T, Wolters B, Knop C, Lass N, Holl RW. Strong effect of pubertal status on metabolic health in obese children: A longitudinal study. *J Clin Endocrinol Metab* (2015) 100(1):301–8. doi: 10.1210/jc.2014-2674

22. Llorente-Cantarero FJ, Aguilar-Gómez FJ, Anguita-Ruiz A, Rupérez AI, Vázquez-Cobela R, Flores-Rojas K, et al. Changes in physical activity patterns from childhood to adolescence: genobox longitudinal study. *Int J Environ Res Public Health* (2020) 17(19):1–13. doi: 10.3390/ijerph17197227

23. Leis R, Jurado-Castro JM, Llorente-Cantarero FJ, Anguita-Ruiz A, Iris-Rupérez A, Bedoya-Carpente JJ, et al. Cluster analysis of physical activity patterns, and relationship with sedentary behavior and healthy lifestyles in prepubertal children: Genobox cohort. *Nutrients* (2020) 12(5):1288. doi: 10.3390/nu12051288

24. Latorre-Millán M, Rupérez AI, González-Gil EM, Santaliestra-Pasías A, Vázquez-Cobela R, Gil-Campos M, et al. Dietary patterns and their association with body composition and cardiometabolic markers in children and adolescents: Genobox cohort. *Nutrients* (2020) 12(11):1–18. doi: 10.3390/nu12113424

25. Llorente-Cantarero FJ, Aguilar-Gómez FJ, Leis R, Bueno G, Rupérez AI, Anguita-Ruiz A, et al. Relationship between physical activity, oxidative stress, and total plasma antioxidant capacity in spanish children from the genobox study. *Antioxidants* (2021) 10(2):1–14. doi: 10.3390/antiox10020320

26. Rupérez AI, Mesa MD, Anguita-Ruiz A, González-Gil EM, Vázquez-Cobela R, Moreno LA, et al. Antioxidants and oxidative stress in children: Influence of puberty and metabolically unhealthy status. *Antioxidants* (2020) 9(7):1–19. doi: 10.3390/antiox9070618

27. Rupérez AI, Olza J, Gil-Campos M, Leis R, Mesa MD, Tojo R, et al. Association of genetic polymorphisms for glutathione peroxidase genes with obesity in Spanish children. *J Nutrigenet Nutrigenomics* (2014) 7(3):130–42. doi: 10.1159/000368833

28. Rupérez AI, López-Guarnido O, Gil F, Olza J, Gil-Campos M, Leis R, et al. Paraoxonase 1 activities and genetic variation in childhood obesity. *Br J Nutr* (2013) 110(9):1639–47. doi: 10.1017/S0007114513001967

29. Rupérez AI, Olza J, Gil-Campos M, Leis R, Mesa MD, Tojo R, et al. Are catalase -844A/G polymorphism and activity associated with childhood obesity? *Antioxidants Redox Signal* (2013) 19(16):1970–5. doi: 10.1089/ars.2013.5386

30. Arie S. Revision of Helsinki declaration aims to prevent exploitation of study participants. *BMJ* (2013) 347(October):6401. doi: 10.1136/bmj.f6401

31. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: International survey. *Br Med J* (2000) 320(7244):1240–3. doi: 10.1136/bmj.320.7244.1240

32. Ferrández Longás Á, Baguer L, Labarta J, Al. E. Longitudinal study of normal Spanish children from birth to adulthood. anthropometric, puberty, radiological and intellectual. *Pediatr Endocrinal Rev* (2005) 2:425–642.

33. Tanner J. Growth and endocrinology of the adolescent. In: Gardner L, editor. *Endocrine and genetic diseases of childhood*. Philadelphia: W. B. Saunders Company (1969). p. 19–69.

34. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics* (2017) 140(3):e20171904. doi: 10.1542/peds.2017-1904

35. Olza J, Aguilera C, Gil-Campos M, Leis R, Bueno G, Valle M. A continuous metabolic syndrome score is associated with specific biomarkers of inflammation and CVD risk in prepubertal children. *Ann Nutr Metab* (2015) 66:72–9. doi: 10.1159/000369981

36. *National cholesterol education program (NCEP). report of the expert panel on blood cholesterol levels in children and adolescents*. Bethesda, Md: National Heart, Lung, and Blood Institute (1991) p. 91–2732. National Institute of Health, NIH.

37. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA* (2001) 285(19):2486–97. doi: 10.1001/jama.285.19.2486

38. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. *Circulation* (2002) 106(25):3143–421. doi: 10.1161/circ.106.25.3143

39. The Lipid Research Clinics Program Epidemiology Committee. Plasma lipid distributions in selected north american populations: The lipid research clinics program prevalence study. the lipid research clinics program epidemiology committee. *Circulation* (1979) 60(2):427–39. doi: 10.1161/01.cir.60.2.427

40. Briefel RR, Carroll MD, Rifkind BM, Cleeman JI, Maurer KR, Johnson CL, et al. Distributions and trends of serum lipid levels among united states children and adolescents ages 4-19 years: Data from the third national health and nutrition examination survey. *Prev Med* (1998) 27:879–90:6. doi: 10.1006/pmed.1998.0376

41. Rupérez AI, Olza J, Gil-Campos M, Leis R, Bueno G, Aguilera CM, et al. Cardiovascular risk biomarkers and metabolically unhealthy status in prepubertal children: Comparison of definitions. *Nutr Metab Cardiovasc Dis* (2018) 28(5):524–30. doi: 10.1016/j.numecd.2018.02.006

42. Anguita-Ruiz A, Mendez-Gutierrez A, Ruperez AI, Leis R, Bueno G, Gil-Campos M, et al. The protein S100A4 as a novel marker of insulin resistance in prepubertal and pubertal children with obesity. *Metabolism* (2020) 105:154187. doi: 10.1016/j.metabol.2020.154187

43. Phillips ND, Neth H, Woike JK, Gaissmaier W. FFTrees: A toolbox to create, visualize, and evaluate fast-and-frugal decision trees. *Judgm Decis Mak* (2017) 12 (4):344–68.

44. Wegwarth O, Gaissmaier W, Gigerenzer G. Smart strategies for doctors and doctors-in-training: Heuristics in medicine. *Med Educ* (2009) 43(8):721–8. doi: 10.1111/j.1365-2923.2009.03359.x

45. Fischer JE, Steiner F, Zucol F, Berger C, Martignon L, Bossart W, et al. Use of simple heuristics to target macrolide prescription in children with community-acquired pneumonia. *Arch Pediatr Adolesc Med* (2002) 156(10):1005–8. doi: 10.1001/archpedi.156.10.1005

46. Molinaro A, Simon R, Pfeiffer R. Prediction error estimation: a comparison of resampling methods. *Bioinformatics* (2005) 21(15):3301–7. doi: 10.1093/bioinformatics/bti499

47. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* (2013) 36(SUPPL.1):67–74. doi: 10.2337/dc13-S067

48. Caprio S, Santoro N, Weiss R. Childhood obesity and the associated rise in cardiometabolic complications. *Nat Metab* (2020) 2(3):223–32. doi: 10.1038/s42255-020-0183-z

49. Wang LX, Filipp SL, Urbina EM, Gurka MJ, DeBoer MD. Longitudinal associations of metabolic syndrome severity between childhood and young adulthood: The bogalusa heart study. *Metab Syndr Relat Disord* (2018) 16 (5):208–14. doi: 10.1089/met.2017.0160

50. Kuschnir MCC, Bloch KV, Szklo M, Klein CH, Barufaldi LA, De Azevedo Abreu G, et al. ERICA: Prevalence of metabolic syndrome in Brazilian adolescents. *Rev Saude Publica* (2016) 50(suppl 1):1s–13s. doi: 10.1590/S01518-8787.2016050006701

51. Katzmarzyk PT, Pérusse L, Malina RM, Bergeron J, Després JP, Bouchard C. Stability of indicators of the metabolic syndrome from childhood and adolescence to young adulthood: The Quebec family study. *J Clin Epidemiol* (2001) 54(2):190–5. doi: 10.1016/S0895-4356(00)00315-2

52. Reinehr T. Metabolic syndrome in children and adolescents: a critical approach considering the interaction between pubertal stage and insulin resistance. *Curr Diabetes Rep* (2016) 16(1):1–9. doi: 10.1007/s11892-015-0695-1

53. Reinehr T, Tittel SR, Holle R, Wiegand S, Gellhaus I, Hebebrand J, et al. Comparison of cardiovascular risk factors between children and adolescents with classes III and IV obesity: findings from the APV cohort. *Int J Obes (Lond)* (2021) 45(5):1061–73. doi: 10.1038/s41366-021-00773-x

54. Burns TL, Letuchy EM, Paulos R, Witt J. Childhood predictors of the metabolic syndrome in middle-aged adults: the muscatine study. *J Pediatr* (2009) 155(3):S5.e17–26. doi: 10.1016/j.jpeds.2009.04.044

55. De Boer MD. Assessing and managing the metabolic syndrome in children and adolescents. *Nutrients* (2019) 11(8):1788. doi: 10.3390/nu11081788

56. Utzschneider KM, Van De Lagemaat A, Faulenbach MV, Goedecke JH, Carr DB, Boyko EJ, et al. Insulin resistance is the best predictor of the metabolic syndrome in subjects with a first-degree relative with type 2 diabetes. *Obesity* (2010) 18(9):1781–7. doi: 10.1038/oby.2010.77

57. Song P, Yu J, Chang X, Wang M, An L. Prevalence and correlates of metabolic syndrome in Chinese children: The China health and nutrition survey. *Nutrients* (2017) 9(1):79. doi: 10.3390/nu9010079

58. Kim S, So WY. Prevalence of metabolic syndrome among Korean adolescents according to the national cholesterol education program, adult treatment panel III and international diabetes federation. *Nutrients* (2016) 8 (10):588. doi: 10.3390/nu8100588

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 05 January 2023
DOI 10.3389/fendo.2022.1061682

OPEN ACCESS

EDITED BY
Aneta Monika Gawlik,
Medical University of Silesia, Poland

REVIEWED BY
Edgar Zenteno,
National Autonomous University of Mexico, Mexico
Tamer Coskun,
Eli Lilly, United States

*CORRESPONDENCE
Daniel Weghuber
d.weghuber@salk.at

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 04 October 2022
ACCEPTED 13 December 2022
PUBLISHED 05 January 2023

CITATION
Pixner T, Stummer N, Schneider AM, Lukas A, Gramlinger K, Julian V, Thivel D, Mörwald K, Mangge H, Dalus C, Aigner E, Furthner D, Weghuber D and Maruszczak K (2023) The relationship between glucose and the liver-alpha cell axis – A systematic review. *Front. Endocrinol.* 13:1061682. doi: 10.3389/fendo.2022.1061682

The relationship between glucose and the liver-alpha cell axis – A systematic review

Thomas Pixner[1,2], Nathalie Stummer[2,3], Anna Maria Schneider[2,3], Andreas Lukas[1,2], Karin Gramlinger[1], Valérie Julian[4], David Thivel[5], Katharina Mörwald[2,3], Harald Mangge[6], Christopher Dalus[2,3], Elmar Aigner[2,7], Dieter Furthner[1,2], Daniel Weghuber[2,3]* and Katharina Maruszczak[2,3]

[1]Department of Pediatric and Adolescent Medicine, Salzkammergutklinikum Voecklabruck, Voecklabruck, Austria, [2]Obesity Research Unit, Paracelsus Medical University, Salzburg, Austria, [3]Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria, [4]Department of Sport Medicine and Functional Explorations, Diet and Musculoskeletal Health Team, Human Nutrition Research Center (CRNH), INRA, University Hospital of Clermont-Ferrand, University of Clermont Auvergne, Clermont-Ferrand, France, [5]Laboratory of Metabolic Adaptations to Exercise under Physiological and Pathological Conditions (AME2P), University of Clermont Auvergne, Clermont-Ferrand, France, [6]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria, [7]First Department of Medicine, Paracelsus Medical University, Salzburg, Austria

Until recently, glucagon was considered a mere antagonist to insulin, protecting the body from hypoglycemia. This notion changed with the discovery of the liver-alpha cell axis (LACA) as a feedback loop. The LACA describes how glucagon secretion and pancreatic alpha cell proliferation are stimulated by circulating amino acids. Glucagon in turn leads to an upregulation of amino acid metabolism and ureagenesis in the liver. Several increasingly common diseases (e.g., non-alcoholic fatty liver disease, type 2 diabetes, obesity) disrupt this feedback loop. It is important for clinicians and researchers alike to understand the liver-alpha cell axis and the metabolic sequelae of these diseases. While most of previous studies have focused on fasting concentrations of glucagon and amino acids, there is limited knowledge of their dynamics after glucose administration. The authors of this systematic review applied PRISMA guidelines and conducted PubMed searches to provide results of 8078 articles (screened and if relevant, studied in full). This systematic review aims to provide better insight into the LACA and its mediators (amino acids and glucagon), focusing on the relationship between glucose and the LACA in adult and pediatric subjects.

KEYWORDS

liver-alpha cell axis, glucagon, glucose, liver, OGTT, pediatric

1 Introduction

The prevalence of obesity, non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) are steadily rising in adults as well as in pediatric populations worldwide (1). NAFLD is considered as the most common chronic liver disease across all age groups (2–6).

Research on the liver-alpha cell axis (LACA) has shed new light on the pathophysiology of NAFLD and proposed glucagon and amino acids as pivotal drivers (7–9). Glucagon is predominantly known for its counter-regulatory mechanism of insulin and the ability to coordinate blood glucose homeostasis. While hypoglycemia stimulates the secretion of glucagon from pancreatic alpha cells to promote gluconeogenesis and glycogenolysis in the liver, glucagon's role is more diverse (10). It has been demonstrated that glucagon administration leads to a reduction in plasma amino acids (AAs) and to increased AA-uptake *via* the liver and consequently to conversion into gluconeogenic precursors both in animals and humans (11, 12). In contrast, evidence that the liver and the pancreatic alpha cells are linked in a feedback-cycle is recent (8, 13). In 2015, Solloway et al. demonstrated that inhibiting the glucagon-receptor increases AA concentrations, reduces AA turnover, and promotes pancreatic alpha cell proliferation (14–16). Further research by Kim et al. in 2017 involving antibody-mediated blockage of the glucagon-receptor led to the discovery of slc38a5. This gene is upregulated in the liver and pancreas during the blockade and encodes an AA transporter favoring neutral amino acids (e.g., glutamine). Slc38a5-deficient mice showed diminished alpha cell proliferation in response to glucagon receptor blockade (17). Furthermore, mTORC1 regulates the slc38a5 expression, and inhibits mTORC1 by rapamycin blocked AA-induced alpha cell proliferation (7, 18, 19). Holst, Wewer Albrechtsen, and Pedersen postulated a feedback regulation mechanism that links the liver and the pancreatic alpha cell known as LACA (8, 9).

The LACA describes, under physiological conditions, how a rise of plasmatic AAs induces glucagon secretion from the pancreatic alpha cells. Postprandial glucagon, in turn, controls hepatic AA metabolism and induces ureagenesis, resulting in decreasing levels of plasmatic AAs. When levels of plasmatic AAs return to their normal range, so does glucagon (see Figure 1A) (9, 20, 21). It is of special interest that the LACA seems to be disturbed in obesity and associated conditions. Besides genetic defects and pharmacological interventions to inhibit glucagon signaling, hepatic disorders (e.g., NAFLD and NASH (9, 22, 23)) and certain metabolic conditions (e.g., obesity (24, 25), T2DM (22)) have been associated with impaired glucagon signaling and hence disrupted LACA (26, 27). Under such conditions, the weakened effect of glucagon on AA turnover results in reduced ureagenesis and hyperaminoacidemia. Elevated levels of plasmatic AAs, in turn, lead to hyperglucagonemia and proliferation of pancreatic alpha cells. This process creates a vicious cycle of metabolic imbalance (see Figure 1B) (10, 11, 14, 17, 22, 23, 28).

While amino acids are drivers of the LACA, study results on specific AAs remain controversial. Most AAs stimulate glucagon and insulin secretion; however, some are more potent (29–34). Glucagon is the second mediator of the LACA, and its secretion is a complex process in itself and regulated by multiple interactions. These include changes in blood glucose, vegetative (sympathetic and parasympathetic) and other neural signals, amino acids, fatty acids, endocrine and paracrine functions (18, 35–39). A disturbed LACA seems to mark glucagon resistance in the hormone AA-pathway (15, 22), resulting in elevated plasmatic levels of amino acids, while the glucose pathway appears to remain intact (10, 13, 14, 17, 20, 21, 40–42). Hyperglucagonemia results in increased hepatic glucose production (43) *via* maintained glycogenolysis (21), and increased activity of gluconeogenesis' key enzymes (9). In obesity, NAFLD, and T2DM, fasting hyperglucagonemia is common and stimulates hepatic glucose production, predisposing to elevated blood glucose levels (44–47). The diabetogenic role of glucagon is widely accepted and confirmed in patients with type 2 diabetes (48–50). If not counterbalanced, hyperglycemia may result in insulin resistance, worsening of NAFLD and the development of T2DM and metabolic syndrome. Hence, understanding how metabolic risk factors such as (increased) glucose intake may affect the LACA is essential. Additionally, AA and glucagon levels during oral glucose tolerance test (OGTT) may hold predictive/diagnostic information on metabolically associated diseases.

Besides providing an overview of the LACA, the aim of this systematic review is to summarize the relationship between glucose and the mediators of the LACA (i.e., glucagon and AAs) under physiological conditions and in conditions of metabolical impairment. This review is structured to report data of the mediators` interactions with glucose and each other as well as their dynamics during experimental glucose administration including results from the pediatric population.

2 Methods

2.1 Design

This systematic review was conducted and written following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (49). Inclusion criteria were quantitative studies, peer reviewed-papers, basic research, and clinical research. We excluded non-English publications and studies with a focus other than human medicine (e.g., veterinary medicine).

2.2 Search strategy

Three authors independently searched the PubMed database and cooperated in screening the results. The purpose of the search was to identify data on LACA and its relationship with glucose. As glucagon and amino acids are the LACA's main mediators, these were of central interest concering glucose-interaction. The first search was conducted on December 13th and 14th, 2021. The first search included the terms "liver AND alpha cell AND axis," "liver-alpha cell," "Liver-islet axis," "Glucagon AND liver," and "Glucose and amino acids" without any parameters (e.g., age) in all articles up to December 1st, 2021. A second search on December 22nd, 2021, included "OGTT Amino acids" (articles from 1.1.2016-12.1.2021) and "OGTT Glucagon" (articles from 1.1.2016-12.1.2021) without additional filters. The rationale for the search period was the keystone study on glucagon-receptor blockade and alpha cell hyperplasia by Solloway et al. in July 2015, the report of hyperglucagonemia in NAFLD in 2016 and postulation of LACA in 2018 (14, 15, 20). As part of the second search ("OGTT Amino acids" and "OGTT Glucagon") studies on pediatric cohorts (0-18 years) published between 1.1.2000-12.31.2021 were searched. The search period for pediatric results was extended until the beginning of the year 2000 due to the lack of pediatric studies and difficulties with glucagon measurements in older studies (51, 52). For the second search inclusion criteria were the same as in the first search attempt. However, exclusion criteria were extended to pregnancy, medication/pharmacological studies, cancer, pancreatectomy, and severe inborn metabolic diseases (e.g., PKU, SMA). Figure 1 shows the two searches and the number of papers remaining after screening and excluding doubles.

2.3 Screening and data extraction

The two authors in charge of the search screened the first 6866 articles by title and abstract. The title or abstract had to provide information associated with at least one of the following topics: liver-alpha cell axis (LACA), amino acids (AAs), glucagon, or glucose metabolism. One hundred ninety-one articles qualified for the full-text article analysis. After the second analysis, 35 of 1073 articles qualified for full-text analysis and were included in this study. After screening 8078 publications, 88 articles (first search: 67 plus second search: 21) were included (see Figure 2). Secondary literature from these articles was studied as well.

2.4 Data synthesis

Information on amino acids, glucagon, glucose, and other relevant data, were used to draft the manuscript. As the studies and reviews were significantly heterogeneous concerning populations and methods, we opted for a narrative literature synthesis rather than a meta-analytical approach for interpreting findings.

3 Results

3.1 Characteristics of literature search

In total 88 articles (first search: 67 plus second search: 21) were included in this review. Secondary literature from these articles was also analyzed and referenced. The literature research *via* PubMed identified 19 articles with standardized glucose administration (i.e., OGTT or intravenous (i.v.) glucose). One article investigated both glucagon and AAs (20). In contrast, 12 other articles examined the effect of glucose administration on glucagon (7, 53–62), and six other articles studied the effect of an OGTT on AAs (63–68). The articles by Knop (7), Junker (54) and Wang et al. (55) were the only trials to include i.v. glucose infusions. Junker (54) mentioned 50g of glucose for OGTT, while the other authors administered 75g glucose OGTT (or 1.75g/kg body weight respectively) (7, 20, 53, 54, 56–68). Six articles included pediatric subjects (22, 62, 68–72). Of these, four reported amino acid levels in children with obesity (22, 68, 69, 72).

3.2 Glucose and glucagon under physiological conditions

Under normal conditions, glucagon and insulin counterbalance each other in order to maintain euglycemia (73). A decrease in blood glucose levels simultaneously determines a rise in glucagon levels and a decline in insulin levels (74). Glucagon regulation is complex and controlled by multiple factors (glycemic, paracrine, endocrine, and neural) (39). Hence the results section will initially focus on the isolated glucagon reactions. In hypoglycemia glucagon is secreted from the pancreatic alpha cell (15, 73). Pancreatic alpha cells are well-vascularized islet of Langerhans, which sense glucose concentrations (75). Glucose uptake is mediated by the glucose transporter 1 (GLUT1) in the alpha cell (76, 77). After glucose is taken up by the alpha cell, it is converted to ATP and water in the cell's mitochondria. Under low blood glucose conditions, ATP concentration drops (18). Alpha cells also contain ATP-sensitive potassium channels (K_{ATP}). Variations in intracellular ATP are linked to changes in membrane potential *via* K_{ATP} (78). In low glucose conditions, the K_{ATP} channels in alpha cells are closed or show only mild activation (78). Thus, the opening of voltage-gated Na- and Ca2-channels generate a membrane potential of about ~60 mV. The opening of the Na- and Ca2-channels cause influx of Na and Ca2 and in turn cause the release of glucagon *via* exocytosis and glucagon from glucagon-containing granules (18, 75, 78). The purpose of glucagon secretion during low blood

10.3389/fendo.2022.1061682

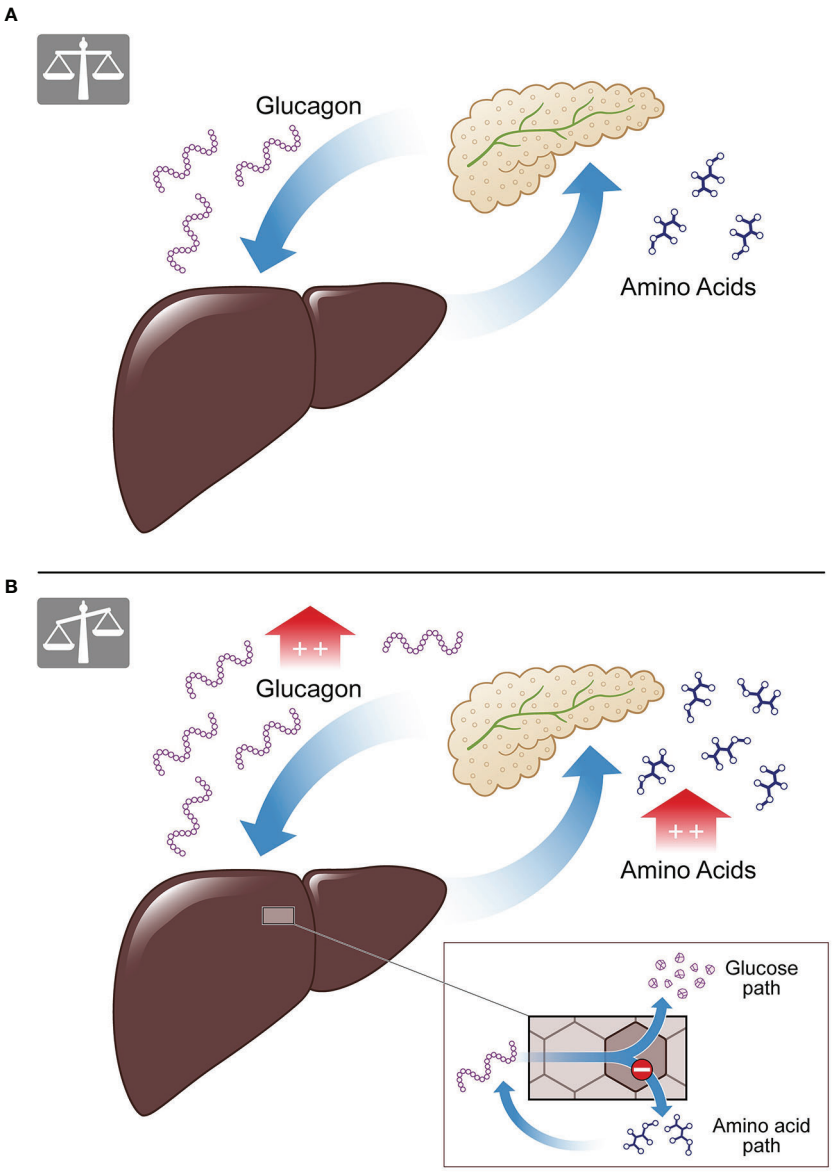


FIGURE 1
The liver-alpha cell axis. **(A)** Under physiological conditions amino acids lead to glucagon secretion from the pancreatic alpha cell, causing amino acid degradation and ureagenesis in the liver. The result is a balanced feedback cycle, termed the liver-alpha cell axis. **(B)** In conditions of metabolical impairment (e.g., NAFLD, T2D) the cycle is malfunctioning. Amino acids cannot be properly degraded as the amino acid pathway is disrupted, while the glucose pathway of glucagon is functioning (see insert). This results in hyperaminoacidemia and hyperglucagonemia.

glucose is to increase hepatic glucose production *via* stimulation of glycogenolysis and gluconeogenesis and suppression of glycogenesis and glycolysis (74, 79). The ability to promote the activity of key enzymes responsible for gluconeogenesis (phosphorylase kinase, fructose 1,6-bisphosphatase, glucose 6-phosphatase, phosphoenolpyruvate carboxykinase) has been widely demonstrated (9). Glucagon levels vary along a circadian rhythm. During the physiological morning fasting period, plasma glucagon levels are lower and significant changes in blood glucose levels are required stimuli (80). Hypoglycemia also induces the activity of pancreatic sympathetic innervation and triggers the release of adrenaline from the adrenal medulla. In rodent studies, adrenaline directly stimulates glucagon secretion by binding to alpha1- or beta-adrenergic receptors on the alpha cell (81–84).

The ability of glucagon to elevate blood glucose levels seems to depend on the duration of fasting. The isolated effect of glucagon on gluconeogenesis is short-lasting as it cannot provide

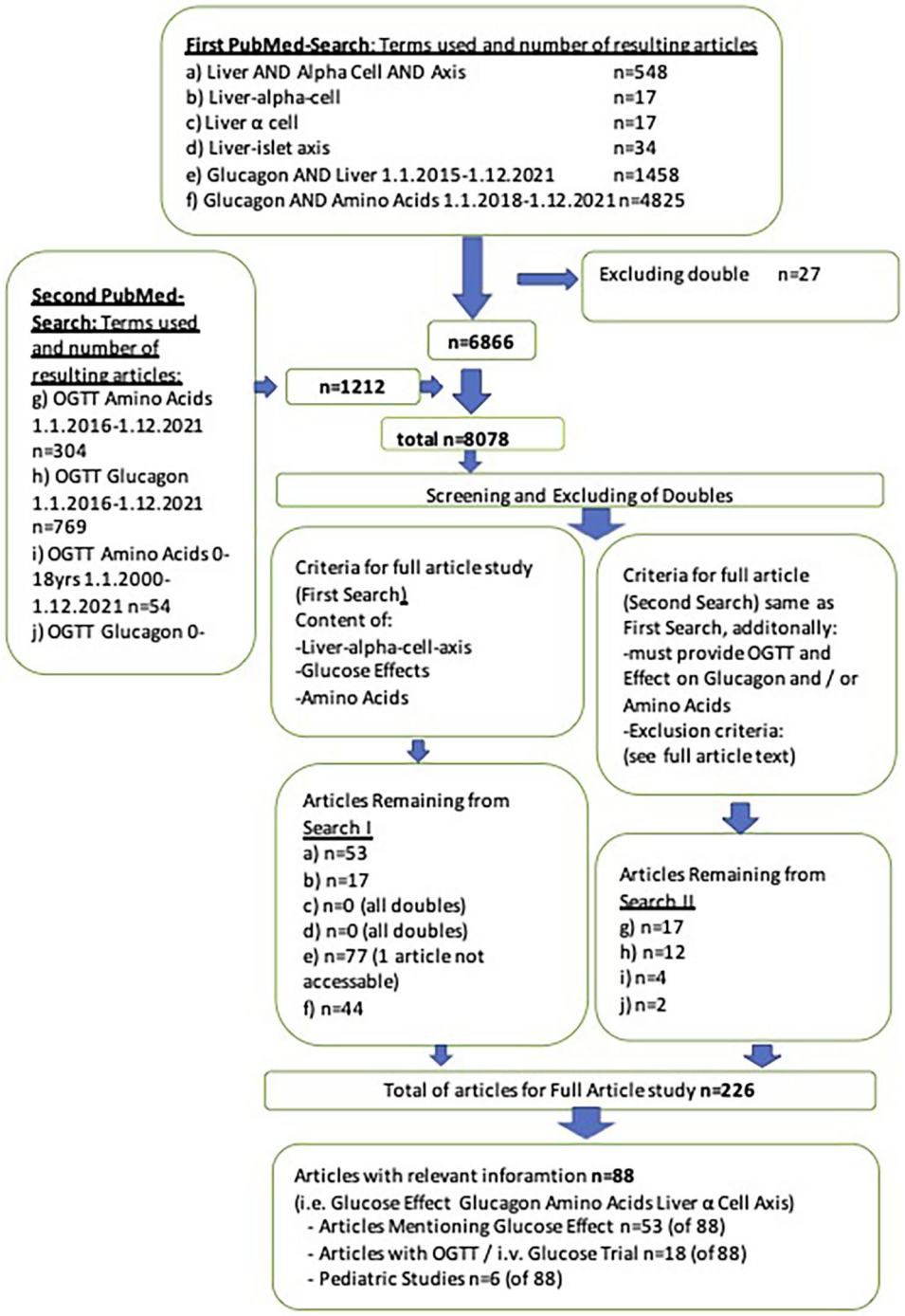


FIGURE 2
PRISMA-flow chart of literature search.

substrates (85) and the effect of glucagon subsides after extended fasting periods (22, 86–89). During longer fasting intervals, blood glucose levels mainly depend on cortisol and amino-acids (9, 48). Despite their decreased effectiveness, glucagon levels are elevated in prolonged fasting and starvation (80, 90). The synthesis of new glucogenic AAs from endogenous sources (e.g., muscle, liver) additionally boosts glucagon levels (and induces ureagenesis) (32, 91). In keeping with this, Holst et al.

listed the glucose-alanine cycle. Alanine is released from the muscle into the circulatory system during prolonged fasting and then converted into pyruvate in the liver (9, 91). Glucagon regulates alanine transferase (responsible for alanine conversion to pyruvate) and the key enzymes responsible for gluconeogenesis. The cycle, however, depends on the alanine supply rather than glucagon action (9). In periods of starvation, insulin secretion is scarce, and may not inhibit the effects of glucagon (and cortisol) (32).

The suppression of glucagon secretion during hyperglycemia remains only partially understood (92–95). In mixed meals, glucagon levels rise together with blood glucose and insulin levels due to the proteins ingested (80). Conversely, glucagon levels plunge close to zero in healthy individuals following high-carbohydrate diets (8). The previously mentioned cellular mechanism works the other way in hyperglycemia, resulting in high intracellular ATP levels and low levels of ADP in the pancreatic alpha cell. The high ATP causes KATP channels to depolarize the membrane potential, thus inactivating Ca^{2+} and Na^{+}-channels. An absent influx of Ca^{2+} and Na^{+} and their lower intracellular concentration hinder glucagon exocytosis and the release of glucagon into the general circulation (78). As glucagon secretion is not only regulated by Ca^{2+} and Na^{+} but also by cAMP, reducing cAMP is presumably involved in inhibiting glucagon secretion (96–99). Yu et al. demonstrated that glucose decreases the cAMP concentration beneath the plasma membrane *via* a direct glycemic effect on pancreatic alpha cells. The mechanism is unclear, but it is reported in mouse and human alpha cells (97). It has been reported that glucagon secretion shows a maximal inhibition at ~5 mM glucose. When glucose levels exceed this limit, so does glucagon secretion paradoxically (38, 100).

3.3 Glucose and glucagon under conditions of metabolic impairment

In individuals with type-2-diabetes-mellitus (T2D), the body's ability to respond to hypoglycemia appears severely attenuated or completely lost (101, 102). While precise mechanisms remains unclear, paracrine and central pathways have been suggested (103). Normal glucose response in T2D can be restored if glucose control is maintained, as shown by Bolli et al. *via* insulin clamping (pancreatic clamp with somatostatin). This study supports the hypothesis that failure of glucose sensing in T2D is not caused by an intrinsic defect of the alpha cells, but by a lack of metabolic control (104). In T2D, fasting hyperglucagonemia, as well as inadequate suppression of glucagon following carbohydrate ingestion, have been described (44). Hyperglucagonemia is common but not pathognomonic for T2D, as some patients also display normal glucagon level (15). In T2D, glucagon hypersecretion contributes to hyperglycemia (105–109). Therefore, glucagon receptor antagonists have gained attention as potential treatment for T2D (109–112). As with T2D, fasting hyperglucagonemia and increased hepatic glucose production have been observed in other conditions of metabolic impairment (15, 48–50). This includes non-diabetic subjects with obesity, non-diabetic subjects with NAFLD, prediabetic patients, and individuals with metabolic syndrome (15, 45–47, 113). Persistent hyperglycemia on the other hand may directly affect the pancreatic alpha cell *via* intracellular acidification, impaired mitochondrial function, and a resulting dysregulated K_{ATP} channel activity in the alpha cells (114). Of particular note, patients with NAFLD show significantly higher fasting plasma glucagon levels (either normoglycemic or T2D) than normoglycemic and T2D control subjects without NAFLD (113). Also, individuals with hepatic cirrhosis presented with hyperglucagonemia, hyperaminoacidemia, and impaired ureagenesis in response to glucagon (115–117).

3.4 Effects of experimental oral & intravenous glucose administration on glucagon

Literature research revealed thirteen articles (see Table 1) that investigated the effects of administration of glucose on glucagon (7, 20, 53–62, 71).

Ten articles studying the effect of glucose on glucagon were performed exclusively using an OGTT. Wewer Albrechtsen et al. examined the influence of an OGTT on glucagon and amino acids, demonstrating the relationship between AAs and glucagon in a feedback loop through the liver-alpha cell axis. Elevation of fasting glucagon was associated with lower concentrations of alanine, tyrosine, glutamine, and increased concentrations of BCAAs. Higher tertiles for HOMA-IR were associated with elevated fasting glucagon and a steeper decline in glucagon levels during OGTT than in lower tertiles while maintaining higher levels after 120 minutes (20). In an observational cohort study by Koopman et al. among 121 nondiabetic individuals, glucagon responses at baseline and glucose levels seven years later were investigated (56). During OGTT, glucagon levels dropped in the first 60 minutes, to reach baseline levels again after about 120 minutes (fasting plasma glucagon 9.4 ± 2.9; glucagon $iAUC_{0\text{-}30min}$ OGTT −0.23 ± 0.7; glucagon $iAUC_{30\text{-}120min}$ OGTT −2.80 ± 3.8; glucagon $iAUC_{0\text{-}120min}$ OGTT −2.94 ± 4.5 [all pmol/L, mean ± SD]). Deteriorating glycemic control over time was associated with insufficient glucagon-suppression during OGTT (56, 60) and an early lack of glucagon suppression exists in prediabetes (118). In keeping with this, Ichikawa et al. found glucagon-levels to be less suppressed during OGTT in T2D and prediabetes than in individuals with normal glucose tolerance (NGT) as well as higher fasting glucagon (prediabetes: 34.4 ± 4.6, T2D 44.1 ± 5.0, NGT: 20.6 ± 3.6, all pg/mL). This decrease of glucagon

TABLE 1 Studies on the dynamics of glucagon after administration of glucose.

Studies on the dynamics of glucagon after administration of glucose										
Authors	Year	Aim of study/article	Sample size	Male/ Female	Age (years)	OGTT/ i.v. glucose	(Measured) Metabolites	Timepoints of measurement (minutes)	Results	Authors' conclusion
Knop FK (7)	2018	To summarize the physiological regulation of glucagon secretion in humans	n.a	n.a.	n.a.	both	Glucagon, GLP-1, GLP-2, insulin, 'gastrointestinally mediated glucose disposal' (GIGD)	n.a.	Glucagon was suppressed normally by i.v. glucose in all groups (independently of glucose tolerance). Glucagon response to oral glucose increases from normal glucose tolerance over impaired glucose tolerance to overt diabetic glucose tolerance. Increased glucagon responses to oral glucose. Hyperglucagonaemia in T2D is aggravated by OGTT, but suppressed similarly as in healthy subjects during IIGI. Elevated fasting plasma glucagon levels contribute to increased basal rate of hepatic glucose production. Demonstration of pivotal role of fasting hyperglucagonaemia in pathogenesis of fasting hyperglycaemia in T2D.	Fasting hyperglucagonaemia is unrelated to the diabetic state, but strongly correlates with obesity, liver fat content and circulating amino acids. Postabsorptive hyperglucagonaemia occurs as a consequence of gut-derived glucagon secretion and/ or glucagonotropic factors. Hyperglucagonaemia seems to occur independently of the diabetic state and rather to be related to obesity-associated disruption of the emerging liver–alpha cell axis (hepatic glucagon resistance) involving amino acids as essential mediators of liver–alpha cell cross-talk (triggering compensatory glucagon secretion from alpha cells). Extrapancreatic glucagon exists.
Wewer-Albrechtsen et al. (20)	2018	Hypothesis was that hepatic insulin resistance (secondary to hepatic steatosis) via defective glucagon signaling/ glucagon resistance would lead to impaired ureagenesis and, hence, increased plasma concentrations of glucagonotropic amino acids and, subsequently, glucagon.	1408	53% males, 47% females	66.2 (mean)	OGTT	AAs (alanine, histidine, tyrosine, glutamine, phenylalanine, isoleucine, leucine, valine) and glucagon, Plasma insulin, ALT, GGT, HOMA-IR, glucagon-alanine-index	0, 30, 120	Plasma concentrations of glucagon and alanine decreased during the OGTT. Absolute concentrations of alanine differed by tertile of HOMA-IR. For middle and upper tertiles of HOMA-IR, fasting plasma concentrations of alanine were higher (0.27 ± 0.06 and 0.28 ± 0.06 mmol/l, respectively), and in these two tertiles alanine concentration decreased in response to glucose intake (change in alanine 120 min after the OGTT: −0.010 [95% CI −0.014, −0.007] and −0.018 mmol/l [95% CI −0.022, −0.015 mmol/l], respectively; $p < 0.001$ for both). Increasing plasma concentrations of BCAAs associated with increasing plasma concentrations of glucagon.	Plasma concentrations of both glucagon and non-BCCAs (e.g. alanine) during an OGTT were affected by increasing HOMA-IR. Observations support hypothesis that impaired hepatic glucagon signaling, potentially due to fat accumulation in the liver and resulting hepatic insulin resistance. This impairs glucagon's ability to lower plasma levels of non-BCAAs. Study supports the existence of a liver alpha cell axis in humans: glucagon regulates plasma levels of amino acids, which in turn feedback to regulate the secretion of glucagon. With hepatic insulin resistance, reflecting hepatic steatosis, the feedback cycle is disrupted, leading to hyperaminoacidaemia and hyperglucagonaemia. The glucagon-alanine index is suggested as a relevant marker for hepatic glucagon signaling.
Wagner et al. (53)	2017	To investigate the change in glucagon during oral glucose tolerance tests (OGTTs), hypothesizing that higher postchallenge glucagon levels are observed in subjects with	Cross-sectional: 4194 total ; Longitudinal study: n=50	n.a.	n.a.	OGTT	Glucagon	0, 30, 60, 90, 120 (TUEF and TULIP cohort) ; 0 and	66–79% of participants showed suppression of glucagon at 120 min (fold change glucagon120/0 <1) during OGTT, whereas 21–34% presented with	Lower glucagon suppression after oral glucose administration is associated with a

(Continued)

10.3389/fendo.2022.1061682

TABLE 1 Continued

Studies on the dynamics of glucagon after administration of glucose										
Authors	Year	Aim of study/article	Sample size	Male/ Female	Age (years)	OGTT/ i.v. glucose	(Measured) Metabolites	Timepoints of measurement (minutes)	Results	Authors' conclusion
		impaired glucose tolerance (in three cohorts of non-diabetic individuals).	(after life-style intervention)					120 (MDCS and PPP-Botnia cohort)	increasing glucagon levels (fold change glucagon120/0 ‡1). Participants with non- suppressed glucagon120 had a lower risk of IGT in all cohorts (odds ratio 0.44–0.53, P < 0.01). They were also leaner and more insulin sensitive and had lower liver fat contents.	metabolically healthier phenotype.
Junker AE (54)	2015	Summarizes three studies (co-)authored by author. 1) assessed the impact of NAFLD on the incretin effect in patients with or without type 2 diabetes. 2) investigated the influence of cirrhosis on incretin physiology 3) examined the glucagonostatic effect of GLP-1 and its potential glucose- dependency in non-diabetic patients with NAFLD - excluded from this review as glucose-levels were kept at fasting levels.	1) NGT and NAFLD n=10; T2D and NAFLD n=10; T2D only n=8; healthy controls n=10 ; 2) Cirrhosis: n=10; Healthy controls: n=10	1) n.a. ; 2) Cirrhosis: 5/ 5; Healthy controls: 5/5	1) NGT and NAFLD: 56 (39–63); T2D and NAFLD: 64 (54–65); T2D only: 59 (50–67); healthy controls: 57 (49–60); (mean (span)) ; 2) Cirrhosis: 54 ±15; Healthy controls: 57 ±15	1,2) OGTT/ IIGI	1,2)Glucagon, GLP-1, GIP, insulin, plasma glucose, incretin effect	1,2) -15 to +240	1) Fasting glucagon levels were similarly low inpatients with T2D only [4.5 pmol/ L(3.0–6.0 pmol/L)] and controls[3.4 pmol/L(1.8–6.0 pmol/L)]. Patients with normal glucose tolerance and NAFLD exhibited immediate glucagon suppression during the first hour of the OGTT and the IIGI [-83 pmol/L (-70to 16 pmol/L) vs.-133 pmol/L(-225 to79 pmol/L)9min,P=0.037]. Controls showed similar immediate glucagon suppression during the first hour of the OGTT and the IGII. Patients with T2D only had delayed and impaired glucagon suppression during the first hour of the OGTT compared to the IIGI[22 pmol/L (-146 to 73 pmol/L) vs. -45 pmol/L (-116 to 27 pmol/L)9min,P=0.027 and 35 pmol/L (-35 to 87 pmol/L)vs.-94 pmol/L (-115 to 43 pmol/L) 9min,P=0.039]. Impaired glucagon suppression was most pronounced in patients with T2D and NAFLD. 2) Patients with cirrhosis and healthy controls had similar incremental changes in glucagon. Plasma glucagon dropped abruptly in both groups following oral as well as intravenous glucose and continued to decrease the following 90 min. A more pronounced suppression of glucagon was seen during IIGI than OGTT.	1) Patients with NAFLD, despite having normal glucose tolerance are characterized by reduced incretin effect, fasting hyperglucagonaemia and impaired handling of ingested glucose. Study emphasizes the role of NAFLD in metabolic dysregulation and suggests an important role for the liver in the regulation of glucagon secretion. 2) In spite of fasting hyperglucagonemia, patients with cirrhosis suppressed glucagon during both OGTT and IIGI, indicating preserved alpha cell sensi-tivity to GLP-1.
Wang et al. (55)	2019	To investigate the progression of obesity-related type 2 diabetes mellitus (T2DM) in rhesus monkeys, especially dynamic changes in insulin and glucagon. Intravenous glucose tolerance test was performed every 6 months to evaluate dynamic changes in glucose, insulin and glucagon levels over 7 years.	52 rhesus monkeys	all males	n.a.	IVGTT	Glucagon, insulin, blood glucose	1, 3, 5, 10, 15, 20, 30, 45, 60	During IVGTTs, glucagon remained consistently elevated in the T2DM group with obesity in all tests, while it increased gradually in the non-T2DM group with obesity and became significantly higher than the lean group in the 7th year test. AUC for glucagon in all tests in the obese T2DM group were significantly higher than the lean and the non-T2DM groups with obesity in year 1, 3 and 5. In the lean group, glucagon immediately decreased after glucose was administered and returned to baseline within an hour. However, in the T2DM group, glucagon decreased after glucose challenge, but then dramatically increased to a level much higher than baseline 1 h later.	Hyperglucagonemia plays an important role in the development of T2D.
Koopman et al. (56)	2019	To examine the association between glucagon responses at baseline and fasting glucose levels 7 years later.	121 (NGT: n=109, IFG: n=12)	50% females	54.1±6.6 at baseline	OGTT	Glucagon (AUCs)	AUCs (0-30 and 30-120)	Glucagon response was differentiated for early (0-30 minutes), late (30-120 minutes) for OGTT. Authors observed an association for the early lack of response and not the late or total glucagon in participants with prediabetes. In NGT Glucagon-level after OGTT steadily declined and reached its lowest level at 60 minutes of OGTT	Within a population without diabetes, relative lack of glucagon suppression early after a meal was associated with increase of glucose levels over time, suggesting a role of insufficient glucagon suppression in

(Continued)

10.3389/fendo.2022.1061682

TABLE 1 Continued

Studies on the dynamics of glucagon after administration of glucose										
Authors	Year	Aim of study/article	Sample size	Male/ Female	Age (years)	OGTT/ i.v. glucose	(Measured) Metabolites	Timepoints of measurement (minutes)	Results	Authors' conclusion
									before returning to baseline at around 120 minutes.	the deterioration of glycemic control.
Engelbrechtsen et al. (57)	2018	To investigate if mutations of the human Ether-á-go-go- related gene (hERG) potassium channel alter glucose homeostasis.	6816 (1329 individuals from the ADDITION-PRO Cohort (NGT: n=708, IFG: n=254, IGT: n=103, IFG and IGT: n=116, T2D: n=148) and 5487 individuals from the Inter 99 cohort)	48% females in ADDITION-PRO cohort and 51% females in Inter 99 cohort	66.3±6.9	OGTT	Glucagon, insulin, blood glucose, GIP, GLP-1	0, 30, 120	The Kv11.1 voltage-gated hERG potassium channel (minor G-allele of rs1805123) was associated with 0.95 pmol/L (− 1.52; − 0.38)) lower fasting glucagon (p = 0.003) and decreased glucagon AUC at 0–30 and 0–120 min (β=−2.73 (−4.73, −0.70), p=0.009 and β= −2.27 (−4.25, − 0.24), p = 0.029, respectively).	Common missense polymorphisms of the Kv11.1 voltage-gated hERG potassium channel (minor G-allele of rs1805123) are associated with alterations in circulating levels of glucagon, suggesting hERG potassium channels play a role in fasting and glucose-stimulated release of glucagon.
Gar et al. (58)	2018	To investigate if glucagon patterns were homogeneous within certain metabolic phenotypes.	285 total (3 metabolic phenotypes: 1) healthy control: n=93; 2) normoglycemic high-risk: n=121; 3) prediabetes/ screening-diagnosed type 2 diabetes: n=71	all females	1) 35.3±4.2; 2) 35.2±4.5; 3) 35.9±4.5	OGTT	Glucagon	0, 30, 60, 90, 120	Fasting plasma glucagon was significantly elevated, and early glucagon suppression was diminished in the prediabetes/diabetes group compared with the control group. (Early-suppression glucagon (0–30) (%): 1) 47.6 (32.8–57.9), 2) 41.3 (22.9–58.3), 3) 32.0 (14.5–51.3) - p=0.0055 ; Late-suppression glucagon (30–120) (%): 1) 31.8 (8.9–49.6), 2) 40.9 (14.9–56.7), 3) 47.4 (33.3–63.6) - p=,0.0001.) Late and overall glucagon suppression was smaller in women with isolated IFG compared with both other groups. Total glucagon suppression was similar in all three groups. The five-point glucagon curves in response to oral glucose were heterogeneous between individuals.	Fasting hyperglucagonemia and delayed glucagon-suppression are strongly linked to obesity and metabolic syndrome. Rising glucagon during OGTT may be a rare phenomenon. It occurs in insulin-sensitive individuals with a tendency toward hypoglycemia, but does not necessarily indicate metabolic health.
Kosuda et al. (59)	2022	To investigate glucagon dynamics in patients with postprandial syndrome.	n=14	3 males/11 females	40 (30–49)	OGTT	Glucagon, insulin, blood glucose	0, 30, 60, 90, 120, 180, 240	Plasma glucagon was immediately suppressed by nearly 70% in patients with postprandial syndrome. Authors state that in healthy persons glucagon is reportedly suppressed by approximately 50%.	In patients with postprandial syndrome glucagon suppression is greater than in healthy subjects.
Ichikawa et al. (60)	2019	To clarify the involvement of glucagon in the pathophysiology of DM. OGTT was performed in subjects with NGT, preDM and DM and changes in glucagon and metabolites pre- and post-OGTT were compared.	NGT:n=25, preDM: n=15, DM: n=13	NGT: 12/13 , preDM: 8/7 , DM: 9/4	NGT: 36.±14.69 7 preDM: 54.1 ±11.5, DM: 60.8±4.8	OGTT	Glucagon, plasma glucose, insulin, glucagon and active GLP-1	0, 30, 60, 90, 120	During OGTT, glucagon levels were less suppressed in DM and preDM than in NGT, whereas no apparent relationship was observed between glucagon and GLP-1 secretion.	Subjects with mild T2D showed elevated fasting glucagon and paradoxical glucagon increase after oral glucose load compared to subjects with normal glucose tolerance.
Jonsson et al. (61)	2021	To examine the impact of gene variants associated with T2D on glucagon-levels during OGTT.	1899 (1346 from ADDITION-Pro cohort, 553 from RigCoh)	709/637 in ADDITION-Pro cohort, 264/289 in RigCoh	66.3±6.9 in ADDITION-Pro cohort, 22.0±2.2 in RigCoh	OGTT	Glucagon	0, 30, 120	*EYA2-variant* was associated with higher 30 min plasma glucagon levels during the OGTT. Authors also identified novel gene locus associated with reduced suppression of early glucagon secretion.	EYA2 locus is associated with increased plasma glucagon levels at 30 min during OGTT, while other variants influence glucagon levels without conferring an increased type 2 diabetes risk.
Manell et al. (62)	2016	Investigation of fasting and postprandial proglucagon derived hormones (e.g. glucagon) in adolescents with obesity along the spectrum of glucose tolerance.	NGT n=23, IGT n=19, T2DM n=4, and age-matched lean	32 males/33 females	10-18	OGTT	Glucagon, GLP-1 and glicentin	-5, 5, 10, 15, 30, 60, 90, 120	Glucagon during OGTT: levels in lean individuals started to decrease between 5 and 10 minutes with suppression close to the observed maximum suppression after 30 minutes. In adolescents with	In adolescents with obesity, glucagon levels are elevated and the progression to T2DM is related to a further

(Continued)

10.3389/fendo.2022.1061682

TABLE 1 Continued

Studies on the dynamics of glucagon after administration of glucose										
Authors	Year	Aim of study/article	Sample size	Male/ Female	Age (years)	OGTT/ i.v. glucose	(Measured) Metabolites	Timepoints of measurement (minutes)	Results	Authors' conclusion
			adolescents (n=19)						obesity and NGT or IGT, glucagon levels tended to increase during the first 5 minutes of the OGTT. There was no lowering below fasting levels until 30 minutes. In individuals with obesity and T2D, glucagon levels increased during the initial 15 minutes, with no reduction below baseline until 60 minutes.	increase as well as an early-phase hyperglucagonemic response to OGTT.
Kahn et al. (71)	2021	To determine whether hyperresponsiveness of the beta cell and insulin resistance in youth vs. adults in the Restoring Insulin Secretion (RISE) Study are related to increased glucagon release.	Youth: n=66 Adults: n=350	Youth: 47 (71.2) Adults: 182 (52.0)	Youth: 10-19 (14.2 ± 2.0) Adults: 52.7 ± 9.3	OGTT	Glucagon, glucose, C-peptide, insulin	−10, −5, 10, 20, 30, 60, 90, 120, 150, 180	Mean ± SD fasting glucagon (7.63 ± 3.47 vs. 8.55 ± 4.47 pmol/L; P = 0.063) and steady-state glucagon (2.24 ± 1.46 vs. 2.49 ± 1.96 pmol/L, P = 0.234) were not different in youth and adults. Fasting glucose and glucagon were positively correlated in adults (r = 0.133, P = 0.012) and negatively correlated in youth (r = −0.143, P = 0.251). The absolute glucagon concentrations were lower in youth at multiple time points during the test. This was due to a more rapid decline in glucagon in the first 30 min during OGTT in youth than adults. However, iAUC C-peptide relative to dAUC glucagon across the 3-h OGTT was not significantly different in youth and adults.	Youth with IGT or recently diagnosed T2D (drug naive) have hyperresponsive beta cells and lower insulin sensitivity, but their glucagon concentrations are not increased compared with those in adults. Alpha cell dysfunction does not appear to explain the difference in β-cell function and insulin sensitivity in youth versus adults.

AAs (amino-acids), AAAs (aromatic amino-acids), ALT (alanine transaminase), BCAAs (branched-chain amino-acids), CI (confidence interval), DM (diabetes mellitus), EFAs (essential fatty acids), EYA2 (EYA transcriptional coactivator and phosphatase 2), FFAs (free fatty acids), GGT (gamma-glutamyl transferase), GIGD (gastrointestinally mediated glucose disposal), GIP (Gastric inhibitory polypeptide), GLP-1 (glucogon-like peptide 1), GLP-2 (glucogon-like peptide 2), hERG (human Ether-á-go-go- related gene), HOMA-IR (Homeostatic Model Assessment of Insulin Resistance), IGT (impaired glucose tolerance), IFG (impaired fasting glucose), IIGI (isoglycemic intravenous glucose infusion), IR (insulin resistance), i.v. (intravenous), IVGTT (intravenous glucose tolerance test), OGTT (oral glucose tolerance test), NGT (normal glucose tolerance), T2D (type 2 diabetes mellitus).

10.3389/fendo.2022.1061682

suppression was also reported in two articles investigating genetic variants and glucagon secretion during OGTT (57, 61). Engelbrechtsen et al. explored common variants in the human ether-aí-go-go related (hERG) gene resulting in a dysfunctional Kv11.1 voltage-gated potassium channel. Authors reported the minor G-allele of rs1805123 to be associated with decreased fasting glucagon release: if combined with the minor A- allele of rs36210421 a suppressed glucagon response to increased glucose levels during an OGTT was reported (57). Jonsson et al. performed a genome-wide association study to identify novel loci that affected plasma glucagon levels. The authors documented that higher plasma glucagon levels at 30 min during the OGTT (Beta 0.145, SE 0.038, P = 1.2 × 10–4) were significantly associated with a T2D variant in EYA2, noting a 7.4% increase in plasma glucagon level per effect allele. Jonsson et al. identified a marker in the MARCH1 locus, significantly associated with a reduced glucagon suppression during the first 30 min of the OGTT (Beta − 0.210, SE 0.037, P = 1.9 × 10–8), (8.2% less suppression per effect allele). They also found nine additional independent markers, not previously associated with T2D, that demonstrated suggestive associations with a reduced suppression of glucagon during the first 30 min of the OGTT (P < 1.0 × 10–5) (61).

Wagner et al. investigated the glucagon response in three cohorts (TUEF study, Bonita-PPP study, Tulip study) with a total of 4194 subjects. They found no suppression of glucagon during OGTT ($glucagon_{120min}$) in 21-34% of subjects (in all three cohorts). These individuals were leaner, had a higher insulin sensitivity, a lower risk for impaired glucose tolerance (IGT) (OR 0,44-0,53 in all cohorts, p<=0.009) and lower fasting glucagon levels. The authors concluded that there was an association between non-suppressed glucagon at 120 minutes of OGTT and a metabolically healthier phenotype with lower IGT risk (odds ratio [OR] was 0.44–0.53 in all cohorts, p ≤ 0.009) (53). Comparing glucagon suppression between the groups (i.e., NGT, IFG, and IGT) and in the TUEF study at the intervals 0–30, 0–60, 0–90, and 0–120 minutes, only fold change glucagon120/0 minutes was different. In the PPP- Bonita study, of the 98 individuals with incident diabetes, significantly fewer had increasing or stable $glucagon_{120min}$ than subjects without diabetes (25 vs. 39%, P = 0.002). $Glucagon_{120min}$ in NGT was 15.2 median (12.6-18.9 [IQR/95%CI]), while in IGT it was 13.5 median (11.5-18.1 [IQR/95%CI]). The TULIP study assessed glucagon (fasting and 120 minutes during OGTT) before and nine months after a lifestyle intervention. Lifestyle intervention reduced fasting glucagon (19 vs. 17.5 [median] and 15.2-25.6 vs. 14.4-20.7 [IQR/95%CI]) as well as glucagon after 120 minutes (16.2 vs. 15.2 [median] and 12.4-20.4 vs. 13.4 vs. 18.1 [IQR/95%CI], all pg/mL) (53). Wagner et al. compared their results to those by Faerch et al. (118), that had registered lower early suppression of glucagon (minutes 0-30) and higher late suppression of glucagon (minutes 30-120) in patients with prediabetes and incident diabetes, compared to individuals with NGT (118). The authors found comparable data on late glucagon suppression, however, the TUEF study did not show differences between prediabetes and NGT for shorter intervals (30/0, 60/0, and 90/0). Also, glucagon levels were about two-fold higher than those of Faerch et al. (118). Wagner et al. stated that similar AUCs for glucagon levels and additional controlling for fasting glucagon suggest that fasting glucagon does explain the association between glucagon suppression and insulin sensitivity. Additionally, they found a strong inverse association of hepatic triglyceride content with non-suppressed $glucagon_{120min}$ in their data (51). Similarly, Gar et al. found four patterns of glucagon dynamics that did not match metabolic phenotypes in female patients. While Gar et al. reported fasting hyperglucagonemia and delayed glucagon suppression in prediabetes and T2D (median Q1 to Q3 for fasting plasma glucagon: 6.0 [4.6 to 8.2] vs 7.7 [5.6 to 11.2] in controls); early glucagon suppression: 47.6 [32.8 to 57.9] vs 32.0 in controls [14.5 to 51.3, all pmol/L], respectively. This applied to only 21% of cases and 8% of the control group. Delayed glucagon suppression was clearly associated with obesity and metabolic syndrome. One cluster of seven individuals with low fasting glucagon had rising glucagon levels during the OGTT. The women in this cluster were lean, insulin sensitive, and displayed low plasma glucose (58). The study with the smallest population was done by Kosuda et al., who investigated glucagon response to an OGTT in 17 patients with idiopathic postprandial syndrome (IPS). They reported two types of glucagon dynamics. The first was characterized by lower fasting glucagon and further suppression during OGTT, and the second with fasting hyperglucagonemia and drastic decrease during OGTT. The authors concluded that glucagon suppression in patients with IPS is more substantial than in healthy individuals (59).

Two publications addressed glucagon levels during an OGTT in a pediatric collective, which are presented in the section on pediatric data below (62, 71). Of the studies that included intravenous glucose administration, one study by Wang et al. was on rhesus monkeys. They performed i.v. glucose tolerance tests every six months and measured glucagon levels during tests. In the T2D group with obesity, glucagon remained elevated. In the non-T2D group with obesity, it increased gradually to become significantly higher in the 7^{th} year test. In lean monkeys, glucagon immediately decreased after i.v. glucose and returned to baseline within an hour. In the T2D group, glucagon initially decreased after the glucose challenge to increase to a considerably higher level than baseline after 60 minutes (55). In human studies, Knop reports hyperglucagonemia during OGTT in T2D patients specifying they responded with normal suppression of glucagon during an isoglycemic intravenous glucose infusion (IVGI) (7, 119). The author documents the reproduction of this effect in a number of studies (120–122). Knop found the same reaction in other forms of diabetes (e.g., secondary diabetes after pancreatitis) (7). In a publication on incretin hormones and glucagon in liver disease, Junker included a summary of three separate studies. The

first investigated the influence of NAFLD on the previously mentioned hormones in individuals with normal glucose tolerance and or T2D. All patients underwent OGTT (50g glucose) and an isoglycemic intravenous glucose infusion (IVGI). In the first study, only fasting glucagon was measured. Incretin effect in controls was stronger than in NAFLD and T2D. Fasting hyperglucagonemia was associated with NAFLD independent of T2D. Controls and T2D without the liver disease had similar fasting glucagon levels. The second study concentrated on patients with cirrhosis. Fasting hyperglucagonemia was present in cirrhosis and both oral (50g glucose OGTT) and i.v. glucose suppressed plasma glucagon. Junker concluded that cirrhosis complicates the treatment of oral glucose and reduces the incretin effect, possibly contributing to glucose intolerance in cirrhotic patients. The third study included no glucagon measurements (54).

3.5 Effects of glucagon on amino acids and vice versa

Glucagon is known to stimulate the influx of AAs into liver cells to provide substrates for gluconeogenesis (106). In order to do so, glucagon stimulates AA transporters expression in the liver for alanine, glutamine, asparagine, and histidine (123, 124). After the influx of AAs into the hepatocytes, the AAs are processed for gluconeogenesis and ureagenesis (85, 125). Under pharmacological blockade of the glucagon receptor signal, plasma concentrations of AAs increase while ureagenesis decreases (13, 28, 126–131). Inhibition of glucagon signaling reduces the expression of genes involved in hepatic AA uptake and AA metabolism, thus resulting in hyperaminoacidemia (10, 13, 14, 17, 28, 132, 133). Wewer Albrechtsen et al., Longuet et al., Kim et al. and Dean et al. reported that amino acids regulate glucagon secretion and the alpha cell mass and its proliferation (10, 14, 16, 17, 22). Most AAs stimulate glucagon and insulin secretion with varying potency (29–33). Additionally, there also seem to be differences among species (34). Otten et al. found fifteen AAs potentially signaling to the alpha cell to increase glucagon secretion. They proposed alanine as the main signal molecule, arguing that, if administered intravenously, it is a potent stimulator of glucagon secretion (134–136). However, in the fasting state, results for alanine remain unclear. While elevated fasting levels of alanine are associated with elevated fasting glucose (137, 138) alanine was not associated with glucagon (136). Elevated glucagon secretion has also been reported following the administration of arginine, cysteine, lysine, glycine, and proline (136). Results for glutamine remain controversial. In cell culture models, it is known to cause alpha cell proliferation but does not cause glucagon secretion in the perfused mouse pancreas (10, 136). Leucine was associated with alpha cell proliferation in isolated mouse islets (10, 17), but the results on glucagon secretion for BCAAs remain inconclusive. BCAAs were reported to not stimulate glucagon secretion (30, 32, 136, 139), except for one study on a perfused rat pancreas (29). In contrast, another study reported significant correlations between postprandial glucagon and leucine, and isoleucine and valine (134). The prolonged administration of AAs led to alpha cell proliferation as shown in isolated pancreatic mouse islets (14), and certain AAs (alanine, glutamine, glutamate, and leucine) were consistently associated with alpha cell proliferation (10, 17). AA concentrations, as shown with BCAA, vary with disease. Patients with NAFLD presented with increased plasma concentrations of BCAAs, aromatic AAs, glutamate, alanine, and lysine, and decreased glycine and threonine (140). Interestingly plasma concentrations of glucagonotropic amino acids were elevated in patients with NAFLD, and AA concentrations correlated with glucagon concentrations (141).

3.6 Effects of experimental oral glucose administration on amino acids

Seven articles investigated the dynamics of amino acids during isolated glucose intervention (i.e., OGTT) in the context of LACA (20, 63–68). Table 2 lists the articles containing information on amino acid dynamics during OGTT as well as their results and conclusions.

In a previously mentioned study from 2018, Wewer Albrechtsen et al. analyzed glucagon and eight AAs during an OGTT (20). The study included 1408 adult individuals from the Danish ADDITION-PRO study that underwent a 75g OGTT. Blood samples were drawn at 0, 30 and 120 minutes to analyze serum glucose, glucagon, and eight AAs. The AAs were four non-BCAAs (alanine, histidine, glutamine, and tyrosine), three BCAAs (isoleucine, leucine, and valine), and phenylalanine. During the OGTT, plasma concentration for glucagon and alanine decreased. Alanine concentrations differed by HOMA-IR tertile. Individuals in the lower HOMA-IR tertile were not significantly affected (fasting: 0.26 ± 0.06 mmol/L change in alanine 120 min after the OGTT: −0.003 mmol/L [95%CI −0.007, 0.000 mmol/L], p=0.076). Middle and upper tertiles for HOMA-IR showed higher fasting alanine (0.27 ± 0.06 and 0.28 ± 0.06 mmol/L, respectively). In these tertiles, a significant decrease occurred during OGTT (change in alanine 120 min after the OGTT: −0.010 [95% CI −0.014, −0.007] and −0.018 mmol/L [95% CI −0.022, −0.015 mmol/L], respectively; p<0.001 for both). Authors found statistically significant non-linear associations with fasting glucagon levels for phenylalanine, isoleucine, leucine and valine ($p \leq 0.049$). They also found a modifying effect of hepatic insulin resistance on the associations with fasting plasma glucagon ($p \leq 0.040$) for alanine, tyrosine, phenylalanine, 'total non-BCAA' (alanine, tyrosine, histidine and glutamine), isoleucine, leucine, and total BCAA. Wewer Albrechtsen et al. found that increasing levels of hepatic insulin resistance (but not peripheral IR) ($p > 0.166$) attenuated the

TABLE 2 Studies on the dynamics of glucagon after administration of glucose.

Studies on the dynamics of amino acids after administration of glucose											
Authors	Year	Aim of study/article	Sample size	Male/ Female	Age (years)	OGTT/ i.v. glucose	Amount of glucose administered	(Measured) Metabolites	Timepoints of measurement (minutes)	Results	Authors' conclusion
Wewer Albrechtsen et al. (20)	2018	Hypothesis was that hepatic IR via defective glucagon signalling/glucagon resistance would cause impaired ureagenesis and increased plasma concentrations of glucagonotropic amino acids and glucagon.	total: n=1408 (Lower tertile: n=469, Middle tertile: n=470, Upper tertile: n=469)	Lower tertile: 49.7% female, Middle tertile: 50.6% female, Upper tertile: 40.7% female	58.9-74.2 (66.2 mean)	OGTT	75 g	Glucose, glucagon, Four non-BCAAs (alanine, histidine, tyrosine and glutamine), Three BCAAs (isoleucine, leucine and valine), Phenylalanine	0, 30, 120	OGTT did not significantly affect alanine for individuals in the lower tertile of HOMA-IR. For middle and upper tertiles of HOMA-IR, fasting plasma concentrations of alanine were higher (0.27 ± 0.06 and 0.28 ± 0.06 mmol/l, respectively), and in these two tertiles alanine concentration decreased in response to OGTT (change in alanine 120 min after the OGTT: −0.010 [95% CI −0.014, −0.007] and −0.018 mmol/l [95% CI −0.022, −0.015 mmol/l], respectively; $p < 0.001$ for both).	Higher fasting plasma glucagon concentrations were associated with lower concentrations of certain non-BCAAs including alanine, tyrosine and glutamine, and with higher concentrations of BCAAs.
Sjögren et al. (63)	2021	To identify differences in circulating and skeletal muscle BCAA levels in response to an OGTT in individuals with normal glucose tolerance or T2D.	total n=61 (NGT: n=32; T2D: n=29)	all male	44-69	OGTT	75 g	BCAAs (isoleucine, leucine and valine), Branched-chain α-keto acids (BCKAs),	fasting, 30, 120	Fasting plasma BCAAs were ~10% higher in T2D. Fasting BCKAs did not differ between NGT and T2D. OGTT decreased circulating levels of BCAA and BCKA in NGT but not in T2D.	Disturbances in the BCAA profile are exacerbated by glucose loading. This reveals, that the metabolic inflexibility that characterises T2D encompasses BCAA catabolism
Wang et al. (64)	2019	To characterize the metabolic changes in response to an oral glucose test (OGTT) and assess the associations of these changes with insulin resistance.	total: n=5340	6 subgroups (male: 33-63%)	46.2-47.2	OGTT	75 g	Total of 78 metabolic markers (incl. Amino-acids, Glucose, insulin, lipids, ketone bodies)	0, 30, 60, 120	NGT: almost all measured AAs were decreased during the OGTT, except for alanine. BCAAs (isoleucine, leucine, and valine) and AAA (phenylalanine and tyrosine) showed stronger decrease during OGTT.	Non-diabetic individuals are exposed to a similar adverse postprandial metabolic milieu, as those with T2D
Li et al. (65)	2016	To investigate changes in postprandial AAs and biogenic amine profiles provoked by an OGTT in hyperlidemia patients using targeted metabolomics.	total: n=70	47/33	37.46-57.62	OGTT	75 g	Total of 21 metabolic markers (incl. Alanine, Arginine, Cystein, Glycine, Glutamic acid, Histidine ,Isoleucine, Leucine, Lysine, Methionine, Phenylalanine,Proline, Serine, Tryptophan, Threonine ,Tyrosine, Valine, Asparagine, Creatine)	0, 120	Healthy controls: 4-hydroxy-L-proline, valine, asparagine, tyrosine decreased significantly. Healthy controls: serine, taurine, cysteine and creatine increased significantly. Hyperlidemia: eucine, isoleucine, serine, histidine, lysine, taurine, cysteine and creatine increased significantly. Six metabolites (methionine, dimethylglycine, aminobutyric acid, niacinamide, allantoin and creatinine) can be used as biomarkers for hyperlidemia:	Elevated fasting and postprandial levels of BCAAs during OGTT revealed significant metabolic alterations in the amino acid-metabolism in hyperlipidemia compared to healthy individuals.
Geidestam et al. (66)	2015	Study changes in OGTT-elicited metabolite patterns in obese subjects during a diet induced weight loss study.	total: n=14	3/11	33,5-40,5	OGTT	75 g	All 21 AAs, 11 FFAs	0, 30, 120	Suppression of AAAs is associated with decreased insulinogenic index observed after weight loss (tyrosine: $r = 0.72$, $p = 0.013$; phenylalanine: $r = 0.63$, $p = 0.039$). Suppression and/or lack of increase in levels of glutamine, isoleucine, leucine and glutamate after OGTT improved towards lean profile. after weight maintenance. Individuals with obesity demonstrated a greater heterogeneity in the OGTT-response before and after weight loss.	Weight loss followed by weight maintenance results in changes of some but not all serum profiles elicitated by OGTT that are different in obese glucose intolerant compared to lean glucose tolerant subjects. Changes coincide with improvements in hepatic or peripheral insulin sensitivity during weight loss and weight maintenance.
Liu et al. (67)	2015	To investigate the metabolic alterations in obesity provoked by an OGTT using targeted metabolomics.	total: n=30	n.a.	18-23	OGTT	n.a.	29 Amino-acids and biogenic amines, 14 FFAs and 14 EFAs	0,30,60,90,120	Compared with the controls, eight amino acids and biogenic amines significantly increased (leucine, valine, isoleucine, phenylalanine, proline, alanine, creatine and asparagine, $P < 0.05$) in the population with obesity and three metabolites, glutamine, glutamic acid and taurine, decreased ($P < 0.05$).	Elevated fasting levels and a delayed decrease in AAs during OGTT are important characteristics of metabolic perturbations in obesity. Correlation between postprandial changes in BCAAs with insulin resistance in obesity of importance.

(Continued)

10.3389/fendo.2022.1061682

TABLE 2 Continued

Studies on the dynamics of amino acids after administration of glucose											
Authors	Year	Aim of study/article	Sample size	Male/ Female	Age (years)	OGTT/ i.v. glucose	Amount of glucose administered	(Measured) Metabolites	Timepoints of measurement (minutes)	Results	Authors' conclusion
Trico et al. (68)	2017	Identify early metabolic features of insulin resistance in youth and whether they predict deterioration of glycemic control	78 non-diabetic adolescents, 16 in follow-up 2 years later	34/44; 2 year follow up: 9/7	8 to 18	OGTT	75 g	BCAAs (i.e. leucine, isoleucine, valine), lactate, alpha-hydroxybutyrate, beta-hydroxybutyrate	0, 30, 60, 90, 120	In adolescents with reduced insulin sensitivity the decline in BCAAs was blunted throughout the course of the OGTT (p<0.03).	During OGTT α-hydroxybutyrate and BCAA concentrations characterize IR-youth and predict worsening of glycemic control.

AAs (amino-acids), AAAs (aromatic amino-acids), BCAAs (branched-chain amino-acids), CI (confidence interval), DM (diabetes mellitus), EFAs (essential fatty acids), FFAs (free fatty acids), GGT (gamma-glutamyl transferase), HOMA-IR (Homeostatic Model Assessment of Insulin Resistance), IR (insulin resistance), i.v. (intravenous), OGTT (oral glucose tolerance test), NGT (normal glucose tolerance), T2D (type 2 diabetes mellitus).

association between glucagon and circulating levels of alanine, glutamine, and tyrosine. This was also significantly associated with hyperaminoacidaemia and hyperglucagonaemia (20). The association between insulin resistance and BCAAs during an OGTT was also investigated by Sjögren et al. They investigated differences in circulating BCAA levels in response to an OGTT between the circulatory system and skeletal muscles (63). For the purpose of this review, the plasma levels of BCAAs were of interest. The study population included thirty-two men with normal glucose tolerance (NGT) and 29 men with T2D. The authors found that the impaired BCAA catabolism in T2D under fasting conditions was exacerbated during OGTT. In NGT, the OGTT resulted in a 37-56% reduction of BCAAs, with no detected changes in patients with T2D. Fasting BCAAs levels (isoleucine, leucine, and valine) were ~10% higher in T2D than in NGT, no significant changes were recorded for corresponding branched-chain α-keto acids (BCKAs). The OGTT decreased circulating levels of BCAA and BCKA in NGT. The authors concluded that a glucose challenge may unmask defects at several steps of BCAA catabolism in T2D. The circulating concentrations of leucine, isoleucine, and derived BCKAs exhibited a positive correlation (r=0.64–0.77) with blood glucose, HOMA-IR, and HbA1c after an OGTT (63). A 2021 study by Wang et al. came to similar conclusions concerning BCAAs. They analyzed 78 metabolic parameters during fasting and during an OGTT. In individuals with NGT almost all measured AAs decreased during the OGTT, except for alanine. BCAAs (isoleucine, leucine, and valine) and aromatic amino acids (AAA) (phenylalanine and tyrosine) showed a more noticeable decrease (15 to 45%) than the other amino acids (not specified by authors) (6 to 10%) at 120 minutes. In insulin resistance (IR), the BCAAs were higher at baseline and had a weaker decrease at 2 hours. Individuals with NGT and IR showed a less favorable metabolic profile than insulin-sensitive individuals with NGT (P<0.0006 and consistent when stratified by sex). Individuals with diabetes or prediabetes showed marginal differences in metabolic responses concerning branched-chain amino acids. Authors concluded, subjects with NGT and IR have a similar metabolic pattern and cardiovascular risk level as in T2D. The article did not include absolute values of AAs (64).

Two studies focused on AA dynamics during OGTT in the context of obesity. Liu et al. measured AAs after an OGTT in a cohort of 15 young adults with obesity (18-23 years) and compared values with those from 15 lean controls. In the group with obesity, baseline (i.e., fasting) BCAAs (leucine, valine, isoleucine) as well as phenylalanine, proline, alanine, creatine, and asparagine were significantly increased (P<0.05), while glutamine, glutamic acid, and taurine were decreased (P<0.05). During the OGTT 2h-glucose was positively associated with leucine (r=0,84, P<0.05) and tryptophan (r=0.77, P<0.05) in controls. In the group with obesity, changes in arginine and histidine were positively associated

with parameters for obesity (P<0.05). Increasing fasting glucose was positively associated with changes in histidine concentrations (P=0.004). HOMA-IR correlated with changes in leucine, isoleucine, phenylalanine, lysine, and histidine during the OGTT (67). The second study, by Geidenstam et al. analyzed 21 AAs during an OGTT in 14 individuals with obesity during weight loss. Results suggested different metabolic patterns during weight loss and weight maintenance, and only a few profile changes towards the lean reference. They reported that after weight-loss a suppression of aromatic amino acids was associated with decreased insulinogenic index (tyrosine: r = 0.72, p = 0.013; phenylalanine: r=0.63, p=0.039). Moreover, OGTT-elicited suppression or lack of increase in levels of glutamine, isoleucine, leucine, and glutamate improved towards the lean profile in weight maintenance following weight loss, and improved glucose tolerance (AUC Glutamine (weight-loss: r=0.76, p=0.003 and weight-maintenance: r=0.85, p=0.0002, respectively). Subjects with obesity's response to OGTT before and after weight loss was more heterogeneous than in lean patients, although reduced during weight maintenance (66). One study was included individuals with known hyperlipidemia and compared their AA dynamics during OGTT to healthy controls. Li et al. investigated 21 metabolic parameters before and after an OGTT (plasma samples at minutes 0 and 120). In healthy controls, the levels of 4-hydroxy-L-proline, valine, asparagine, tyrosine decreased, and the concentrations of four amino acids (serine, taurine, cysteine and creatine) increased significantly after the OGTT. In HLP there were significant increases in leucine, isoleucine, serine, histidine, lysine, taurine, cysteine, and creatine, while reductions in six metabolites (methionine, dimethylglycine, aminobutyric acid, niacinamide, allantoin, and creatinine) were noted after the OGTT. Li et al. reported that their data positively associated the postprandial changes in isoleucine and HOMA-IR. The study only listed fasting values and used graphics for measurements at 120 minutes and their association to clinical parameters (e.g., insulin concentrations) (65). The study on the effect of an OGTT on BCAAs in a pediatric population by Trico et al. is listed in section 3.8. on pediatric data (68).

3.7 Further studies on experimental glucose administration

In their study on the role of glucagon and muscle wasting in critical illness, Thiessen et al. infused critically ill patients with insulin and glucose, failing to lower glucagon, instead raising it with parenteral administration of amino acids. The authors suggested the effect of adrenaline and cortisol to avoid a decline in glucagon levels after glucose administration. They concluded that during critical illness hyperglucagonemia increases hepatic AA-catabolism without affecting muscle wasting or blood glucose (142). A study by Kelly et al. included two mathematical models of glucagon effectiveness and sensitivity from an OGTT (not included in the previous sections. These models were used to calculate how various degrees of patient glucagon-sensitivity and effectiveness might affect serum glucose and glucagon concentrations during IVGTT and insulin infusion tests. These suggested that the models could provide a mathematical platform from which the effect of glucagon during a glucose test may be predicted (143). In another publication, Lund et al. examined the glucagon of ten pancreatectomy patients and ten healthy controls. They found glucagon present in patients without a pancreas, demonstrating the existence of extrapancreatic glucagon. The authors suggested that this gut-derived extrapancreatic glucagon may play an unrecognized role in diabetes secondary to total pancreatectomy (144).

3.8 Pediatric data

Four studies focused on amino acids in a pediatric population. The study by Suzuki et al. included 26 children with obesity (15 male, age 122.2 ± 4.2 months; 11 female, age 122.9 ± 4.1 months). Elevated branched-chain AAs (BCAAs) leucine, isoleucine, and valine are associated with impaired glucose tolerance and hyperuricemia at early stages of pediatric obesity (72). In this study, HOMA-IR positively correlated with BCAAs, phenylalanine, tryptophan, methionine, threonine, lysine, alanine, tyrosine, glutamate, proline, arginine, and ornithine. In children with obesity and decreased HOMA-IR, levels of BCAAs, aspartic acid, alanine, tyrosine, glutamate, and proline decreased, but levels of glycine and serine increased after six months of lifestyle intervention (i.e., nutrition and exercise, no medication). After intervention in children with obesity and high HOMA-IR, all AA-levels declined (72). Cosentino et al. reported that leucine (19% (p=0.015)), isoleucine (21% (p=0.024)), and valine (21% [p=0.025]) as well as aromatic AAs (AAA, i.e., phenylalanine and tyrosine) were more elevated in children with obesity compared to healthy controls. A lifestyle-intervention program only showed negligible differences in BCAA- and AAA-changes (p>0.05) (145). Goffredo et al. found that plasmatic BCAAs negatively correlated with peripheral and hepatic insulin sensitivity. Dysregulation of BCAAs in adolescents with obesity was considered a characteristic of NAFLD and, therefore, a predictor of an increase in liver fat content (69). The only study to report changes in amino acid levels in a pediatric population during an OGTT was by Trico et al. (see Table 2). The study reports BCAA levels and included 78 non-diabetic children and adolescents aged 8-18 who underwent an OGTT (1,75g/kg body weight, up to 75g). Sixteen participants underwent a second OGTT two years later. Insulin sensitivity was estimated from the OGTT using Whole-body insulin sensitivity index (WBISI). In adolescents with a lower WBISI, fasting BCAA levels had increased. During the OGTT, BCAAs showed a more blunted decline after glucose administration in individuals with a

lower WBISI (WBISI effect p<0.03 for all BCAAs). BCAA curves were not significantly different between insulin-sensitive and insulin-resistant individuals during the OGTT (146).

We found two publications on glucagon dynamics during an OGTT in a pediatric population. The study by Manell et al. investigated the plasma levels of glucagon, GLP-1, and glicentin in adolescents with obesity and T2D. In line with adult data, the authors found that adolescents with T2D and obesity had fasting hyperglucagonemia twice the level of the NGT group. Adolescents with obesity and NGT had 30% higher fasting glucagon than controls, glucagon levels increased with a decline in glucose tolerance. Fasting glucagon did not differ between NGT and IGT. During the OGTT, glucagon levels in lean adolescents decreased between minutes 5 to 10. Maximum suppression was achieved after 30 minutes. In adolescents with obesity (both NGT and IGT), glucagon levels showed an increasing trend in the first five minutes without declining below baseline in the first 30 minutes. In adolescents with obesity and T2D there was an increase in the first 15 minutes and no decline below baseline until 60 minutes after the start of the OGTT. Boxplots and fasting glucose concentrations are indicated for each subpopulation (62). The second study's objective was to determine how the hyperresponsiveness of the beta cell and the insulin resistance in youth compared to adults (both with IGT or T2D) was due to an increased glucagon release. Data was gathered from the RISE study (66 youth and 350 adults), and patients on antidiabetic drugs were excluded. The younger population was 10-19 years of age with a Tanner stage >1. Fasting glucagon and steady-state glucagon did not differ between younger and adult participants. While fasting glucose and glucagon were positively correlated in adults (r=0.133, p=0.012), they negatively correlated in the younger group (r= -0.143, p=0.251). At comparable fasting glucagon levels, the 10-19 years olds had higher C-peptide levels and a lower insulin sensitivity. During a hyperglycemic clamp (blood glucose at around 11.1. mmol/L and >25 mmol/L), glucagon suppression was similar between the pediatric and the adult collectives. In the course of the OGTT, glucagon decreased in both groups, although absolute glucagon levels were lower in the young patients at multiple time points. Notably, the decline was steeper in the younger population in the first 30 minutes, but AUC for glucagon did not differ significantly between the two groups. Following an arginine infusion, glucagon levels in this study increased in youth and adults, but the response was significantly lower in the pediatric population. Higher fasting glucagon concentrations were associated with lower insulin sensitivity for both age groups. The authors had hypothesized that hyperglucagonemia would significantly contribute to the hyperresponsiveness of beta cells and insulin resistance in youth. As glucagon concentrations were lower in youth, the authors dismissed their hypothesis of direct beta cell stimulation *via* glucagon, suggesting a greater beta cell sensitivity towards glucagon in youths (71).

4 Discussion

The LACA is an established concept and disruption of the LACA may trigger hyperglucagonemia and hyperaminoacidemia. To this day, the underlying mechanisms are incompletely understood (22). As amino acids are considered to be drivers of the LACA (i.e., they stimulate glucagon secretion and alpha cell proliferation), their dynamics during glucose administration is of interest (29–33).

In healthy adults without steatosis and with NGT, fasting amino acid levels are normal and AA levels decrease readily during OGTT. However, while results for specific AAs are partly inconclusive (e.g., alanine) (22, 64, 140), BCAAs have consistently been linked to metabolic impairment (e.g., HOMA-IR as surrogate for hepatic insulin resistance) (147). Fasting BCAA-levels are elevated in adults with obesity, NAFLD, and T2D and are also associated with cardiovascular diseases (148). In individuals with diabetes and prediabetes, BCAA catabolism is impaired and plasmatic levels decrease slower during OGTT as in individuals with normal glucose metabolism (20, 64, 67). Hence, the OGTT might be useful in demasking defects in BCAA-catabolism (e.g. T2D) (63). BCAA catabolism involves a reversible and an irreversible process. The first takes place either in the cytosol or the mitochondria and is catalyzed *via* a branched-chain aminotransferase (BCAT). The second process is catalyzed in the mitochondria by branched-chain keto-acid-dehydrogenase (BCKDH), leading to formation of substrates that enter the Krebs cycle (149–151). In individuals with diabetes, BCAT and BCKDH expression may be decreased due to genetic variants (152, 153). However, a deranged function of the two enzymes has also been related to increased levels of insulin, fatty acids, and proinflammatory mediators, linking these hallmarks of obesity-associated metabolic impairment to BCAA catabolism and the LACA (154).

While a number of publications on fasting glucagon exist, there is very limited data on its dynamics after glucose administration. In general, glucagon levels are expected to decline when blood glucose levels rise. In adults, this response has been constantly observed after i.v. glucose administration, while responses to oral glucose vary. Of note, T2D is not only attributed to beta-cell dysfunction but also alpha-cell dysfunction. In keeping with this, hyperglucagonemia was repeatedly reported during OGTT in T2D and has been attributed to an incretin effect as well as to intestinal glucagon secretion (7, 119). Additionally, endogenous glucose production is higher during OGTT than after i.v. glucose administration (7). The lack of early glucagon suppression, especially within the first 30-60 minutes, may be an early and reliable hallmark of prediabetes and IGT in adults (56, 60, 118), although contradictory results question this (53). It is of interest that increased glucagon levels upon oral glucose challenge can also be found in healthy individuals and has even been associated with a

lower risk for impaired glucose tolerance (IGT) (53). A potential explanation may be a protective effect of glucagon against acute hypoglycemia to counterbalance a strong insulin response to the glucose load and genetic variants involved in ion-channels regulating glucagon secretion (55, 56, 58, 155). Still, higher fasting glucagon and late glucagon-suppression during OGTT are consistent findings in prediabetes, T2D, obesity and the metabolic syndrome (53). The degree of obesity has been positively associated with glucagon levels, independent of insulin-resistance (25) and individuals suffering from hepatic dysfunctions display fasting hyperglucagonemia without altered glucose tolerance (156). As NAFLD has been demonstrated to be even more strongly associated with hyperglucagonemia than T2D, Wewer-Albrechtsen et al. considered the different degrees of liver fat in patients with T2D the reason for varying glucagon levels. However, the extent to which the steatotic liver is also partially resistant to the hyperglycemic actions of glucagon is unknown (22). As hyperglucagonemia is associated with fasting hyperglycemia and elevated HbA1c-levels in adults (22), the question arises if hyperglucagonemia precedes the impairment in glucose metabolism. In adults, the glucagon receptor seems to remain functional for the glucose pathway while the AA-pathway is impaired (157). Glucagon effects are mainly exerted *via* glycogenolysis *via* molecular pathways that are clearly separated from those of gluconeogenesis and ureagenesis (22). In *in vitro* studies, glucagon modulated beta cell function as well as endocrine hormonal function and parasympathetic levels (158).

Of note, dysregulated glucagon secretion is not as significant in adolescents as in adults with T2D. This speaks against a causal role of alpha cell dysfunction in the gradual progression from normal to impaired glucose metabolism and eventually T2D (159). Moreover, contrary to adults, glucagon was negatively associated with fasting glucose in a study by Stinson et al., as they investigated the influence of childhood obesity in 4012 Danish individuals (age 6-10 years). While fasting glucagon was associated with cardiometabolic risk markers (e.g., BMI SDS, body fat percentage, liver fat percentage, triglycerides, blood pressure), it was not associated with hyperglycemia. Thus, hyperglucagonemia might precede impairments in glucose regulation (160). This finding is in line with the pediatric data from the RISE study (71). Kahn et al. also reported an inverse relationship between glucagon and fasting glucose in their collective of 10-19 year old patients. In the RISE study there was no difference in glucagon suppression during OGTT between the age groups, however, arginine administration resulted in significantly lower glucagon levels in the pediatric population. In youth with IGT or newly diagnosed T2D, hypersecretion of beta cells and reduced insulin sensitivity have been described. At comparable glucagon levels as adults have, they also display higher C-peptide levels. Kahn et al. concluded that alpha cell dysfunction in youth does not explain beta cell dysfunction but suggests a greater glucagon-sensitivity in the pediatric population (71). In a pediatric population with obesity and T2D, glucagon-levels showed an early rise during OGTT (62). Manell et al. concluded that insulin or glucose were not responsible for hyperglucagonemia, but gut-derived glucagon or an altered glucagonotropic response to GIP. Additionally, a blunted GLP-1 response to affect glucagon was ruled out, as neither the response of GLP-1, nor insulin correlated with the glucagon-response. In this study, the lower GLP-1 response in adolescents with obesity, independent of glucose tolerance, corroborates previous findings from adult studies (48, 161). Pediatric studies only reported fasting concentrations of AA levels (22, 68, 69, 72). In accordance with adult data, elevated fasting BCAAs correlated with obesity and insulin resistance (22, 72) and were predictors of liver fat content (69). This indicates similarities of BCAAs in the role of metabolic diseases between pediatric and adult patients.

The limitations of this study were a relative paucity of studies on glucose and the LACA, the small number of pediatric studies, and the type of study design (i.e., mainly cross-sectional). Only one article by Dean included a single specific paragraph on the effect of glucose on the LACA (36). Most studies focused on fasting levels of glucagon and/or AAs. One challenge in studies involving the measurement of glucagon is quantifying exact plasma levels. Measurements have conventionally been performed with radioimmunoassay (RIA), deemed unreliable, especially at lower concentrations (51, 52, 60). Wagner at al. reported glucagon levels two times higher than Faerch et al. (118), both studies were performed in 2016 using different assays (53). The enzyme-linked immunosorbent assay (ELISA) may produce more accurate results (60). The main findings of this systematic review include a synopsis on what is known on the relationship of glucose and the liver-alpha cell axis. Certain aspects (e.g., inverse association between glucagon and glucose in youth vs. adults, change of AA-levels with age) hint towards potential differences between adults and children concerning LACA and pathophysiology and need further investigation (69, 71, 162, 163). Many aspects of glucagon-secretion (e.g., in hyperglycemia) are still incompletely understood (92–95). While hyperglycemia might directly attenuate alpha cell function, the consequences for the LACA remain unclear (114). Impaired glucagon response during OGTT is not only present in patients with T2D, but also in healthy individuals (58). The results on AAs in the fasting state remain partly inconclusive, but BCAAs are associated with metabolic impairments and show different dynamics in an altered LACA (63). In conclusion, the concept of the LACA is of great interest but still understudied. The LACA may provide better insight into metabolic diseases, and the dynamics of glucagon and amino acids during standardized glucose challenge tests may hold a predictive or diagnostic value.

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.

Author contributions

Conceptualization, TP, DF and DW; Methodology, DW, TP, AL and NS; Analysis, TP, DF, KG and AS; Writing—Original Draft Preparation, TP, DF, VJ and DT; Writing—Review and Editing, TP, KG, KMö, KMa, HM, EA, CDand NS; Supervision, DW; Project Administration, TP and DF. All authors discussed the results and commented on the manuscript. All authors have read and agreed to the published version of the manuscript.

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 handling editor AMG declared a past co-authorship with the authors DT and DW.

Publisher's note

References

1. Vajro P, Mandato C, Franzese A, Ciccimarra E, Lucariello S, Savoia M, et al. Vitamin e treatment in pediatric obesity-related liver disease: a randomized study. *J Pediatr Gastroenterol Nutr* (2004) 38(1):48–55. doi: 10.1097/00005176-200401000-00012

2. (NCD-RisC) NRFC. 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(10113):2627–42.

3. Welsh JA, Karpen S, Vos MB. Increasing prevalence of nonalcoholic fatty liver disease among united states adolescents, 1988-1994 to 2007-2010. *J Pediatr* (2013) 162(3):496–500.e1. doi: 10.1016/j.jpeds.2012.08.043

4. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. *PloS One* (2015) 10(10):e0140908. doi: 10.1371/journal.pone.0140908

5. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* (2006) 118 (4):1388–93. doi: 10.1542/peds.2006-1212

6. Lobstein T, Brinsden H, Neveux M, Cavalcanti OB, Barquera S, Baur L, et al. *World obesity atlas 2022*. (2022). Available at: https://www.worldobesityday.org/assets/downloads/World_Obesity_Atlas_2022_Web.pdf

7. Knop FK. EJE PRIZE 2018: A gut feeling about glucagon. *Eur J Endocrinol* (2018) 178(6):R267–R80. doi: 10.1530/EJE-18-0197

8. Janah L, Kjeldsen S, Galsgaard KD, Winther-Sørensen M, Stojanovska E, Pedersen J, et al. Glucagon receptor signaling and glucagon resistance. *Int J Mol Sci* (2019) 20(13):3314. doi: 10.3390/ijms20133314

9. Holst JJ, Wewer Albrechtsen NJ, Pedersen J, Knop FK. Glucagon and amino acids are linked in a mutual feedback cycle: The liver-α-Cell axis. *Diabetes* (2017) 66(2):235–40. doi: 10.2337/db16-0994

10. Dean ED, Li M, Prasad N, Wisniewski SN, Von Deylen A, Spaeth J, et al. Interrupted glucagon signaling reveals hepatic α cell axis and role for l-glutamine in α cell proliferation. *Cell Metab* (2017) 25(6):1362–73.e5. doi: 10.1016/j.cmet.2017.05.011

11. Boden G, Master RW, Rezvani I, Palmer JP, Lobe TE, Owen OE. Glucagon deficiency and hyperaminoacidemia after total pancreatectomy. *J Clin Invest.* (1980) 65(3):706–16. doi: 10.1172/JCI109717

12. Muller WA, Berger M, Suter P, Cüppers HJ, Reiter J, Wyss T, et al. Glucagon immunoreactivities and amino acid profile in plasma of duodenopancreatectomized patients. *J Clin Invest.* (1979) 63(5):820–7. doi: 10.1172/JCI109381

13. Winther-Sørensen M, Galsgaard KD, Santos A, Trammell SAJ, Sulek K, Kuhre RE, et al. Glucagon acutely regulates hepatic amino acid catabolism and the effect may be disturbed by steatosis. *Mol Metab* (2020) 42:101080. doi: 10.1016/j.molmet.2020.101080

14. Solloway MJ, Madjidi A, Gu C, Eastham-Anderson J, Clarke HJ, Kljavin N, et al. Glucagon couples hepatic amino acid catabolism to mTOR-dependent regulation of α-cell mass. *Cell Rep* (2015) 12(3):495–510. doi: 10.1016/j.celrep.2015.06.034

15. Suppli MP, Lund A, Bagger JI, Vilsbøll T, Knop FK. Involvement of steatosis-induced glucagon resistance in hyperglucagonaemia. *Med Hypotheses.* (2016) 86:100–3. doi: 10.1016/j.mehy.2015.10.029

16. Longuet C, Robledo AM, Dean ED, Dai C, Ali S, McGuinness I, et al. Liver-specific disruption of the murine glucagon receptor produces α-cell hyperplasia: evidence for a circulating α-cell growth factor. *Diabetes* (2013) 62(4):1196–205. doi: 10.2337/db11-1605

17. Kim J, Okamoto H, Huang Z, Anguiano G, Chen S, Liu Q, et al. Amino acid transporter Slc38a5 controls glucagon receptor inhibition-induced pancreatic α cell hyperplasia in mice. *Cell Metab* (2017) 25(6):1348–61.e8. doi: 10.1016/j.cmet.2017.05.006

18. Gromada J, Franklin I, Wollheim CB. Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. *Endocr Rev* (2007) 28(1):84–116. doi: 10.1210/er.2006-0007

19. Reaven GM, Chen YD, Golay A, Swislocki AL, Jaspan JB. Documentation of hyperglucagonemia throughout the day in nonobese and obese patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* (1987) 64 (1):106–10. doi: 10.1210/jcem-64-1-106

20. Wewer Albrechtsen NJ, Færch K, Jensen TM, Witte DR, Pedersen J, Mahendran Y, et al. Evidence of a liver-alpha cell axis in humans: hepatic insulin resistance attenuates relationship between fasting plasma glucagon and glucagonotropic amino acids. *Diabetologia* (2018) 61(3):671–80. doi: 10.1007/s00125-017-4535-5

21. Galsgaard KD. The vicious circle of hepatic glucagon resistance in non-alcoholic fatty liver disease. *J Clin Med* (2020) 9(12):4049. doi: 10.3390/jcm9124049

22. Wewer Albrechtsen NJ, Pedersen J, Galsgaard KD, Winther-Sørensen M, Suppli MP, Janah L, et al. The liver-α-Cell axis and type 2 diabetes. *Endocr Rev* (2019) 40(5):1353–66. doi: 10.1210/er.2018-00251

23. Mu J, Qureshi SA, Brady EJ, Muise ES, Candelore MR, Jiang G, et al. Anti-diabetic efficacy and impact on amino acid metabolism of GRA1, a novel small-molecule glucagon receptor antagonist. *PloS One* (2012) 7(11):e49572. doi: 10.1371/journal.pone.0049572

24. Junker AE, Gluud L, Holst JJ, Knop FK, Vilsbøll T. Diabetic and nondiabetic patients with nonalcoholic fatty liver disease have an impaired incretin effect and fasting hyperglucagonaemia. *J Intern Med* (2016) 279(5):485–93. doi: 10.1111/joim.12462

25. Ferrannini E, Muscelli E, Natali A, Gabriel R, Mitrakou A, Flyvbjerg A, et al. Association of fasting glucagon and proinsulin concentrations with insulin resistance. *Diabetologia* (2007) 50(11):2342–7. doi: 10.1007/s00125-007-0806-x

26. Hayashi Y, Seino Y. Regulation of amino acid metabolism and α-cell proliferation by glucagon. *J Diabetes Investig* (2018) 9(3):464–72. doi: 10.1111/jdi.12797

27. John AM, Schwartz RA. Glucagonoma syndrome: a review and update on treatment. *J Eur Acad Dermatol Venereol.* (2016) 30(12):2016–22. doi: 10.1111/jdv.13752

28. Galsgaard KD, Winther-Sørensen M, Ørskov C, Kissow H, Poulsen SS, Vilstrup H, et al. Disruption of glucagon receptor signaling causes hyperaminoacidemia exposing a possible liver-alpha-cell axis. *Am J Physiol Endocrinol Metab* (2018) 314(1):E93–E103. doi: 10.1152/ajpendo.00198.2017

29. Assan R, Attali JR, Ballerio G, Boillot J, Girard JR. Glucagon secretion induced by natural and artificial amino acids in the perfused rat pancreas. *Diabetes* (1977) 26(4):300–7. doi: 10.2337/diab.26.4.300

30. Kuhara T, Ikeda S, Ohneda A, Sasaki Y. Effects of intravenous infusion of 17 amino acids on the secretion of GH, glucagon, and insulin in sheep. *Am J Physiol* (1991) 260(1 Pt 1):E21–6. doi: 10.1152/ajpendo.1991.260.1.E21

31. Pipeleers DG, Schuit FC, Van Schravendijk CF, Van de Winkel M. Interplay of nutrients and hormones in the regulation of glucagon release. *Endocrinology* (1985) 117(3):817–23. doi: 10.1210/endo-117-3-817

32. Rocha DM, Faloona GR, Unger RH. Glucagon-stimulating activity of 20 amino acids in dogs. *J Clin Invest.* (1972) 51(9):2346–51. doi: 10.1172/JCI107046

33. Unger RH, Ohneda A, Aguilar-Parada E, Eisentraut AM. The role of aminogenic glucagon secretion in blood glucose homeostasis. *J Clin Invest.* (1969) 48(5):810–22. doi: 10.1172/JCI106039

34. Wendt A, Eliasson L. Pancreatic α-cells - the unsung heroes in islet function. *Semin Cell Dev Biol* (2020) 103:41–50. doi: 10.1016/j.semcdb.2020.01.006

35. Ahrén B. Autonomic regulation of islet hormone secretion–implications for health and disease. *Diabetologia* (2000) 43(4):393–410. doi: 10.1007/s001250051322

36. Dean ED. A primary role for α-cells as amino acid sensors. *Diabetes* (2020) 69(4):542–9. doi: 10.2337/dbi19-0021

37. Briant LJB, Dodd MS, Chibalina MV, Rorsman NJG, Johnson PRV, Carmeliet P, et al. CPT1a-dependent long-chain fatty acid oxidation contributes to maintaining glucagon secretion from pancreatic islets. *Cell Rep* (2018) 23 (11):3300–11. doi: 10.1016/j.celrep.2018.05.035

38. Walker JN, Ramracheya R, Zhang Q, Johnson PR, Braun M, Rorsman P. Regulation of glucagon secretion by glucose: paracrine, intrinsic or both? *Diabetes Obes Metab* (2011) 13 Suppl 1:95–105. doi: 10.1111/j.1463-1326.2011.01450.x

39. Zeigerer A, Sekar R, Kleinert M, Nason S, Habegger KM, Müller TD. Glucagon's metabolic action in health and disease. *Compr Physiol* (2021) 11 (2):1759–83. doi: 10.1002/cphy.c200013

40. Ellenbroek JH, Töns HAM, Hanegraaf MAJ, Rabelink TJ, Engelse MA, Carlotti F, et al. Pancreatic α-cell mass in obesity. *Diabetes Obes Metab* (2017) 19 (12):1810–3. doi: 10.1111/dom.12997

41. Pedersen JS, Rygg MO, Kristiansen VB, Olsen BH, Serizawa RR, Holst JJ, et al. Nonalcoholic fatty liver disease impairs the liver-alpha cell axis independent of hepatic inflammation and fibrosis. *Hepatol Commun* (2020) 4(11):1610–23. doi: 10.1002/hep4.1562

42. Gar C, Haschka SJ, Kern-Matschilles S, Rauch B, Sacco V, Prehn C, et al. The liver-alpha cell axis associates with liver fat and insulin resistance: a validation study in women with non-steatotic liver fat levels. *Diabetologia* (2021) 64(3):512–20. doi: 10.1007/s00125-020-05334-x

43. Ramnanan CJ, Edgerton DS, Kraft G, Cherrington AD. Physiologic action of glucagon on liver glucose metabolism. *Diabetes Obes Metab* (2011) 13 Suppl 1:118–25. doi: 10.1111/j.1463-1326.2011.01454.x

44. Mitrakou A, Kelley D, Veneman T, Jenssen T, Pangburn T, Reilly J, et al. Contribution of abnormal muscle and liver glucose metabolism to postprandial hyperglycemia in NIDDM. *Diabetes* (1990) 39(11):1381–90. doi: 10.2337/diab.39.11.1381

45. Knop FK, Aaboe K, Vilsbøll T, Vølund A, Holst JJ, Krarup T, et al. Impaired incretin effect and fasting hyperglucagonaemia characterizing type 2 diabetic subjects are early signs of dysmetabolism in obesity. *Diabetes Obes Metab* (2012) 14(6):500–10. doi: 10.1111/j.1463-1326.2011.01549.x

46. Færch K, Pilgaard K, Knop FK, Hansen T, Pedersen O, Jørgensen T, et al. Incretin and pancreatic hormone secretion in Caucasian non-diabetic carriers of the TCF7L2 rs7903146 risk T allele. *Diabetes Obes Metab* (2013) 15(1):91–5. doi: 10.1111/j.1463-1326.2012.01675.x

47. Mitrakou A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J, et al. Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med* (1992) 326(1):22–9. doi: 10.1056/NEJM199201023260104

48. Wewer Albrechtsen NJ. Glucagon receptor signaling in metabolic diseases. *Peptides* (2018) 100:42–7. doi: 10.1016/j.peptides.2017.11.016

49. Holst JJ, Holland W, Gromada J, Lee Y, Unger RH, Yan H, et al. Insulin and glucagon: Partners for life. *Endocrinology* (2017) 158(4):696–701. doi: 10.1210/en.2016-1748

50. Kazda CM, Ding Y, Kelly RP, Garhyan P, Shi C, Lim CN, et al. Evaluation of efficacy and safety of the glucagon receptor antagonist LY2409021 in patients with type 2 diabetes: 12- and 24-week phase 2 studies. *Diabetes Care* (2016) 39(7):1241–9. doi: 10.2337/dc15-1643

51. Wewer Albrechtsen NJ, Hartmann B, Veedfald S, Windeløv JA, Plamboeck A, Bojsen-Møller KN, et al. Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? *Diabetologia* (2014) 57 (9):1919–26. doi: 10.1007/s00125-014-3283-z

52. Bak MJ, Albrechtsen NW, Pedersen J, Hartmann B, Christensen M, Vilsbøll T, et al. Specificity and sensitivity of commercially available assays for glucagon and oxyntomodulin measurement in humans. *Eur J Endocrinol* (2014) 170(4):529–38. doi: 10.1530/EJE-13-0941

53. Wagner R, Hakaste LH, Ahlqvist E, Heni M, Machann J, Schick F, et al. Nonsuppressed glucagon after glucose challenge as a potential predictor for glucose tolerance. *Diabetes* (2017) 66(5):1373–9. doi: 10.2337/db16-0354

54. Junker AE. The role of incretin hormones and glucagon in patients with liver disease. *Dan Med J* (2017) 64(5):B5363.

55. Wang C, Xiao Y, Wang J, Hou N, Cui W, Hu X, et al. Dynamic changes in insulin and glucagon during disease progression in rhesus monkeys with obesity-related type 2 diabetes mellitus. *Diabetes Obes Metab* (2019) 21(5):1111–20. doi: 10.1111/dom.13624

56. Koopman ADM, Beulens JW, van der Heijden A, Elders P, Dekker JM, Alssema M, et al. A prospective study on glucagon responses to oral glucose and mixed meal and 7-year change in fasting glucose. *Clin Endocrinol (Oxf).* (2019) 91 (1):82–6. doi: 10.1111/cen.13977

57. Engelbrechtsen L, Mahendran Y, Jonsson A, Gjesing AP, Weeke PE, Jørgensen ME, et al. Common variants in the hERG (KCNH2) voltage-gated potassium channel are associated with altered fasting and glucose-stimulated plasma incretin and glucagon responses. *BMC Genet* (2018) 19(1):15. doi: 10.1186/s12863-018-0602-2

58. Gar C, Rottenkolber M, Sacco V, Moschko S, Banning F, Hesse N, et al. Patterns of plasma glucagon dynamics do not match metabolic phenotypes in young women. *J Clin Endocrinol Metab* (2018) 103(3):972–82. doi: 10.1210/jc.2017-02014

59. Kosuda M, Watanabe K, Koike M, Morikawa A, Saito H, Kohno G, et al. Glucagon response to glucose challenge in patients with idiopathic postprandial syndrome. *J Nippon Med Sch.* (2022) 89(1):102–7. doi: 10.1272/jnms.JNMS.2022_89-205

60. Ichikawa R, Takano K, Fujimoto K, Motomiya T, Kobayashi M, Kitamura T, et al. Basal glucagon hypersecretion and response to oral glucose load in prediabetes and mild type 2 diabetes. *Endocr J* (2019) 66(8):663–75. doi: 10.1507/endocrj.EJ18-0372

61. Jonsson A, Stinson SE, Torekov SS, Clausen TD, Færch K, Kelstrup L, et al. Genome-wide association study of circulating levels of glucagon during an oral glucose tolerance test. *BMC Med Genomics* (2021) 14(1):3. doi: 10.1186/s12920-020-00841-7

62. Manell H, Staaf J, Manukyan L, Kristinsson H, Cen J, Stenlid R, et al. Altered plasma levels of glucagon, GLP-1 and glicentin during OGTT in adolescents with obesity and type 2 diabetes. *J Clin Endocrinol Metab* (2016) 101(3):1181–9. doi: 10.1210/jc.2015-3885

63. Sjögren RJO, Rizo-Roca D, Chibalin AV, Chorell E, Furrer R, Katayama S, et al. Branched-chain amino acid metabolism is regulated by ERRα in primary human myotubes and is further impaired by glucose loading in type 2 diabetes. *Diabetologia* (2021) 64(9):2077–91. doi: 10.1007/s00125-021-05481-9

64. Wang Q, Jokelainen J, Auvinen J, Puukka K, Keinänen-Kiukaanniemi S, Järvelin MR, et al. Insulin resistance and systemic metabolic changes in oral glucose tolerance test in 5340 individuals: an interventional study. *BMC Med* (2019) 17 (1):217. doi: 10.1186/s12916-019-1440-4

65. Li Q, Gu W, Ma X, Liu Y, Jiang L, Feng R, et al. Amino acid and biogenic amine profile deviations in an oral glucose tolerance test: A comparison between healthy and hyperlipidaemia individuals based on targeted metabolomics. *Nutrients* (2016) 8(6):379. doi: 10.3390/nu8060379

66. Geidenstam N, Danielsson AP, Spégel P, Ridderstråle M. Changes in glucose-elicited blood metabolite responses following weight loss and long term weight maintenance in obese individuals with impaired glucose tolerance. *Diabetes Res Clin Pract* (2016) 113:187–97. doi: 10.1016/j.diabres.2015.12.024

67. Liu L, Feng R, Guo F, Li Y, Jiao J, Sun C. Targeted metabolomic analysis reveals the association between the postprandial change in palmitic acid, branched-chain amino acids and insulin resistance in young obese subjects. *Diabetes Res Clin Pract* (2015) 108(1):84–93. doi: 10.1016/j.diabres.2015.01.014

68. Tricò D, Prinsen H, Giannini C, de Graaf R, Juchem C, Li F, et al. Elevated α-hydroxybutyrate and branched-chain amino acid levels predict deterioration of

glycemic control in adolescents. *J Clin Endocrinol Metab* (2017) 102(7):2473–81. doi: 10.1210/jc.2017-00475

69. Goffredo M, Santoro N, Tricò D, Giannini C, D'Adamo E, Zhao H, et al. A branched-chain amino acid-related metabolic signature characterizes obese adolescents with non-alcoholic fatty liver disease. *Nutrients* (2017) 9(7):642. doi: 10.3390/nu9070642

70. Giannini C, Pietropaoli N, Polidori N, Chiarelli F, Marcovecchio ML, Mohn A. Increased GLP-1 response to oral glucose in pre-pubertal obese children. *J Pediatr Endocrinol Metab* (2016) 29(8):901–6. doi: 10.1515/jpem-2016-0050

71. Kahn SE, Mather KJ, Arslanian SA, Barengolts E, Buchanan TA, Caprio S, et al. Hyperglucagonemia does not explain the β-cell hyperresponsiveness and insulin resistance in dysglycemic youth compared with adults: Lessons from the RISE study. *Diabetes Care* (2021) 44(9):1961–9. doi: 10.2337/dc21-0460

72. Suzuki Y, Kido J, Matsumoto S, Shimizu K, Nakamura K. Associations among amino acid, lipid, and glucose metabolic profiles in childhood obesity. *BMC Pediatr* (2019) 19(1):273. doi: 10.1186/s12887-019-1647-8

73. Christensen M, Vedtofte L, Holst JJ, Vilsbøll T, Knop FK. Glucose-dependent insulinotropic polypeptide: a bifunctional glucose-dependent regulator of glucagon and insulin secretion in humans. *Diabetes* (2011) 60 (12):3103–9. doi: 10.2337/db11-0979

74. Jiang G, Zhang BB. Glucagon and regulation of glucose metabolism. *Am J Physiol Endocrinol Metab* (2003) 284(4):E671–8. doi: 10.1152/ajpendo.00492.2002

75. Müller TD, Finan B, Clemmensen C, DiMarchi RD, Tschöp MH. The new biology and pharmacology of glucagon. *Physiol Rev* (2017) 97(2):721–66. doi: 10.1152/physrev.00025.2016

76. Heimberg H, De Vos A, Pipeleers D, Thorens B, Schuit F. Differences in glucose transporter gene expression between rat pancreatic alpha- and beta-cells are correlated to differences in glucose transport but not in glucose utilization. *J Biol Chem* (1995) 270(15):8971–5. doi: 10.1074/jbc.270.15.8971

77. Yasuda K, Yamada Y, Inagaki N, Yano H, Okamoto Y, Tsuji K, et al. Expression of GLUT1 and GLUT2 glucose transporter isoforms in rat islets of langerhans and their regulation by glucose. *Diabetes* (1992) 41(1):76–81. doi: 10.2337/diab.41.1.76

78. Quesada I, Tudurí E, Ripoll C, Nadal A. Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes. *J Endocrinol* (2008) 199(1):5–19. doi: 10.1677/JOE-08-0290

79. Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. *Nat Rev Endocrinol* (2010) 6 (12):689–97. doi: 10.1038/nrendo.2010.187

80. Bankir L, Bouby N, Speth RC, Velho G, Crambert G. Glucagon revisited: Coordinated actions on the liver and kidney. *Diabetes Res Clin Pract* (2018) 146:119–29. doi: 10.1016/j.diabres.2018.10.004

81. Vieira E, Liu YJ, Gylfe E. Involvement of alpha1 and beta-adrenoceptors in adrenaline stimulation of the glucagon-secreting mouse alpha-cell. *Naunyn Schmiedebergs Arch Pharmacol* (2004) 369(2):179–83. doi: 10.1007/s00210-003-0858-5

82. De Marinis YZ, Salehi A, Ward CE, Zhang Q, Abdulkader F, Bengtsson M, et al. GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of n- and l-type Ca2+ channel-dependent exocytosis. *Cell Metab* (2010) 11(6):543–53. doi: 10.1016/j.cmet.2010.04.007

83. Hamilton A, Zhang Q, Salehi A, Willems M, Knudsen JG, Ringgaard AK, et al. Adrenaline stimulates glucagon secretion by Tpc2-dependent Ca. *Diabetes* (2018) 67(6):1128–39. doi: 10.2337/db17-1102

84. Hevener AL, Bergman RN, Donovan CM. Novel glucosensor for hypoglycemic detection localized to the portal vein. *Diabetes* (1997) 46(9):1521–5. doi: 10.2337/diab.46.9.1521

85. Battezzati A, Simonson DC, Luzi L, Matthews DE. Glucagon increases glutamine uptake without affecting glutamine release in humans. *Metabolism* (1998) 47(6):713–23. doi: 10.1016/S0026-0495(98)90036-1

86. Brand CL, Jørgensen PN, Svendsen I, Holst JJ. Evidence for a major role for glucagon in regulation of plasma glucose in conscious, nondiabetic, and alloxan-induced diabetic rabbits. *Diabetes* (1996) 45(8):1076–83. doi: 10.2337/diab.45.8.1076

87. Brand CL, Jørgensen PN, Knigge U, Warberg J, Svendsen I, Kristensen JS, et al. Role of glucagon in maintenance of euglycemia in fed and fasted rats. *Am J Physiol* (1995) 269(3 Pt 1):E469–77. doi: 10.1152/ajpendo.1995.269.3.E469

88. Brand CL, Rolin B, Jørgensen PN, Svendsen I, Kristensen JS, Holst JJ. Immunoneutralization of endogenous glucagon with monoclonal glucagon antibody normalizes hyperglycaemia in moderately streptozotocin-diabetic rats. *Diabetologia* (1994) 37(10):985–93. doi: 10.1007/BF00400461

89. Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, et al. Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. *Proc Natl Acad Sci U S A.* (2003) 100 (3):1438–43. doi: 10.1073/pnas.0237106100

90. Hojlund K, Wildner-Christensen M, Eshøj O, Skjaerbaek C, Holst JJ, Koldkjaer O, et al. Reference intervals for glucose, beta-cell polypeptides, and counterregulatory factors during prolonged fasting. *Am J Physiol Endocrinol Metab* (2001) 280(1):E50–8. doi: 10.1152/ajpendo.2001.280.1.E50

91. Felig P. The glucose-alanine cycle. *Metabolism* (1973) 22(2):179–207. doi: 10.1016/0026-0495(73)90269-2

92. Göpel SO, Kanno T, Barg S, Weng XG, Gromada J, Rorsman P. Regulation of glucagon release in mouse -cells by KATP channels and inactivation of TTX-sensitive na+ channels. *J Physiol* (2000) 528(Pt 3):509–20. doi: 10.1111/j.1469-7793.2000.00509.x

93. Ishihara H, Maechler P, Gjinovci A, Herrera PL, Wollheim CB. Islet beta-cell secretion determines glucagon release from neighbouring alpha-cells. *Nat Cell Biol* (2003) 5(4):330–5. doi: 10.1038/ncb951

94. Franklin I, Gromada J, Gjinovci A, Theander S, Wollheim CB. Beta-cell secretory products activate alpha-cell ATP-dependent potassium channels to inhibit glucagon release. *Diabetes* (2005) 54(6):1808–15. doi: 10.2337/diabetes.54.6.1808

95. Olsen HL, Theander S, Bokvist K, Buschard K, Wollheim CB, Gromada J. Glucose stimulates glucagon release in single rat alpha-cells by mechanisms that mirror the stimulus-secretion coupling in beta-cells. *Endocrinology* (2005) 146 (11):4861–70. doi: 10.1210/en.2005-0800

96. Elliott AD, Ustione A, Piston DW. Somatostatin and insulin mediate glucose-inhibited glucagon secretion in the pancreatic α-cell by lowering cAMP. *Am J Physiol Endocrinol Metab* (2015) 308(2):E130–43. doi: 10.1152/ajpendo.00344.2014

97. Yu Q, Shuai H, Ahooghalandari P, Gylfe E, Tengholm A. Glucose controls glucagon secretion by directly modulating cAMP in alpha cells. *Diabetologia* (2019) 62(7):1212–24. doi: 10.1007/s00125-019-4857-6

98. Tengholm A, Gylfe E. cAMP signalling in insulin and glucagon secretion. *Diabetes Obes Metab* (2017) 19 Suppl 1:42–53. doi: 10.1111/dom.12993

99. Hughes JW, Ustione A, Lavagnino Z, Piston DW. Regulation of islet glucagon secretion: Beyond calcium. *Diabetes Obes Metab* (2018) 20 Suppl 2:127–36. doi: 10.1111/dom.13381

100. Ramracheya R, Ward C, Shigeto M, Walker JN, Amisten S, Zhang Q, et al. Membrane potential-dependent inactivation of voltage-gated ion channels in alpha-cells inhibits glucagon secretion from human islets. *Diabetes* (2010) 59 (9):2198–208. doi: 10.2337/db09-1505

101. Gerich JE, Langlois M, Noacco C, Karam JH, Forsham PH. Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. *Science* (1973) 182(4108):171–3. doi: 10.1126/science.182.4108.171

102. Bolli GB, Gerich JE. The "dawn phenomenon"–a common occurrence in both non-insulin-dependent and insulin-dependent diabetes mellitus. *N Engl J Med* (1984) 310(12):746–50. doi: 10.1056/NEJM198403223101203

103. Biggers DW, Myers SR, Neal D, Stinson R, Cooper NB, Jaspan JB, et al. Role of brain in counterregulation of insulin-induced hypoglycemia in dogs. *Diabetes* (1989) 38(1):7–16. doi: 10.2337/diab.38.1.7

104. Bolli G, De Feo P, Perriello G, De Cosmo S, Compagnucci P, Santeusanio F, et al. Mechanisms of glucagon secretion during insulin-induced hypoglycemia in man. role of the beta cell and arterial hyperinsulinemia. *J Clin Invest.* (1984) 73 (4):917–22. doi: 10.1172/JCI111315

105. Lefebvre PJ, Luyckx AS. Glucagon and diabetes: a reappraisal. *Diabetologia* (1979) 16(6):347–54. doi: 10.1007/BF01223153

106. Almdal TP. Importance of glucagon for nitrogen loss in diabetes–via an accelerated hepatic conversion of amino nitrogen to urea nitrogen. *Dan Med Bull* (1991) 38(2):113–20.

107. Li XC, Liao TD, Zhuo JL. Long-term hyperglucagonaemia induces early metabolic and renal phenotypes of type 2 diabetes in mice. *Clin Sci (Lond).* (2008) 114(9):591–601. doi: 10.1042/CS20070257

108. Gosmain Y, Masson MH, Philippe J. Glucagon: the renewal of an old hormone in the pathophysiology of diabetes. *J Diabetes* (2013) 5(2):102–9. doi: 10.1111/1753-0407.12022

109. Lefèbvre PJ, Paquot N, Scheen AJ. Inhibiting or antagonizing glucagon: making progress in diabetes care. *Diabetes Obes Metab* (2015) 17(8):720–5. doi: 10.1111/dom.12480

110. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J Clin Invest.* (2012) 122(1):4–12. doi: 10.1172/JCI60016

111. Shen DM, Lin S, Parmee ER. A survey of small molecule glucagon receptor antagonists from recent patents (2006 - 2010). *Expert Opin Ther Pat.* (2011) 21 (8):1211–40. doi: 10.1517/13543776.2011.587001

112. Scheen AJ, Paquot N, Lefèbvre PJ. Investigational glucagon receptor antagonists in phase I and II clinical trials for diabetes. *Expert Opin Investig Drugs* (2017) 26(12):1373–89. doi: 10.1080/13543784.2017.1395020

113. Junker AE, Gluud LL, van Hall G, Holst JJ, Knop FK, Vilsbøll T. Effects of glucagon-like peptide-1 on glucagon secretion in patients with non-alcoholic fatty liver disease. *J Hepatol* (2016) 64(4):908–15. doi: 10.1016/j.jhep.2015.11.014

114. Knudsen JG, Hamilton A, Ramracheya R, Tarasov AI, Brereton M, Haythorne E, et al. Dysregulation of glucagon secretion by hyperglycemia-induced sodium-dependent reduction of ATP production. *Cell Metab* (2019) 29 (2):430–42.e4. doi: 10.1016/j.cmet.2018.10.003

115. Marchesini G, Forlani G, Zoli M, Angiolini A, Scolari MP, Bianchi FB, et al. Insulin and glucagon levels in liver cirrhosis. relationship with plasma amino acid imbalance of chronic hepatic encephalopathy. *Dig. Dis Sci* (1979) 24(8):594–601. doi: 10.1007/BF01333703

116. Holst JJ, Burcharth F, Kühl C. Pancreatic glucoregulatory hormones in cirrhosis of the liver: portal vein concentrations during intravenous glucose tolerance test and in response to a meal. *Diabete Metab* (1980) 6(2):117–27.

117. Bugianesi E, Kalhan S, Burkett E, Marchesini G, McCullough A. Quantification of gluconeogenesis in cirrhosis: response to glucagon. *Gastroenterology* (1998) 115(6):1530–40. doi: 10.1016/S0016-5085(98)70033-2

118. McGarry JD, Foster DW. Regulation of hepatic fatty acid oxidation and ketone body production. *Annu Rev Biochem* (1980) 49:395–420. doi: 10.1146/annurev.bi.49.070180.002143

119. Knop FK, Vilsbøll T, Madsbad S, Holst JJ, Krarup T. Inappropriate suppression of glucagon during OGTT but not during isoglycaemic i.v. glucose infusion contributes to the reduced incretin effect in type 2 diabetes mellitus. *Diabetologia* (2007) 50(4):797–805. doi: 10.1007/s00125-006-0566-z

120. Knop FK, Vilsbøll T, Højberg PV, Larsen S, Madsbad S, Vølund A, et al. Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? *Diabetes* (2007) 56(8):1951–9. doi: 10.2337/db07-0100

121. Muscelli E, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, et al. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. *Diabetes* (2008) 57(5):1340–8. doi: 10.2337/db07-1315

122. Oh TJ, Kim MY, Shin JY, Lee JC, Kim S, Park KS, et al. The incretin effect in Korean subjects with normal glucose tolerance or type 2 diabetes. *Clin Endocrinol (Oxf).* (2014) 80(2):221–7. doi: 10.1111/cen.12167

123. Kilberg MS, Barber EF, Handlogten ME. Characteristics and hormonal regulation of amino acid transport system a in isolated rat hepatocytes. *Curr Top Cell Regul* (1985) 25:133–63. doi: 10.1016/B978-0-12-152825-6.50009-6

124. Lim SK, Cynober L, De Bandt JP, Aussel C. A na(+)-dependent system a and ASC-independent amino acid transport system stimulated by glucagon in rat hepatocytes. *Cell Biol Int* (1999) 23(1):7–12. doi: 10.1006/cbir.1998.0321

125. Lacey JH, Bradford NM, Joseph SK, McGivan JD. Increased activity of phosphate-dependent glutaminase in liver mitochondria as a result of glucagon treatment of rats. *Biochem J* (1981) 194(1):29–33. doi: 10.1042/bj1940029

126. Vilstrup H, Hansen BA, Almdal TP. Glucagon increases hepatic efficacy for urea synthesis. *J Hepatol* (1990) 10(1):46–50. doi: 10.1016/0168-8278(90)90072-Y

127. Hamberg O, Vilstrup H. Regulation of urea synthesis by glucose and glucagon in normal man. *Clin Nutr* (1994) 13(3):183–91. doi: 10.1016/0261-5614(94)90099-X

128. Petersen KF, Hansen BA, Vilstrup H. Time dependent stimulating effect of glucagon on the capacity of urea-n synthesis in rats. *Horm Metab Res* (1987) 19 (2):53–6. doi: 10.1055/s-2007-1011737

129. Almdal TP, Holst JJ, Heindorff H, Vilstrup H. Glucagon immunoneutralization in diabetic rats normalizes urea synthesis and decreases nitrogen wasting. *Diabetes* (1992) 41(1):12–6. doi: 10.2337/diab.41.1.12

130. Hamberg O, Vilstrup H. Effects of insulin and glucose on urea synthesis in normal man, independent of pancreatic hormone secretion. *J Hepatol* (1994) 21 (3):381–7. doi: 10.1016/S0168-8278(05)80317-4

131. Kraft G, Coate KC, Winnick JJ, Dardevet D, Donahue EP, Cherrington AD, et al. Glucagon's effect on liver protein metabolism in vivo. *Am J Physiol Endocrinol Metab* (2017) 313(3):E263–E72. doi: 10.1152/ajpendo.00045.2017

132. Galsgaard KD, Winther-Sørensen M, Pedersen J, Kjeldsen SAS, Rosenkilde MM, Wewer Albrechtsen NJ, et al. Glucose and amino acid metabolism in mice depend mutually on glucagon and insulin receptor signaling. *Am J Physiol Endocrinol Metab* (2019) 316(4):E660–E73. doi: 10.1152/ajpendo.00410.2018

133. Charlton MR, Adey DB, Nair KS. Evidence for a catabolic role of glucagon during an amino acid load. *J Clin Invest.* (1996) 98(1):90–9. doi: 10.1172/JCI118782

134. Otten J, Stomby A, Waling M, Chorell E, Ryberg M, Svensson M, et al. The liver-alpha-cell axis after a mixed meal and during weight loss in type 2 diabetes. *Endocr Connect.* (2021) 10(9):1101–10. doi: 10.1530/EC-21-0171

135. Müller WA, Faloona GR, Unger RH. The effect of alanine on glucagon secretion. *J Clin Invest.* (1971) 50(10):2215–8. doi: 10.1172/JCI106716

136. Galsgaard KD, Jepsen SL, Kjeldsen SAS, Pedersen J, Wewer Albrechtsen NJ, Holst JJ. Alanine, arginine, cysteine, and proline, but not glutamine, are substrates for, and acute mediators of, the liver-α-cell axis in female mice. *Am J Physiol Endocrinol Metab* (2020) 318(6):E920–E9. doi: 10.1152/ajpendo.00459.2019

137. Stancáková A, Civelek M, Saleem NK, Soininen P, Kangas AJ, Cederberg H, et al. Hyperglycemia and a common variant of GCKR are associated with the levels of eight amino acids in 9,369 Finnish men. *Diabetes* (2012) 61(7):1895–902. doi: 10.2337/db11-1378

138. Gonzalez-Franquesa A, Burkart AM, Isganaitis E, Patti ME. What have metabolomics approaches taught us about type 2 diabetes? *Curr Diabetes Rep* (2016) 16(8):74. doi: 10.1007/s11892-016-0763-1

139. Eisenstein AB, Strack I. Amino acid stimulation of glucagon secretion by perifused islets of high-protein-fed rats. *Diabetes* (1978) 27(4):370–6. doi: 10.2337/diab.27.4.370

140. Gaggini M, Carli F, Rosso C, Buzzigoli E, Marietti M, Della Latta V, et al. Altered amino acid concentrations in NAFLD: Impact of obesity and insulin resistance. *Hepatology* (2018) 67(1):145–58. doi: 10.1002/hep.29465

141. Wewer Albrechtsen NJ, Junker AE, Christensen M, Hædersdal S, Wibrand F, Lund AM, et al. Hyperglucagonemia correlates with plasma levels of non-branched-chain amino acids in patients with liver disease independent of type 2 diabetes. *Am J Physiol Gastrointest. Liver Physiol* (2018) 314(1):G91–G6. doi: 10.1152/ajpgi.00216.2017

142. Thiessen SE, Derde S, Derese I, Dufour T, Vega CA, Langouche L, et al. Role of glucagon in catabolism and muscle wasting of critical illness and modulation by nutrition. *Am J Respir Crit Care Med* (2017) 196(9):1131–43. doi: 10.1164/rccm.201702-0354OC

143. Kelly RA, Fitches MJ, Webb SD, Pop SR, Chidlow SJ. Modelling the effects of glucagon during glucose tolerance testing. *Theor Biol Med Model* (2019) 16 (1):21. doi: 10.1186/s12976-019-0115-3

144. Lund A, Bagger JI, Wewer Albrechtsen NJ, Christensen M, Grøndahl M, Hartmann B, et al. Evidence of extrapancreatic glucagon secretion in man. *Diabetes* (2016) 65(3):585–97. doi: 10.2337/db15-1541

145. Cosentino RG, Churilla JR, Josephson S, Molle-Rios Z, Hossain MJ, Prado WL, et al. Branched-chain amino acids and relationship with inflammation in youth with obesity: A randomized controlled intervention study. *J Clin Endocrinol Metab* (2021) 106(11):3129–39. doi: 10.1210/clinem/dgab538

146. Tricò D, Caprio S, Rosaria Umano G, Pierpont B, Nouws J, Galderisi A, et al. Metabolic features of nonalcoholic fatty liver (NAFL) in obese adolescents: Findings from a multiethnic cohort. *Hepatology* (2018) 68(4):1376–90. doi: 10.1002/hep.30035

147. Mahendran Y, Jonsson A, Have CT, Allin KH, Witte DR, Jørgensen ME, et al. Genetic evidence of a causal effect of insulin resistance on branched-chain amino acid levels. *Diabetologia* (2017) 60(5):873–8. doi: 10.1007/s00125-017-4222-6

148. Yang R, Dong J, Zhao H, Li H, Guo H, Wang S, et al. Association of branched-chain amino acids with carotid intima-media thickness and coronary artery disease risk factors. *PloS One* (2014) 9(6):e99598. doi: 10.1371/journal.pone.0099598

149. Adeva-Andany MM, López-Maside L, Donapetry-García C, Fernández-Fernández C, Sixto-Leal C. Enzymes involved in branched-chain amino acid metabolism in humans. *Amino Acids* (2017) 49(6):1005–28. doi: 10.1007/s00726-017-2412-7

150. Bloomgarden Z. Diabetes and branched-chain amino acids: What is the link? *J Diabetes* (2018) 10(5):350–2. doi: 10.1111/1753-0407.12645

151. Zhang S, Zeng X, Ren M, Mao X, Qiao S. Novel metabolic and physiological functions of branched chain amino acids: a review. *J Anim Sci Biotechnol* (2017) 8:10. doi: 10.1186/s40104-016-0139-z

152. Gar C, Rottenkolber M, Prehn C, Adamski J, Seissler J, Lechner A. Serum and plasma amino acids as markers of prediabetes, insulin resistance, and incident diabetes. *Crit Rev Clin Lab Sci* (2018) 55(1):21–32. doi: 10.1080/10408363.2017.1414143

153. Hernández-Alvarez MI, Díaz-Ramos A, Berdasco M, Cobb J, Planet E, Cooper D, et al. Early-onset and classical forms of type 2 diabetes show impaired expression of genes involved in muscle branched-chain amino acids metabolism. *Sci Rep* (2017) 7(1):13850. doi: 10.1038/s41598-017-14120-6

154. Wang Q, Holmes MV, Davey Smith G, Ala-Korpela M. Genetic support for a causal role of insulin resistance on circulating branched-chain amino acids and inflammation. *Diabetes Care* (2017) 40(12):1779–86. doi: 10.2337/dc17-1642

155. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PloS Med* (2009) 6(7):e1000097. doi: 10.1371/journal.pmed.1000097

156. Raddatz D, Rossbach C, Buchwald A, Scholz KH, Ramadori G, Nolte W. Fasting hyperglucagonemia in patients with transjugular intrahepatic portosystemic shunts (TIPS). *Exp Clin Endocrinol Diabetes* (2005) 113(5):268–74. doi: 10.1055/s-2005-837546

157. Suppli MP, Bagger JI, Lund A, Demant M, van Hall G, Strandberg C, et al. Glucagon resistance at the level of amino acid turnover in obese subjects with hepatic steatosis. *Diabetes* (2020) 69(6):1090–9. doi: 10.2337/db19-0715

158. Huising MO. Paracrine regulation of insulin secretion. *Diabetologia* (2020) 63(10):2057–63. doi: 10.1007/s00125-020-05213-5

159. Elder DA, Prigeon RL, Wadwa RP, Dolan LM, D'Alessio DA. Beta-cell function, insulin sensitivity, and glucose tolerance in obese diabetic and nondiabetic adolescents and young adults. *J Clin Endocrinol Metab* (2006) 91 (1):185–91. doi: 10.1210/jc.2005-0853

160. Stinson SE, Jonsson AE, de Retana Alzola IF, Lund MAV, Frithioff-Bøjsøe C, Aas Holm L, et al. Hyperglucagonemia in pediatric adiposity associates with cardiometabolic risk factors but not hyperglycemia. *J Clin Endocrinol Metab* (2022) 107(6):1569–76. doi: 10.1210/clinem/dgac108

161. Færch K, Torekov SS, Vistisen D, Johansen NB, Witte DR, Jonsson A, et al. GLP-1 response to oral glucose is reduced in prediabetes, screen-detected type 2 diabetes, and obesity and influenced by sex: The ADDITION-PRO study. *Diabetes* (2015) 64(7):2513–25. doi: 10.2337/db14-1751

162. Lomenick JP, White JR, Smart EJ, Clasey JL, Anderson JW. Glucagon-like peptide 1 and pancreatic polypeptide responses to feeding in normal weight and overweight children. *J Pediatr Endocrinol Metab* (2009) 22(6):493–500. doi: 10.1515/JPEM.2009.22.6.493

163. Yeow TP, Pacini G, Tura A, Hor CP, Lim SL, Tan FH, et al. Preserved glucagon-like peptide-1 responses to oral glucose, but reduced incretin effect, insulin secretion and sensitivity in young asians with type 2 diabetes mellitus. *BMJ Open Diabetes Res Care* (2017) 5(1):e000352. doi: 10.1136/bmjdrc-2016-000352

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 30 January 2023
DOI 10.3389/fendo.2023.1108618

OPEN ACCESS

EDITED BY
Dénes Molnár,
University of Pécs, Hungary

REVIEWED BY
Daniel Weghuber,
Paracelsus Medical University, Austria
Mirjam Močnik,
University Clinical Centre Maribor, Slovenia

*CORRESPONDENCE
Afif Nakhleh
anakhleh@gmail.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 26 November 2022
ACCEPTED 16 January 2023
PUBLISHED 30 January 2023

CITATION
Nakhleh A, Sakhnini R, Furman E and Shehadeh N (2023) Cardiometabolic risk factors among children and adolescents with overweight and Class 1 obesity: A cross-sectional study. Insights from stratification of Class 1 obesity. *Front. Endocrinol.* 14:1108618. doi: 10.3389/fendo.2023.1108618

Cardiometabolic risk factors among children and adolescents with overweight and Class 1 obesity: A cross-sectional study. Insights from stratification of Class 1 obesity

Afif Nakhleh [1,2*†], Rizan Sakhnini [1,2†], Eyal Furman [1] and Naim Shehadeh [1,2,3]

[1]Maccabi Healthcare Services, Haifa, Israel, [2]The Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel, [3]Ruth & Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

Context: Severe childhood obesity is associated with increased prevalence of cardiometabolic risk factors (CMRFs). Among children with Class 1 obesity, higher BMI may indicate greater cardiometabolic risk. Class 1 obesity reflects a wide spectrum of BMI values. Each 10% increase in BMI above the 95th percentile is equivalent to an average increase of 2.15 kg/m2 and 2.75 kg/m2 in BMI among children and adolescents, respectively. Such increments may be of clinical importance.

Objectives: The study aimed to determine the prevalence and clustering of CMRFs in children and adolescents with BMI 110%-119% of the 95th BMI percentile.

Methods: A cross-sectional analysis of data, from an Israeli health maintenance organization, of children and adolescents (5-17 years) with overweight or Class 1 obesity, and at least one measurement of lipid profile during Jan/2020-May/2021. CMRFs were defined as abnormal lipid profile, elevated alanine aminotransferase, hypertension, and prediabetes or diabetes. Study groups included overweight and Class 1 Obesity-A (BMI < 110%) and Obesity-B (BMI ≥ 110%) of the 95th BMI percentile.

Results: Of 7211 subjects included, 40.2% were overweight, 50.3% obesity-A, and 9.5% obesity-B. Multivariable analyses showed that children and adolescents from the Obesity-B group had increased odds for higher triglycerides, LDL cholesterol, and ALT levels; and lower HDL cholesterol levels, as compared to Obesity-A. The odds of prediabetes (insignificant) tended to be higher in the Obesity-B group, which was associated with increased CMRFs clustering.

Conclusions: Among children and adolescents with Class 1 obesity, BMI ≥ 110% of the 95th percentile was associated with higher prevalence and clustering of CMRFs.

KEYWORDS

cardiometabolic risk factors, children, adolescents, overweight, Class 1 obesity

1 Introduction

Obesity among children and adolescents has become a major public health challenge in the twenty-first century. Over the past decade, the prevalence of pediatric obesity in the United States increased from 17.7% to 21.5% (1). Pediatric obesity leads to a significant increase in cardiometabolic morbidity, including type 2 diabetes, hypertension, dyslipidemia, fatty liver, and cardiovascular complications (2–4). Screening tests for children with obesity include fasting lipids, glycated hemoglobin (HbA1c), fasting or random plasma glucose, and liver enzymes (5).

The clustering of cardiometabolic risk factors (CMRFs) in early childhood is of particular concern, given that the majority of children remain obese in adulthood (3). Skinner et al., using National Health and Nutrition Examination Survey (NHANES) data, demonstrated that severe obesity in children and adolescents was associated with increased prevalence of CMRFs, particularly among males (6). They also showed that, as expected, subjects with Class 1 obesity, defined as having a BMI ≥95th percentile to <120% of the 95th percentile for age and sex, had a higher risk of having most risk factors as compared to those with overweight (6, 7).

Each 10% increase in BMI above the 95th percentile is equivalent to an average increase of 2.1 kg/m^2 and 2.7 kg/m^2 in BMI among male children and adolescents, respectively; and of 2.2 kg/m^2 and 2.8 kg/m^2 among female children and adolescents, respectively (Table S1) (8). Such increments in BMI may be clinically significant. For example, the Princeton Follow-up Study demonstrated that the risk of metabolic syndrome in adulthood increased by 24% for each 10% increase in age-specific BMI in children and youth (9).

The question of whether the increment in BMI above 109% of the 95th percentile translates into a significant difference in the risk of cardiometabolic morbidity within the Class 1 obesity group has not yet been addressed. Therefore, in the present study, we aimed at assessing the prevalence and clustering of CMRFs in Israeli children and adolescents with a BMI of 110%-119% of the 95th BMI percentile, as compared to those with overweight and Class 1obesity.

2 Materials and methods

This non-interventional, cross-sectional analysis of data was conducted using the electronic medical database of Maccabi Healthcare Services (MHS), a large health maintenance organization in Israel serving over two million patients. All data were collected from the MHS automated database during May 2021. Clinical data included (1): demographic and anthropometric information on age, gender, socioeconomic status (SES) of the place of residence, weight, BMI percentile, and blood pressure (2); laboratory results including fasting serum total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, plasma glucose, glycated hemoglobin (HbA1c), and alanine aminotransferase (ALT); and (3) data from the MHS diabetes registry. The retrieved data included the last available value in the database recorded during January 2020 - May 2021, except for blood pressure for which we included the last two measurements when available, and fasting plasma glucose and HbA1c for which we retrieved all the available data.

We obtained approval from the MHS institutional review board and ethics committee to access and analyze data. Individual patient informed consent was not required because of the anonymized nature of patient records.

2.1 Study sample and definitions

We performed a cross-sectional analysis of data from children and adolescents 5-17 years of age with overweight or Class 1 obesity, who had at least one measurement of lipid profile during the study period.

Age was expressed in years and rounded to the nearest year (cutoff points are at the midpoint of the child's year). BMI was classified according to percentiles established by the Centers for Disease Control and Prevention (CDC). These were validated for Israeli children and adolescents, for age (by month) and sex (10). Overweight was defined as a BMI between the 85th and 94th percentile. Obesity-A was defined as a BMI at or greater than the 95th percentile up to 109% of the 95th percentile. A BMI between 110% and 119% of the 95th percentile was defined as Obesity-B. Subjects with a BMI at or greater than 120% of the 95th percentile (or BMI greater than 35 kg/m^2) were not included in this study.

Data were obtained on the socioeconomic status (SES) of the place of residence based on the Israeli Central Bureau of Statistics scoring system: low (score 1-4 out of 10), medium (score 5-8 out of 10), and high (score 9-10 out of 10).

Of 26783 children and adolescents (14744 females and 12039 males) aged 5-17 years with documented BMI ≥85th percentile between January 2020 through May 2021, 7211 subjects had a BMI<120% of the 95th percentile and available lipid profile data and thus were included in the study (Figure S1).

We aimed at evaluating children or adolescents for potential comorbidities associated with overweight and obesity. Table 1

TABLE 1 Definitions of comorbidities and abnormal values for cardiometabolic variables.

Variable	Age group, yr	Number of subjects evaluated	Definition of comorbidity or abnormal value
Total cholesterol	5-17	7211	≥200 mg/dL
LDL cholesterol	5-17	7211	≥130 mg/dL
HDL cholesterol	5-17	7211	≤35 mg/dL
Triglycerides	5-17	7211	≥150 mg/dL
ALT	5-17	6869	>25 U/L (boys) >22 U/L (girls)
Prediabetes	5-17	7211	≥2 FPG tests 100-125 mg/dL or ≥1 HbA1c test 5.7%-6.4%
Diabetes mellitus	5-17	7211	Inclusion in MHS diabetes registry
Systolic BP	5-12	1226	≥95th percentile (adjusted to the 50th percentile of age- and gender-specific height)
	13-17	3773	≥130 mm Hg
Diastolic BP	5-12	1226	≥95th percentile (adjusted to the 50th percentile of age- and gender-specific height)
	13-17	3773	≥80 mm Hg

ALT, alanine aminotransferase; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MHS, Maccabi Healthcare Services.

summarizes characteristics of the sample age groups and the definition of comorbidities and abnormal values for cardiometabolic variables.

Abnormal lipid profile values were defined using standard cutoff values for levels of total cholesterol (≥200 mg/dL), LDL cholesterol (≥130 mg/dL), HDL cholesterol (<35 mg/dL), and triglycerides (≥150 mg/dL). Serum ALT concentrations above the 95th percentile (>25 U/L for boys and >22U/L for girls) were regarded as abnormal, as proposed by Schwimmer JB et al. (11). In our sample, 6869 children and adolescents had at least one documented ALT value.

Blood pressure was recorded as the mean value of up to two measurements or as a single measurement (3810 of the children and adolescents had two measurements, 1189 had only one measurement, and 2212 had no measurement). For children aged 5–12 years, we used standardized blood pressure tables in which abnormal values were determined according to age and gender and adjusted to the 50th percentile of height; abnormal values were defined as any value at or above the 95th percentile in Table S2. For children aged ≥13 years, we used cutoffs of ≥130 mm Hg for systolic blood pressure and ≥80 mm Hg for diastolic blood pressure (12).

We defined individuals as having prediabetes if they fulfilled the following criteria: at least two separate fasting plasma glucose values between 100 mg/dL and 125mg/dL, or a single measurement of HbA1c between 5.7% and 6.4%; and the person was not included in the MHS diabetes registry. We used data from MHS automated patients' registry to diagnose diabetes (13). This registry is based on a validated algorithm that collects data from electronic medical records, laboratory results (HbA1c and fasting plasma glucose), dispensed medications (oral glucose-lowering agents or insulin), and clinical diagnoses (more details are available in the Supplementary file: Methods).

2.2 Statistical analysis

Continuous data are presented as mean ± standard deviation (SD) or median (interquartile range). Categorical data are presented as absolute numbers and percentages. Prevalence is reported as a percentage with a 95% confidence interval.

The chi-square test or Fisher's exact test was used to assess the association between categorical variables. T-test or ANOVA were used to assess the association between continuous variables.

To examine the effect of weight on CMRFs we performed multivariable analyses using generalized linear models (GLMs) with a logarithmic link. We reported the odds ratios (after exponentiation of the coefficients). We also performed logistic regression analyses to evaluate the effect of weight on the clustering of CMRFs. Models were adjusted when appropriate for age and sex. A p-value < 0.05 was considered significant.

All statistical analyses were performed using STATA, version 17 (Stata Corp., Texas, USA).

3 Results

Among 7211 children and adolescents with a BMI at the 85th percentile or higher, 40.2% were classified as overweight, 50.3% as obesity-A, and 9.5% as obesity-B (Table 2). Figure S2 depicts median BMI values classified by weight categories and age. No significant correlation was found between SES and severity of obesity (Table S3).

Table S4 shows the mean values for each cardiometabolic variable for all subjects. These values increased with the severity of obesity, except for HDL cholesterol.

Table 3 and Figure 1 show the prevalence of CMRFs among children and adolescents, classified by weight category. Except for total cholesterol, diastolic blood pressure, and diabetes, the prevalence of CMRFs (including prediabetes) consistently increased with the severity of obesity in adolescents (Tables 3, S5). This trend was evident albeit to a lesser extent among children for whom only systolic blood pressure and ALT levels differed significantly between weight groups (Tables 3, S6).

10.3389/fendo.2023.1108618

TABLE 2 Distribution of weight groups by age and sex.

	Overweight	Obesity-A	Obesity-B	Total
All subjects (5-17) n, %	2902 (40.2)	3624 (50.3)	685 (9.5)	7211 (100)
Male	1086 (37.4)	1506 (41.6)	294 (42.9)	2886 (40)
Female	1816 (62.6)	2118 (58.4)	391 (57.1)	4325 (60)
Children (5-11) n, %	489 (33.3)	806 (54.9)	174 (11.8)	1469 (20.4)
Male	175 (35.8)	280 (34.7)	63 (36.2)	518 (35.3)
Female	314 (64.2)	526 (65.3)	111 (63.8)	951 (64.7)
Adolescents (12-17) n, %	2413 (42)	2818 (49.1)	511 (8.9)	5742 (79.6)
Male	911 (37.8)	1226 (43.5)	231 (45.2)	2368 (41.2)
Female	1502 (62.2)	1592 (56.5)	280 (54.8)	3374 (58.8)

Table 4 shows a direct comparison of CMRFs between weight groups of children and adolescents in multivariable generalized linear models that adjusted for age and sex. Children and adolescents in the obesity-B group had greater odds for higher triglycerides, LDL cholesterol, and ALT and lower HDL cholesterol levels, as compared to the obesity-A group. These differences were significant in females except for the difference in ALT levels, the only observation that was significant in males. The odds of prediabetes tended to be higher in the obesity-B group but did not reach significance. Overweight children and adolescents had lower odds for most of the CMRFs.

TABLE 3 Prevalence of comorbidities and abnormal values for cardiometabolic variables by weight among children and adolescents.

Risk factor variable and weight category	All subjects			Children			Adolescents		
	Subjects, n	Prevalence, % (95% CI)	P value	Subjects, n	Prevalence, % (95% CI)	P value	Subjects, n	Prevalence, % (95% CI)	P value
Triglycerides			<0.001			0.11			<0.001
Overweight	2902	8.9(7.8-9.9)		489	7.4 (5-9.7)		2413	9.2 (8-10.3)	
Obesity-A	3624	12.7 (11.6-13.8)		806	9.2 (7.2-11.2)		2818	13.7 (12.4-15)	
Obesity-B	685	15.5 (12.8-18.2)		174	12.6 (7.7-17.6)		511	16.4 (13.2-19.7)	
HDL cholesterol			<0.001			0.62			<0.001
Overweight	2902	4.6 (3.8-5.3)		489	1.8 (0.6-3)		2413	5.1 (4.3-6)	
Obesity-A	3624	6.4 (5.6-7.2)		806	2.6 (1.5-3.7)		2818	7.5 (6.5-8.5)	
Obesity-B	685	8.5 (6.4-10.6)		174	2.9 (0.4-5.4)		511	10.4 (7.7-13)	
LDL cholesterol			0.01			0.55			0.015
Overweight	2902	6.2 (5.3-7)		489	7.2 (4.9-9.4)		2413	6 (5-6.9)	
Obesity-A	3624	7 (6.2-7.8)		806	6.8 (5.1-8.6)		2818	7.1 (6.1-8)	
Obesity-B	685	9.3 (7.2-11.5)		174	9.2 (4.9-13.5)		511	9.4 (6.9-11.9)	
Total cholesterol			0.54			0.33			0.57
Overweight	2902	9.4 (8.4-10.5)		489	12.7 (9.7-15.6)		2413	8.8 (7.7-9.9)	
Obesity-A	3624	9.9 (8.9-10.9)		806	10.8 (8.7-12.9)		2818	9.6 (8.5-10.7)	

(Continued)

TABLE 3 Continued

Risk factor variable and weight category	All subjects			Children			Adolescents		
	Subjects, n	Prevalence, % (95% CI)	P value	Subjects, n	Prevalence, % (95% CI)	P value	Subjects, n	Prevalence, % (95% CI)	P value
Obesity-B	685	10.8 (8.5-13.1)		174	14.4 (9.2-19.6)		511	9.6 (7-12.1)	
Systolic BP			<0.001			<0.001			<0.001
Overweight	2059	7.3 (6.2-8.5)		215	11.2 (7-15.4)		1844	6.9 (5.7-8)	
Obesity-A	2490	13.5 (12.1-14.8)		339	23.3 (18.8-27.8)		2151	11.9 (10.5-13.3)	
Obesity-B	450	15.3 (12-18.7)		70	25.7 (15.5-36)		380	13.4 (10-16.8)	
Diastolic BP			0.10			0.28			0.28
Overweight	2059	11.4 (10-12.7)		215	11.2 (7-15.4)		1844	11.4 (9.9-12.8)	
Obesity-A	2490	13.3 (12-14.6)		339	15.9 (12-19.8)		2151	12.9 (11.5-14.3)	
Obesity-B	450	13.8 (10.6-17)		70	15.7 (7.2-24.2)		380	13.4 (10-16.8)	
Prediabetes			<0.001			0.61			<0.001
Overweight	2902	7.8 (6.8-8.7)		489	3.5 (1.9-5.1)		2413	8.6 (7.5-9.7)	
Obesity-A	3624	9.2 (8.3-10.2)		806	3.1 (1.9-4.3)		2818	11 (9.8-12.1)	
Obesity-B	685	11.2 (8.7-13.4)		174	4.6 (1.5-7.7)		511	13.3 (10.4-16.3)	
Diabetes			0.09			0.87			0.22
Overweight	2902	1.1 (0.7-1.4)		489	0.6 (0-1.3)		2413	1.2 (0.7-1.6)	
Obesity-A	3624	0.7 (0.5-1)		806	0.5 (0-1)		2818	0.8 (0.5-1.1)	
Obesity-B	685	0.3 (0-0.7)		174	0		511	0.4 (0-0.9)	
ALT			<0.001			<0.001			<0.001
Overweight	2783	13.6 (12.3-14.9)		471	15.3 (12-18.5)		2312	13.3 (11.9-14.7)	
Obesity-A	3440	21.2 (19.8-22.6)		759	22.7 (19.7-25.6)		2681	20.8 (19.2-22.3)	
Obesity-B	646	28.2 (24.7-31.6)		165	30.9 (23.9-38)		481	27.2 (23.3-31.2)	

P value for comparison among three groups. A p value < 0.05 was considered significant.
ALT, alanine aminotransferase; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

The number of CMRFs increased with the severity of obesity in children and adolescents (Table S7; Figure 2). The prevalence of clustering of ≥2 and ≥3 CMRFs were 12.1% and 6.3%, respectively; and 19.8% and 6.7% among children and adolescents from the obesity-B group, respectively (Figure 2). Table 5 shows the adjusted odds ratios for the number of CMRFs across weight groups of children and adolescents. Compared with the obesity-A group, subjects from the obesity-B group were at 52% and 76% higher risk of clustering of ≥ 2 and ≥3 CMRFs, respectively (Table 5).

4 Discussion

The present study shows a higher prevalence of most of the cardiovascular risk factors in children and adolescents with a BMI between 110%-119% of the 95th BMI percentile (obesity-B group) as compared to individuals with lower degree of obesity. This ten percent increment in BMI within the Class 1 obesity group translates into significantly higher odds for clustering of CMRFs.

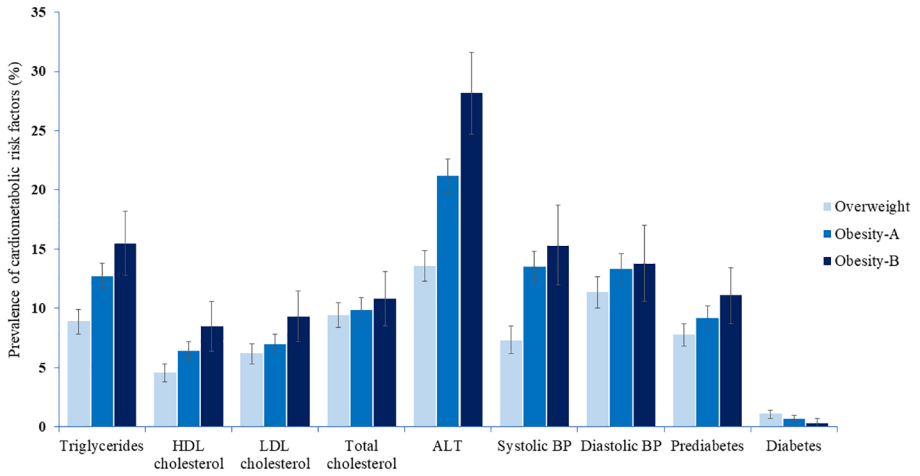


FIGURE 1
The prevalence of cardiometabolic risk factors among children and adolescents by weight category.

Severe obesity in childhood is associated with significant metabolic and cardiovascular morbidity (14). In our study, we aimed at assessing children and adolescents with lower degrees of obesity. Class 1 obesity reflects a wide spectrum of BMI values. Each 10% increase in BMI above the 95th percentile is equivalent to an average increase of 2.15 kg/m^2 and 2.75 kg/m^2 in BMI among children and adolescents, respectively (Table S1) (8). Such increments may be of high clinical importance. For example, the Princeton Follow-up Study demonstrated that the risk of metabolic syndrome in adulthood increased by 24% for each 10% increase in age-specific BMI in childhood (9). In young adults, each 1-kg/m^2 increase in BMI was associated with a 6% higher risk of developing type 2 diabetes before the age of 45 years (15). In a cohort of Danish and Finnish subjects, each z-score increase in BMI at 7 years of age (equivalent to a 1.5 to 2.5 kg/m^2 increment) was associated with a 5%-10% greater risk of coronary heart disease in adulthood (16). Moreover, the risk of mortality increases significantly throughout the overweight and obesity range. In adults younger than 50 years of age, every five units higher BMI above 25 kg/m^2 was associated with an approximate 52% higher risk of premature death (17). These findings emphasize the importance of losing relative weight at a young age.

We subclassified Class 1 obesity into two groups to "zoom in" and better understand the cardiometabolic morbidity associated with lower degrees of obesity in childhood.

In our study, the prevalence of most CMRFs increased with the severity of obesity, except for diastolic blood pressure, diabetes, and total cholesterol (Table 3).

Our results are in line with Sorof et al. that showed a progressive increase in systolic blood pressure with each increase in BMI percentile, whereas diastolic blood pressure showed no association (18). In our study, the prevalence of prediabetes was higher in the obesity groups as expected. However, the prevalence of diabetes was low with no difference seen between the groups. These registry-derived diagnoses include patients with all forms of diabetes, including type 1 diabetes, which might explain the higher (though insignificant) trend of having diabetes in the overweight group (Table 4). However, Twig et al. showed that the cumulative incidence of early-onset type 2 diabetes was more than 2-fold higher among adolescents with severe obesity than among those with Class 1 obesity (4). This relationship should be regarded as a continuum in which each increment above the 95th BMI increases the risk of prediabetes and the transition toward type 2 diabetes.

Obesity is associated with elevated ALT levels. We defined abnormal serum ALT concentrations as >25 U/L for boys and >22U/L for girls (11). This is because liver biopsy specimens from children with normal or mildly elevated ALT (≥26 to 50 U/L for boys and ≥23 to 44 U/L for girls) were histologically abnormal, including advanced fibrosis (19). In our study, an increment in BMI above 109% of the 95th percentile more than doubled the risk for abnormal ALT values in children and adolescents as compared to those with overweight (Table S5, S6). Despite being nonspecific when used alone, the increased plasma concentrations of ALT with higher severity of obesity might reflect the presence of nonalcoholic fatty liver disease (NAFLD) in a substantial proportion of these children.

There were differences observed between children and adolescents in terms of their lipid and glucose profiles, with children showing nonsignificant differences across the weight groups (Table 3). This suggests that the duration of obesity may play a stronger role than BMI alone.

There were important differences between male and female subjects in our study. It should be noted that girls constitute 60% of the study sample, although the prevalence of obesity in the general population is greater among boys (20, 21). Given the cross-sectional nature of the study and the eligibility for inclusion based on available data, this gender disparity may have occurred by chance. The present study demonstrates clearly that an increment in BMI above 109% of the 95th percentile is clinically significant, particularly among females. This increment increases the odds of higher triglycerides and LDL cholesterol and lower HDL cholesterol levels as compared to in females with lower degree of obesity (Table 4). This markedly unfavorable lipid profile relative to weight gain in girls has been reported in a previous study which demonstrated slightly stronger indirect effects of weight gain, through childhood adiposity, in girls as compared with boys (22). There was a nonsignificant trend towards increased odds for prediabetes and higher ALT levels in females with a BMI above 109% of the 95th percentile as compared to those with lower

10.3389/fendo.2023.1108618

TABLE 4 Odds ratios for cardiometabolic risk factors among children and adolescents by sex and weight.

Risk factor variable and weight category	All subjects			Female subjects			Male subjects		
	Subjects, n	Odds ratio, (95% CI)	P value	Subjects, n	Odds ratio, (95% CI)	P value	Subjects, n	Odds ratio, (95% CI)	P value
Triglycerides									
Overweight	2902	0.66 (0.56-0.78)	<0.001	1816	0.75 (0.61-0.92)	0.006	1086	0.51 (0.39-0.68)	<0.001
Obesity-A	3624	Reference		2118	Reference		1506	Reference	
Obesity-B	685	1.28 (1.01-1.61)	0.04	391	1.36 (1.01-1.82)	0.04	294	1.16 (0.80-1.69)	0.42
HDL cholesterol									
Overweight	2902	0.66 (0.53-0.83)	<0.001	1816	0.69 (0.50-0.96)	0.03	1086	0.65 (0.48-0.89)	0.006
Obesity-A	3624	Reference		2118	Reference		1506	Reference	
Obesity-B	685	1.44 (1.06-1.95)	0.02	391	1.65 (1.07-2.53)	0.02	294	1.24 (0.80-1.91)	0.32
LDL cholesterol									
Overweight	2902	0.86 (0.71-1.05)	0.15	1816	0.96 (0.75-1.23)	0.79	1086	0.69 (0.49-0.97)	0.03
Obesity-A	3624	Reference		2118	Reference		1506	Reference	
Obesity-B	685	1.37 (1.03-1.83)	0.03	391	1.49 (1.03-2.15)	0.03	294	1.20 (0.76-1.92)	0.42
Total Cholesterol									
Overweight	2902	0.94 (0.80-1.11)	0.49	1816	1.01 (0.83-1.24)	0.88	1086	0.77 (0.57-1.05)	0.09
Obesity-A	3624	Reference		2118	Reference		1506	Reference	
Obesity-B	685	1.10 (0.85-1.43)	0.47	391	1.22 (0.87-1.70)	0.21	294	0.92 (0.58-1.46)	0.725
Systolic BP									
Overweight	2059	0.56 (0.46-0.67)	<0.001	1282	0.58 (0.45-0.75)	<0.001	777	0.53 (0.41-0.69)	<0.001
Obesity-A	2490	Reference		1413	Reference		1077	Reference	
Obesity-B	450	1.11 (0.85-1.44)	0.43	251	1.29 (0.91-1.85)	0.15	199	0.94 (0.64-1.38)	0.75
Diastolic BP									
Overweight	2059	0.78 (0.66-0.92)	0.003	1282	0.84 (0.69-1.04)	0.10	777	0.71 (0.55-0.92)	0.01
Obesity-A	2490	Reference		1413	Reference		1077	Reference	
Obesity-B	450	0.99 (0.76-1.28)	0.96	251	1.10 (0.78-1.54)	0.59	199	0.85 (0.56-1.28)	0.43
Prediabetes									
Overweight	2902	0.76 (0.64-0.91)	0.003	1816	0.78 (0.61-0.98)	0.04	1086	0.76 (0.58-1.01)	0.06
Obesity-A	3624	Reference		2118	Reference		1506	Reference	
Obesity-B	685	1.29 (0.99-1.69)	0.06	391	1.37 (0.96-1.96)	0.07	294	1.18 (0.78-1.78)	0.43
Diabetes									

(Continued)

TABLE 4 Continued

Risk factor variable and weight category	All subjects			Female subjects			Male subjects		
	Subjects, n	Odds ratio, (95% CI)	P value	Subjects, n	Odds ratio, (95% CI)	P value	Subjects, n	Odds ratio, (95% CI)	P value
Overweight	2902	1.59 (0.95-2.67)	0.08	1816	1.54 (0.80-2.95)	0.20	1086	1.67 (0.72-3.89)	0.23
Obesity-A	3624	Reference		2118	Reference		1506	Reference	
Obesity-B	685	0.40 (0.10-1.71)	0.22	391	0.34 (0.04-2.55)	0.29	294	0.51 (0.07-4.00)	0.52
ALT									
Overweight	2783	0.59 (0.52-0.68)	<0.001	1735	0.56 (0.47-0.68)	<0.001	1048	0.63 (0.52-0.77)	<0.001
Obesity-A	3440	Reference		2007	Reference		1433	Reference	
Obesity-B	646	1.42 (1.18-1.72)	<0.001	368	1.28 (0.98-1.67)	0.07	278	1.61 (1.22-2.12)	<0.001

GLMs that controlled for age and sex were used for these analyses. Obesity-A is the referent group.
A p value < 0.05 was considered significant.
ALT, alanine aminotransferase; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

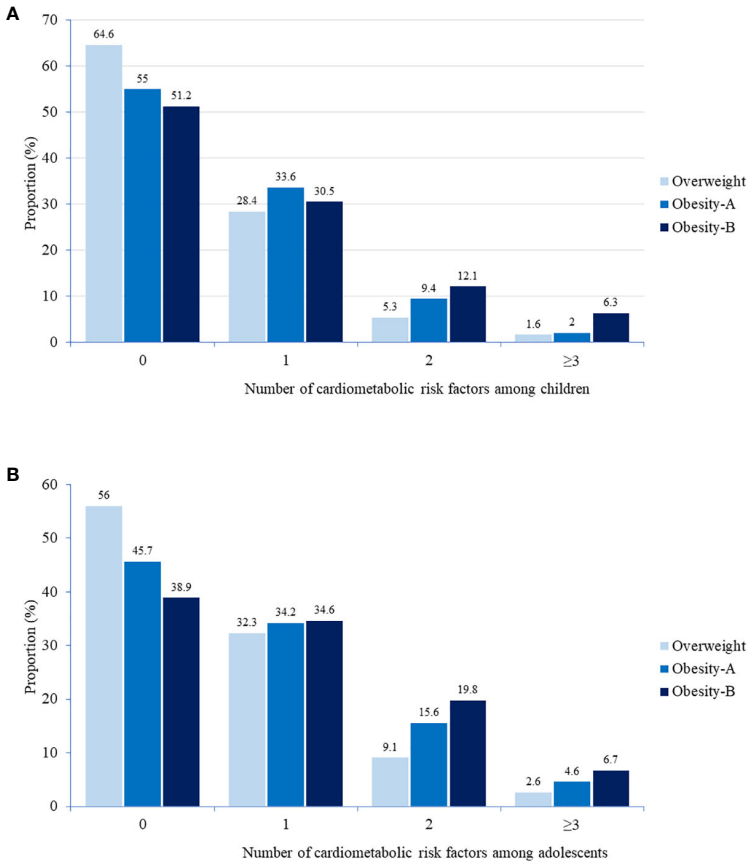


FIGURE 2
Distribution of cardiometabolic risk factors among children **(A)** and adolescents **(B)** by weight category. Cardiometabolic risk factors include hyperglycemia (prediabetes or diabetes), high triglycerides, LDL cholesterol, ALT, and systolic or diastolic BP, or low HDL cholesterol. The number of cardiometabolic risk factors increased with the severity of obesity in children (p<0.001) and adolescents (p<0.001). P value for difference between groups was determined by chi square test.

TABLE 5 Odds ratios of having ≥1, ≥2, and ≥3 (vs zero) cardiometabolic risk factors* by weight category in children and adolescents.

Weight category	≥1 cardiometabolic risk factors		≥2 cardiometabolic risk factors		≥3 cardiometabolic risk factors	
	Odds ratio, (95% CI)	P value	Odds ratio, (95% CI)	P value	Odds ratio, (95% CI)	P value
Overweight	0.64 (0.58-0.71)	<0.001	0.51 (0.44-0.59)	<0.001	0.56 (0.42-0.75)	<0.001
Obesity-A	Reference		Reference		Reference	
Obesity-B	1.29 (1.09-1.53)	0.002	1.52 (1.24-1.85)	<0.001	1.76 (1.25-2.49)	0.001

Cardiometabolic risk factors include hyperglycemia (prediabetes or diabetes), high triglycerides, LDL cholesterol, ALT, and systolic or diastolic BP or low HDL cholesterol.
Logistic regression that controlled for age and sex was used for these analyses. Obesity-A is the referent group.
A p value < 0.05 was considered significant.
ALT, alanine aminotransferase; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

degree of obesity. In males, an increment above 109% of the 95th BMI percentile was associated with increased odds for higher ALT levels. This result is in line with previous studies showing that prevalence was generally higher in boys as compared with girls and increased incrementally with greater BMI (23, 24).

Our findings differ from those in other reports. Some studies showed minimal differences between boys and girls and another study (in which 16.7% of the sample had severe obesity) showed a higher prevalence of risk factors in males (6, 25–27).

The clustering of CMRFs in childhood is of high concern, taking into account the fact that around 80% of them remain obese in adulthood (3). Indeed, this observation justifies the evaluation of cardiometabolic variables at a young age, especially among those who are overweight or obese.

Several definitions of metabolic syndrome in children and adolescents have been proposed (28–33). These definitions are diverse and lack uniformity, and there is no clear consensus on which to use. The American Academy of Pediatrics recommends shifting the focus to the concept of clustering of CMRFs rather than defining metabolic syndrome in children and adolescents (34).

In the Bogalusa Heart Study, the clustering of CMRFs was associated with increased severity of asymptomatic coronary atherosclerotic lesions in young people (35). Since the clustering of risk factors is present in childhood and continues into young adulthood, the presence of multiple risk factors such as hyperglycemia, hypertension, and abnormal lipid profile may indicate a faster progression of atherosclerosis in young people.

Using conservative thresholds for prediabetes, dyslipidemia, and hypertension, a large multi-ancestral cohort demonstrated a direct correlation between the severity of obesity and prevalence of CMRFs, whereby each half-unit increase in the BMI z score increased the risk of having cardiovascular risk factors' clustering by 55% (29). Skinner et al. showed that values for some, but not all, CMRFs were higher with increased severity of obesity in children and adolescents, and demonstrated that greater severity of obesity is associated with a higher risk of lower HDL-cholesterol levels, elevated plasma triglycerides, and high systolic and diastolic blood pressure (6).

In our study, subjects with BMI between 110%-120% of the 95th BMI percentile had a higher prevalence of CMRFs clustering (17.8% and 6.6% for clustering of ≥2 and ≥3 risk factors, respectively) (Table S7). They exhibited a substantially higher risk for CMRFs clustering as compared to those with lower degree of obesity (Table 5). This highlights the need for further stratification of weight groups at a young age to better reflect the risk for metabolic and cardiovascular morbidity.

Previous research has shown that a lower SES during childhood is linked to a higher risk of developing metabolic syndrome in adulthood, even when controlling for other childhood risk factors (36). However, our study did not find a significant association between SES and the level of obesity, thus it cannot be considered a confounding factor.

There are several limitations to our study that should be considered. First, this is a cross-sectional study, which cannot prove the causality between obesity and CMRFs. In addition, we cannot exclude any secondary causes of obesity, such as genetic or hormonal factors. Second, we lacked data on lifestyle, physical activity, and other indicators of obesity such as waist circumference. The latter may be more sensitive than BMI in the context of this study and better define cardiovascular risk in the long term. Third, our sample might differ from the general population in terms of gender representation, as obesity is more common in boys (20). A considerable difference was observed in the number of individuals with obesity-A and obesity-B (50.3% compared to 9.5%). It should be noted that our sample was limited to individuals with available lipid profile data. This limitation, combined with the observational nature of the study, raises the possibility that the representation of obesity-B in our sample may not accurately reflect its prevalence in the general population. Finally, the sample size was relatively small in weight subgroups of children younger than 12 years old, which resulted in some estimates with wide confidence intervals, deeming them insignificant. Thus, some of these estimates should be interpreted with caution.

Our study has certain strengths. The study was based on a relatively large sample of children and adolescents. This permitted the evaluation of a wide variety of CMRFs and the exploration of their clustering among weight subgroups. In conclusion, among children and adolescents with Class 1 obesity, a BMI ≥ 110% of the 95th percentile was associated with a higher prevalence of and greater clustering of CMRFs. The consideration of this group (obesity-B group) in the standard obesity classification may assist in the identification of children and adolescents who could be at a greater risk for abnormal lipid profile, hyperglycemia, and abnormal ALT levels.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Maccabi Healthcare Services institutional review board and ethics committee. Approval number 0183-20-MHS. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

AN and RS were responsible for the design of the study and data acquisition. AN and RS contributed to the analysis and the interpretation of data, drafted the manuscript and revised it critically for important intellectual content. NS and EF contributed to the analysis and the interpretation of data and revised the manuscript critically for important intellectual content. All authors contributed to the article and approved the submitted version.

Funding

The authors declare that this study received funding from Novo Nordisk, Israel. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Acknowledgments

We would like to thank Mrs. Ateret Malachi for her help in this study.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1108618/full#supplementary-material

References

1. Hu K, Staiano AE. Trends in obesity prevalence among children and adolescents aged 2 to 19 years in the US from 2011 to 2020. *JAMA Pediatr* (2022) 176:1037–9. doi: 10.1001/jamapediatrics.2022.2052

2. Weihrauch-Blüher S, Schwarz P, Klusmann JH. Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. *Metabolism* (2019) 92:147–52. doi: 10.1016/j.metabol.2018.12.001

3. Caprio S, Santoro N, Weiss R. Childhood obesity and the associated rise in cardiometabolic complications. *Nat Metab* (2020) 2:223–32. doi: 10.1038/s42255-020-0183-z

4. Twig G, Zucker I, Afek A, Cukierman-Yaffe T, Bendor CD, Derazne E, et al. Adolescent obesity and early-onset type 2 diabetes. *Diabetes Care* (2020) 43:1487–95. doi: 10.2337/dc19-1988

5. Styne DM, Arslanian SA, Connor EL, Farooqi IS, Murad MH, Silverstein JH, et al. Pediatric obesity–assessment, treatment, and prevention: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2017) 102:709–57. doi: 10.1210/jc.2016-2573

6. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic risks and severity of obesity in children and young adults. *N Engl J Med* (2015) 373:1307–17. doi: 10.1056/NEJMoa1502821

7. Kelly AS, Barlow SE, Rao G, Inge TH, Hayman LL, Steinberger J, et al. Severe obesity in children and adolescents: Identification, associated health risks, and treatment approaches. *Circulation* (2013) 128:1689–712. doi: 10.1161/CIR.0b013e3182a5cfb3

8. *Growth charts - data table of BMI-for-age charts* (2001). Available at: https://www.cdc.gov/growthcharts/html_charts/bmiagerev.htm (Accessed 17 October 2022).

9. Morrison JA, Friedman LA, Gray-McGuire C. Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: The Princeton lipid research clinics follow-up study. *Pediatrics* (2007) 120:340–5. doi: 10.1542/peds.2006-1699

10. Goldstein A, Haelyon U, Krolik E, Sack J. Comparison of body weight and height of Israeli schoolchildren with the tanner and centers for disease control and prevention growth charts. *Pediatrics* (2001) 108:E108. doi: 10.1542/peds.108.6.e108

11. Schwimmer JB, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* (2010) 138:1357–64, 1364.e1-2. doi: 10.1053/j.gastro.2009.12.052

12. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. SUBCOMMITTEE ON SCREENING AND MANAGEMENT OF HIGH BLOOD PRESSURE IN CHILDREN. clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics* (2017) 140:e20171904. doi: 10.1542/peds.2017-1904

13. Chodick G, Heymann AD, Shalev V, Kookia E. The epidemiology of diabetes in a large Israeli HMO. *Eur J Epidemiol.* (2003) 18:1143–6. doi: 10.1023/B:EJEP.0000006635.36802.c8

14. Bendor CD, Bardugo A, Pinhas-Hamiel O, Afek A, Twig G. Cardiovascular morbidity, diabetes and cancer risk among children and adolescents with severe obesity. *Cardiovasc Diabetol* (2020) 19:1–14. doi: 10.1186/s12933-020-01052-1

15. Hillier TA, Pedula KL. Characteristics of an adult population with newly diagnosed type 2 DiabetesThe relation of obesity and age of onset. *Diabetes Care* (2001) 24:1522–7. doi: 10.2337/diacare.24.9.1522

16. Andersen LG, Ängquist L, Eriksson JG, Forsen T, Gamborg M, Osmond C, et al. Birth weight, childhood body mass index and risk of coronary heart disease in adults: Combined historical cohort studies. *PloS One* (2010) 5:e14126. doi: 10.1371/journal.pone.0014126

17. Di Angelantonio E, Bhupathiraju SN, Wormser D, Gao P, Kaptoge S, de Gonzalez AB, et al. Body-mass index and all-cause mortality: Individual-participant-data meta-analysis of 239 prospective studies in four continents. *Lancet* (2016) 388:776–86. doi: 10.1016/S0140-6736(16)30175-1

18. Sorof JM, Lai D, Turner J, Poffenbarger T, Portman RJ. Overweight, ethnicity, and the prevalence of hypertension in school-aged children. *Pediatrics* (2004) 113:475–82. doi: 10.1542/peds.113.3.475

19. Molleston JP, Schwimmer JB, Yates KP, Murray KF, Cummings OW, Lavine JE, et al. Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels. *J Pediatr* (2014) 164:707–713.e3. doi: 10.1016/j.jpeds.2013.10.071

20. Shah B, Tombeau Cost K, Fuller A, Birken CS, Anderson LN. Sex and gender differences in childhood obesity: Contributing to the research agenda. *BMJ Nutr Prev Heal* (2020) 3:387. doi: 10.1136/bmjnph-2020-000074

21. *WHO European regional obesity report 2022* (2022). Copenhagen: WHO Regional Office for Europe; 2022. Licence: CC BY-NC-SA 3.0 IGO.WHO EUROPEAN REGIONAL OBESITY REPORT 2022. Available at: https://apps.who.int/iris/bitstream/handle/10665/353747/9789289057738-eng.pdf (Accessed 8 November 2022).

22. Van Hulst A, Barnett TA, Paradis G, Roy-Gagnon MH, Gomez-Lopez L, Henderson M. Birth weight, postnatal weight gain, and childhood adiposity in relation to lipid profile and blood pressure during early adolescence. *J Am Heart Assoc* (2017) 6: e006302. doi: 10.1161/JAHA.117.006302

23. Villanueva-Ortega E, Garcés-Hernández MJ, Herrera-Rosas A, López-Alvarenga JC, Laresgoiti-Servitje E, Escobedo G, et al. Gender-specific differences in clinical and metabolic variables associated with NAFLD in a Mexican pediatric population. *Ann Hepatol* (2019) 18:693–700. doi: 10.1016/j.aohep.2019.04.012

24. Anderson EL, Howe LD, Jones HE, Higgins JPT, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. *PloS One* (2015) 10:e0140908. doi: 10.1371/journal.pone.0140908

25. Freedman DS, Mei Z, Srinivasan SR, Berenson GS, Dietz WH. Cardiovascular risk factors and excess adiposity among overweight children and adolescents: The bogalusa heart study. *J Pediatr* (2007) 150:12–17.e2. doi: 10.1016/j.jpeds.2006.08.042

26. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: The bogalusa heart study. *Pediatrics* (1999) 103:1175–82. doi: 10.1542/peds.103.6.1175

27. Lambert M, Delvin EE, Levy E, O'Loughlin J, Paradis G, Barnett T, et al. Prevalence of cardiometabolic risk factors by weight status in a population-based sample of Quebec children and adolescents. *Can J Cardiol* (2008) 24:575–83. doi: 10.1016/S0828-282X(08)70639-1

28. Noubiap JJ, Nansseu JR, Lontchi-Yimagou E, Nkeck JR, Nyaga UF, Ngouo AT, et al. Global, regional, and country estimates of metabolic syndrome burden in children and adolescents in 2020: A systematic review and modelling analysis. *Lancet Child Adolesc Heal* (2022) 6:158–70. doi: 10.1016/S2352-4642(21)00374-6

29. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* (2004) 350:2362–74. doi: 10.1056/NEJMoa031049

30. Zimmet P, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents - an IDF consensus report. *Pediatr Diabetes.* (2007) 8:299–306. doi: 10.1111/j.1399-5448.2007.00271.x

31. De Ferranti SD, Gauvreau K, Ludwig DS, Neufeld EJ, Newburger JW, Rifai N. Prevalence of the metabolic syndrome in American adolescents: Findings from the third national health and nutrition examination survey. *Circulation* (2004) 110:2494–7. doi: 10.1161/01.CIR.0000145117.40114.C7

32. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: Findings from the third national health and nutrition examination survey, 1988-1994. *Arch Pediatr Adolesc Med* (2003) 157:821–7. doi: 10.1001/archpedi.157.8.821

33. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: An American heart Association/ National heart, lung, and blood institute scientific statement. *Circulation* (2005) 112:2735–52. doi: 10.1161/CIRCULATIONAHA.105.169404

34. Magge SN, Goodman E, Armstrong SC. COMMITTEE ON NUTRITION; SECTION ON ENDOCRINOLOGY; SECTION ON OBESITY. the metabolic syndrome in children and adolescents: Shifting the focus to cardiometabolic risk factor clustering. *Pediatrics* (2017) 140:e20171603. doi: 10.1542/peds.2017-1603

35. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. the bogalusa heart study. *N Engl J Med* (1998) 338:1650–6. doi: 10.1056/NEJM199806043382302

36. Puolakka E, Pahkala K, Laitinen TT, Magnussen CG, Hutri-Kähönen N, Tossavainen P, et al. Childhood socioeconomic status in predicting metabolic syndrome and glucose abnormalities in adulthood: the cardiovascular risk in young finns study. *Diabetes Care* (2016) 39(12):2311–7. doi: 10.2337/dc16-1565

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 20 April 2023
DOI 10.3389/fendo.2023.1143755

OPEN ACCESS

EDITED BY
Dénes Molnár,
University of Pécs, Hungary

REVIEWED BY
Elzbieta Petriczko,
Pomeranian Medical University, Poland
Semra Çaglar Çetinkaya,
University of Health Sciences, Türkiye

*CORRESPONDENCE
Maria Krajewska
mkrajewska@wum.edu.pl

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 13 January 2023
ACCEPTED 06 April 2023
PUBLISHED 20 April 2023

CITATION
Krajewska M, Witkowska-Sędek E, Rumińska M, Kucharska AM, Stelmaszczyk-Emmel A, Sobol M, Majcher A and Pyrżak B (2023) The link between vitamin D, chemerin and metabolic profile in overweight and obese children - preliminary results. *Front. Endocrinol.* 14:1143755. doi: 10.3389/fendo.2023.1143755

The link between vitamin D, chemerin and metabolic profile in overweight and obese children - preliminary results

Maria Krajewska[1]*, Ewelina Witkowska-Sędek[1], Małgorzata Rumińska[1], Anna M. Kucharska[1], Anna Stelmaszczyk-Emmel[2], Maria Sobol[3], Anna Majcher[1] and Beata Pyrżak[1]

[1]Department of Paediatrics and Endocrinology, Medical University of Warsaw, Warsaw, Poland, [2]Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Warsaw, Poland, [3]Department of Biophysics, Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland

Background: Vitamin D affects adipogenesis, oxidative stress, inflammation, secretion of adipocytokines, lipid metabolism and thermogenesis. Some researchers postulate that those effects could be exerted by the influence of vitamin D on chemerin levels.

Aim of the study: We aimed to investigate if there is a link between serum 25-hydroksyvitamin D [25(OH)D], chemerin and metabolic profile in overweight and obese children before and after vitamin D supplementation.

Material and methods: The prospective study included 65 overweight and obese children aged 9.08-17.5 years and 26 peers as a control. None of the patients in the study group had received vitamin D within the last twelve months before the study.

Results: The study group had lower baseline 25(OH)D ($p<0.001$) and higher chemerin ($p<0.001$), triglycerides (TG, $p<0.001$), triglycerides/high density lipoprotein cholesterol (TG/HDL-C, $p<0.001$), C-reactive protein (CRP, $p<0.05$), fasting insulin ($p<0.001$), Homeostasis Model Assessment - Insulin Resistance (HOMA-IR, $p<0.001$), alanine aminotransferase (ALT, $p<0.001$) and uric acid ($p<0.001$) compared to the control group. Baseline vitamin D was related to fasting insulin ($R=-0.29$, $p=0.021$), HOMA-IR ($R=-0.30$, $p=0.016$), HDL-C ($R=0.29$, $p=0.020$) and uric acid ($R=-0.28$, $p=0.037$) in the study group. Baseline chemerin was related to insulin at 30' ($R=0.27$, $p=0.030$), 60' ($R=0.27$, $p=0.033$), 90' ($R=0.26$, $p=0.037$) and 120' ($R=0.26$, $p=0.040$) during the oral glucose tolerance test (OGTT) and ALT ($R=0.25$, $p=0.041$) in the study group. Correlation between vitamin D and chemerin ($R=-0.39$, $p=0.046$) was found only in the control group. After six months of vitamin D supplementation a decrease in CRP ($p<0.01$), total cholesterol ($p<0.05$), ALT ($p<0.01$), glucose at 150' OGTT ($p<0.05$) was observed. Moreover, we noticed a tendency for negative association between 25(OH)D and chemerin levels ($p=0.085$). Multivariable

backward linear regression models were build using baseline vitamin D, baseline chemerin and six months chemerin as the dependent variables.

Conclusions: Our study confirmed that vitamin D has positive effect on metabolic profile in overweight and obese children. The relationship between vitamin D and chemerin is not clear, nevertheless we have observed a tendency to decrease chemerin concentrations after improving vitamin D status, even without a significant reduction in body fat mass.

KEYWORDS

obesity, children, vitamin D, chemerin, C-reactive protein, glucose, insulin, lipid profile

Introduction

Adipose tissue as a highly metabolically active organ has been intensively studied over the last decades (1, 2). It secretes a number of adipokines and pro-inflammatory cytokines which link excess body fat with chronic inflammation and atherosclerosis, contributing to the development of obesity-related metabolic disorders (1, 3–5). Vitamin D is also involved in those processes (6, 7). The development of obesity and vitamin D status are mutually dependent (6). It has been confirmed that vitamin D can affect both genomic and nongenomic responses in adipose tissue. Its effect includes the impact on adipogenesis and apoptosis, on the development of oxidative stress and inflammation, the regulation of the secretion of adipokines, pro-inflammatory and anti-inflammatory cytokines, the influence on lipid metabolism and thermogenesis (8). Some studies reported the beneficial effect of vitamin D supplementation on reducing cardiometabolic risk factors in childhood obesity (9–11). Some researchers postulated that the protective effect of vitamin D on metabolic profile could be exerted by the influence on chemerin levels (12–14). Chemerin is one of the most important multifunctional adipokines, generated mainly in subcutaneous and visceral adipose tissue by elastase and tryptase which activate prochemerin. Chemerin mRNA is expressed also in fibroblasts, chondrocytes, epithelial cells, platelets and in a number of organs such as liver, female reproductive organs, adrenal glands, lungs, kidneys and pancreas (15–18). Chemerin is involved in glucose homeostasis, lipid metabolism, maintenance of energy balance, adipogenesis, angiogenesis, inflammatory and autoimmune processes (1, 18–22). Recent studies indicate that it could be also used as a marker of tumours (23, 24). Increased chemerin levels, typical for obesity, are associated with adiposity-related dyslipidaemia, insulin resistance, low-grade inflammation and hypertension (25–29). Recent evidence suggest that chemerin, similarly to other adipokines e.g. leptin, may influence bone metabolism. Experimental data indicate that chemerin promotes osteoclastogenesis (30–35).

The aim of these study was to investigate the link between serum 25-hydroksyvitamin D [25(OH)D], chemerin and metabolic profile in overweight and obese children before and after vitamin D supplementation.

Material and methods

The prospective study included 65 children (51 obese and 14 overweight, aged 9.08-17.5 years) with mean body mass index (BMI) 30.9 ± 4.8 and 26 peers as a control with mean BMI 18.3 ± 2.6 age- and sex- matched. None of the patients in the study group had received vitamin D within the last twelve months before the study. The study protocol was approved by the Bioethics Committee at the Medical University of Warsaw (decision number KB/257/2013) and conducted in the Department of Paediatrics and Endocrinology at the Medical University of Warsaw (Poland). At the time of blood collection, children in both the study and the control group were healthy, without infection or chronic diseases and were not taking any medication. During the study period the participants did not change their diet or the level of physical activity. Serum 25(OH)D, chemerin, C-reactive protein (CRP), glucose and insulin during the oral glucose tolerance test (OGTT), uric acid, aminotransferases (aspartate aminotransferase – AST and alanine aminotransferase - ALT), lipid profile (total cholesterol, low density lipoprotein cholesterol - LDL-C, high density lipoprotein cholesterol - HDL-C, triglycerides - TG) and glycated haemoglobin (HbA1c) were determined at baseline (both in the study and in the control group) and after six months of vitamin D supplementation (in the study group). Following indices were calculated: TG/HDL-C ratio, Homeostasis Model Assessment - Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI) at baseline and after six months of vitamin D supplementation. The aim of vitamin D administration was to achieve the reference serum 25(OH)D levels between 30 and 50 ng/ml after six months of intervention (36). Depending on the serum 25(OH)D levels, the doses of vitamin D ranged from 2000 to 4000 units per day. Serum 25 (OH)D concentrations were assessed every month, which allowed us to control compliance and to modify administered vitamin D doses to achieve reference values after six months of the study.

Anthropometric parameters (height, weight, waist and hip circumference) were measured using standardized methods. Based on these measurements BMI, waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were calculated. The skinfold thickness (mm) was measured under the triceps brachii muscle and under the inferior scapular angle. Body fat percentage (%FAT skinfolds) was calculated in the study group and in the control group using Slaughter formula (37). Additionally, in the study group the percentage of fat was measured using a bioimpedance analysis (% FAT BIA) device (Maltron Body FAT Analyzer BF-905). Height and weight were evaluated according to polish 2010 growth references for school-aged children and adolescents (38). The degree of obesity expressed as BMI standard deviation score (SDS) was calculated using the LMS method to normalize skewness of the distribution of BMI (38, 39). Obesity was defined as BMI SDS ≥ 2, and overweight as BMI SDS ≥ 1 and < 2 (40).

Biochemical analyses

Blood samples were collected after overnight fasting and analysed by standard methods. Serum 25(OH)D levels (ng/ml) were determined by immunoassay method using Architect Analyzer (Abbott Diagnostics, Lake Forest, USA). Serum levels of chemerin (pg/ml) were evaluated by ELISA (Mediagnost, Reutlingen, Germany) using Asys UVM 340 Analyzer. The concentrations of fasting glucose (mg/dl) and glucose in the oral glucose tolerance test (OGTT 1.75g of glucose/kg body weight, no more than 75g; blood samples taken at 0', 30', 60', 90', 120', 150', 180') were determined in blood serum by glucose oxidase colorimetric method using Vitros 5600 Analyzer (Ortho Clinical Diagnostic, New Jersey, USA). The concentrations of HbA1c (%) were measured in whole blood by ion-exchange high-performance liquid chromatography using D-10 Hemoglobin Analyzer (BIO-RAD). The concentrations of insulin (uIU/ml) were measured in serum by immunoassay using IMMULITE 2000 Xpi Analyzer (Siemens). Serum levels of total cholesterol (mg/dl), LDL-C (mg/dl), HDL-C (mg/dl), TG (mg/dl), ALT (U/l), AST (U/l), CRP (mg/dl) and uric acid (mg/dl) were determined in blood serum using Vitros 5600 Analyzer (Ortho Clinical Diagnostic, New Jersey, USA). The HOMA-IR was calculated as follows: HOMA-IR = (fasting glucose mg/dl) x (fasting insulin uIU/ml)/405. The QUICKI was calculated as follows: QUICKI = 1/[log(fasting insulin uIU/ml) + log(fasting glucose mg/dl)] (41). The levels of uric acid were classified as normal or increased according to the reference values provided by the manufacturer. The values of lipid parameters were classified as normal or increased/decreased (increased total cholesterol, LDL-C, TG and decreased HDL-C) according to the reference values used in paediatric population (42).

Statistical analysis

Statistical analysis was performed using Statistica 13.3. Data distribution was checked using the Shapiro–Wilk test. Data were presented as means with standard deviation or the median and interquartile ranges, as appropriate. Comparisons between baseline data of the study group and the control group were made using the T-test for parametric data or using the U Mann-Whitney test for non-parametric data. Analysis of changes of the same parameter at baseline and after six months of vitamin D supplementation were provided using the T-test or the Wilcoxon test, as appropriate. Correlation analysis was performed using Spearman correlation coefficient. In further analysis, we used multivariable stepwise regression analysis to determine which factors (model first: body mass SDS, hip circumference, BMI SDS, waist circumference or model second: fasting insulin, HOMA-IR, HDL-C, uric acid) were associated with baseline 25(OH)D (as dependent variable). We also analysed which parameters (model third: %FAT BIA, %FAT skinfolds, WHtR, BMI SDS or model fourth: insulin at 60' and 120' during the OGTT, ALT, TG/HDL-C) were associated with baseline chemerin levels (as dependent variable). In model fifth: WHR, WHtR, %FAT skinfolds and model sixth: TG, TG/HDL-C, ALT we investigated which factors were associated with chemerin values after six months of vitamin D supplementation.

Results

Baseline anthropometric and biochemical parameters in the study group and in the control group

Baseline anthropometric and biochemical characteristics of the study group and of the control group are presented in Tables 1, 2. As expected, baseline 25(OH)D levels were significantly lower in children with excess body weight compared to the control group (median values 16.0 vs. 25.7 ng/ml, p<0.001), whereas baseline chemerin levels were significantly higher in the study group than in the control group (median 212.0 vs. 147.1 pg/ml, p<0.001). We noticed also that the study group had higher TG (p<0.001), TG/HDL-C ratio (p<0.001), CRP (p<0.05), fasting insulin (p<0.001), HOMA-IR (p<0.001), ALT activity (p<0.001) and uric acid (p<0.001) compared to the control group. The levels of HDL-C were significantly lower (p<0.001) in overweight and obese children.

Correlation analysis revealed that baseline 25(OH)D levels correlated significantly with nutritional status parameters of the study group such as body weight SDS (R=-0.27, p=0.032), BMI SDS (R=-0.27, p=0.028), hip circumference (R=-0.27, p=0.030). In the control group none associations between baseline vitamin D status and anthropometric parameters were observed.

Analysing baseline metabolic profile of both groups we found that vitamin D status was related significantly to fasting insulin (R=-0.29, p=0.021), HOMA-IR (R=-0.30, p=0.016, Figure 1), QUICKI (R=0.29, p=0.020), HDL-C (R=0.29, p=0.020) and uric acid (R=-0.28, p=0.037) in the study group. These associations were not found in the control group. We did not find any associations between 25(OH)D levels and CRP, fasting glucose or glucose levels in OGTT, HbA1c, aminotransferase activity both in the study group and the control group at baseline. Interestingly, we noticed, but only in the control group, significant negative correlation between

10.3389/fendo.2023.1143755

TABLE 1 Baseline anthropometric measurements in the study group and in the control group.

	STUDY GROUP (n = 65)	CONTROL GROUP (n 26)	p value
Age (years)	13.4 ± 2.11	13.5 ± 2.39	ns
Height SDS	0.6 ± 1.25	-0.7 ± 1.47	< 0.001
Weight SDS	2.3 ± 0.69	-0.4 ± 1.15	< 0.001
WC (cm)	90.9 ± 10.27	61.6 ± 6.53	< 0.001
HC (cm)	106.2 ± 10.60	77.1 ± 8.35	< 0.001
WHR	0.9 ± 0.06	0.8 ± 0.04	< 0.001
WHtR	0.56 (0.52-0.60)	0.41 (0.39-0.44)	< 0.001
% FAT (skinfolds)	35.7 (32.1-39.7)	23.1 (19.6-24.7)	< 0.001
BMI SDS	2.3 ± 0.47	-0.3 ± 0.83	< 0.001

Data are presented as means ± standard deviations score or as median with interquartile range, as appropriate. SDS, standard deviation score; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; % FAT (skinfolds), percentage of body fat estimated from skinfolds; BMI, body mass index; ns, not significant.

baseline vitamin D level and baseline chemerin (R=-0.39, p=0.046, Figure 2).

Baseline chemerin levels were related to WHtR (R=0.32, p=0.010), %FAT BIA (R=0.25, p=0.045), %FAT skinfolds (R=0.26, p=0.042), insulin at 30' (R=0.27, p=0.030), 60' (R=0.27, p=0.033), 90' (R=0.26, p=0.037) and 120' during the OGTT (R=0.26, p=0.040) and ALT activity (R=0.25, p=0.041) in the study group. In the control group we found only positive relationship between baseline chemerin level and WHR (R=0.46, p=0.045).

Anthropometric and biochemical parameters after six months of vitamin D supplementation in the study group

Comparison between anthropometric and biochemical parameters in the study group at baseline and after six months of vitamin D supplementation is presented in Tables 3, 4. After six months of vitamin D supplementation the levels of 25(OH)D significantly increased in the study group compared to baseline values (median 16.0 vs. 27.1 ng/ml, p<0.001), while BMI SDS and chemerin levels did not change significantly. We found significant decrease in CRP (p<0.01), total cholesterol (p<0.05), ALT (p<0.01) and AST (p<0.01). We also noticed a decrease in glucose at 150' during the OGTT (p<0.05) and a tendency for lower glucose levels at 120' during the OGTT (p=0.079).

Although we did not find any relationship between vitamin D status after six months of its supplementation and anthropometric or biochemical parameters at six months, we noticed a tendency for

TABLE 2 Baseline biochemical parameters in the study group and in the control group.

	STUDY GROUP (n = 65)	CONTROL GROUP (n = 26)	p value
25(OH)D (ng/ml)	16.0 (12.6 - 20.0)	25.7 (18.6 - 31.1)	< 0.001
Total cholesterol (mg/dl)	161.0 (142.0 - 181.0)	160.5 (138.0 - 170.0)	ns
Triglycerides (TG) (mg/dl)	108.0 (83.0 - 161.0)	68.0 (53.0 - 93.0)	< 0.001
TG/HDL-C	2.5 (2.0 - 4.2)	1.1 (0.7 - 1.7)	< 0.001
LDL-C (mg/dl)	94.3 ± 27.1	84.2 ± 29.1	ns
HDL-C (mg/dl)	43.4 ± 11.1	60.3 ± 10.7	< 0.001
CRP (mg/dl)	0.5 (0.5 - 0.7)	0.5 (0.5 - 0.5)	< 0.01
Glucose fasting (mg/dl)	86.0 (82.0 - 91.0)	84.0 (78.0 - 86.0)	ns
Insulin fasting (uIU/ml)	14.7 (9.1 - 25.6)	7.2 (2.9 - 11.7)	< 0.001
HOMA-IR	3.0 (1.9 - 5.4)	1.5 (0.6 - 2.6)	< 0.001
QUICKI	0.32 (0.30 - 0.35)	0.36 (0.33 - 0.42)	< 0.001
HbA1c (%)	5.2 (5.0 - 5.35)	5.1 (4.9 - 5.3)	ns
chemerin (pg/ml)	212.0 (177.5 - 238.3)	147.1 (128.6 - 171.3)	< 0.001
AST (U/I)	28.0 (23.0 - 35.0)	25.0 (22.0 - 33.5)	ns
ALT (U/I)	27.0 (21.0 - 36.0)	18.5 (13.5 - 22.0)	< 0.001
Uric acid (mg/dl)	5.85 (5.3 - 6.8)	4.6 (3.9 - 5.2)	< 0.001

Data are presented as means ± standard deviations score or as median with interquartile range, as appropriate. 25(OH)D, 25-hydroxyvitamin D; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CRP, C-reactive protein; HOMA-IR, Homeostasis Model Assessment- Insulin Resistance, QUICKI, Quantitative Insulin Sensitivity Check Index; HbA1c, Glycated Hemoglobin, AST, aspartate aminotransferase; ALT, alanine aminotransferase; ns, not significant.

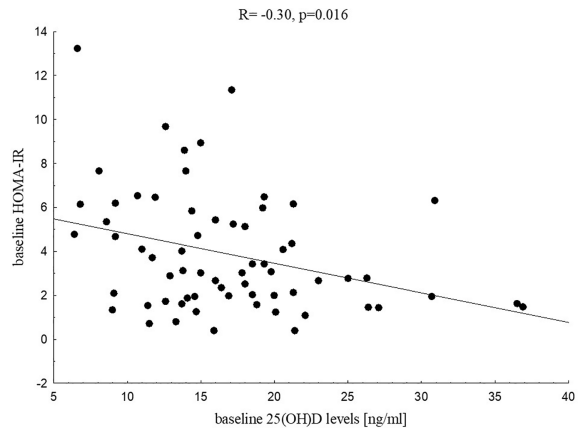


FIGURE 1
Correlation between 25(OH)D levels and HOMA-IR in the study group at baseline.

negative association between 25(OH)D and chemerin levels (p=0.085).

In further analysis we aimed to investigate if changes in vitamin D status during its supplementation influence metabolic profile in children with excess body mass. We found that changes in 25(OH)D levels were related negatively to changes in fasting glucose (R=-0.32, p=0.009, Figure 3) and changes in insulin levels at 120' during the OGTT (R=-0.26, p=0.036).

After six months of vitamin D supplementation chemerin levels correlated significantly with WHR (R=0.42, p=0.002), WHtR (R=0.38, p=0.006) and tended to be positively associated with % FAT skinfolds (p=0.052) and waist circumference (p=0.068). Among biochemical parameters six months chemerin levels correlated significantly with insulin at 30' during the OGTT (R=0.25, p=0.043), TG (R=0.25, p=0.042), TG/HDL-C ratio (R=0.25, p=0.044) and ALT (R=0.39, p=0.012, Figure 4) in that period.

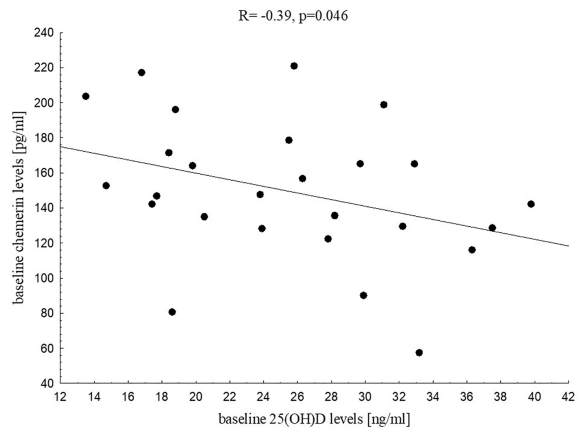


FIGURE 2
Correlation between 25(OH)D and chemerin levels in the control group at baseline.

We investigated also how many patients in the study group presented dyslipidaemia and hyperuricemia, and if there were any significant associations between vitamin D and chemerin in these subgroups. At baseline dyslipidaemia was observed in 87% of children and hyperuricemia in 37,5%. After six months of vitamin D supplementation dyslipidaemia was found in 83% of the study group and hyperuricemia in 30%. Unfortunately, we did not find any statistically significant relationships.

Summary of the multivariable backward linear regression models build for the study group

Based on findings of Spearman correlation analysis we build some multivariable backward linear regression models for the study group using baseline 25(OH)D values, baseline chemerin and six months chemerin values as the dependent variables, as appropriate.

The first model included baseline vitamin D (as dependent variable) and baseline anthropometric parameters such as body mass SDS, hip circumference, BMI SDS and waist circumference as independent variables. This model was statistically significant with cumulative R^2 = 0.10, p=0.011 and identified hip circumference as the parameter significantly negatively associated with baseline 25(OH)D levels. The received correlation coefficient was equal β=-0.320 ± 0.122 with 95% CI from -0.564 to -0.075.

The second model included baseline vitamin D (as dependent variable) and baseline fasting insulin, HOMA-IR, HDL-C and uric acid as independent variables. The model was statistically significant with cumulative R^2 = 0.20, p=0.001 and identified only HDL-C level as the parameter significantly positively associated with baseline 25(OH)D level. The received correlation coefficient was equal β=0.443 ± 0.122 with 95% CI from 0.199 to 0.688.

Next two models included baseline chemerin level as dependent variable and respectively baseline % FAT BIA, % FAT skinfolds, WHtR and BMI SDS (third model) and baseline insulin at 60' during the OGTT, insulin at 120' during the OGTT, ALT and TG/HDL-C as independent variables (fourth model). The third model was statistically significant with cumulative R^2 = 0.09, p=0.023. In that model WHtR was identified as one factor significantly positively associated with baseline chemerin. The received correlation coefficient was equal β=0.294 ± 0.126 with 95% CI from 0.043 to 0.545. The fourth model wasn't statistically significant with cumulative R^2 = 0.03, p=0.814.

Chemerin values after six months of vitamin D supplementation were used as dependent variable in the fifth (WHR, WHtR, % FAT skinfolds at six months as independent variables) and sixth model (TG, TG/HDL-C, ALT at six months as independent variables). The fifth model was statistically significant with cumulative R^2 = 0.14, p=0.007 and identified WHtR at six months of vitamin D supplementation as a parameter significantly positively associated with chemerin level in that period. The received correlation coefficient was equal β=0.379 ± 0.135 with 95% CI from 0.108 to 0.651. The sixth model was statistically significant with cumulative R^2 = 0.14, p=0.016 and revealed ALT as

10.3389/fendo.2023.1143755

TABLE 3 Comparison between anthropometric parameters in the study group at baseline and after six months of vitamin D supplementation.

	BASELINE	SIX MONTHS	p value
Height SDS	0.6 ± 1.25	0.6 ± 1.25	ns
Weight SDS	2.3 ± 0.67	2.2 ± 0.76	ns
WC (cm)	90.9 ± 10.27	89.7 ± 11.07	ns
HC (cm)	106.2 ± 10.60	106.6 ± 11.80	ns
WHR	0.9 ± 0.06	0.9 ± 0.05	ns
WHtR	0.56 (0.5-0.6)	0.54 (0.5-0.6)	< 0.001
% FAT (skinfolds)	35.7 (32.1-39.7)	34.3 (29.9-38.1)	< 0.001
% FAT BIA	40.4 ± 7.61	38.3 ± 8.93	ns
BMI SDS	2.3 ± 0.47	2.2 ± 0.56	ns

Data are presented as means ± standard deviations score or as median with interquartile range, as appropriate. SDS, standard deviation score; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; %FAT (skinfolds), percentage of body fat estimated from skinfolds; %FAT BIA, percentage of body fat estimated using bioelectrical impedance analysis method; BMI, body mass index; ns, not significant.

a factor significantly positively associated with chemerin at six months. The received correlation coefficient was equal β=0.378 ± 0.150 with 95% CI from 0.073 to 0.682.

Discussion

In our study we aimed to investigate the relationships between vitamin D status, chemerin and metabolic prolife parameters among overweight and obese children. Our analysis confirmed significantly lower vitamin D levels coincided with higher chemerin levels in patients with excess body fat compared to the controls, but we did not find any direct relationships between vitamin D status and chemerin in those children. This results stand in line with previous studies (25, 43–46). We noticed that baseline vitamin D was related mainly to fasting insulin, insulin resistance indices, HDL-C and uric acid, whereas baseline chemerin was positively associated with insulin secretion after glucose intake in OGTT and ALT activity in the study group. Both vitamin D and chemerin were related to nutritional status parameters, vitamin D mainly to BMI SDS and hip circumference, while chemerin was related to WHtR and fat mass expressed as %FAT BIA and %FAT skinfolds. Interestingly, in the control group with normal nutritional status parameters, significant negative relationship between 25(OH)D and chemerin was found. In overweight and obese children we observed a tendency for negative association between chemerin and 25(OH)D levels, but only after improvement in vitamin D status after six months of supplementation.

The relationships between excess body fat, insulin resistance, inflammation, dyslipidaemia and vitamin D deficit are intensively studied. Obesity-related insulin resistance and low-grade chronic inflammation seem to be the most important predictive factors for the development of complications of overweight and obesity such as metabolic syndrome, type 2 diabetes mellitus, cardiovascular disease and osteopenia/osteoporosis (47). The associations between vitamin D status and insulin sensitivity parameters in obese pediatric population have been previously reported (12, 48–53). The effects of vitamin D on glucose homeostasis, exerted mainly by its active form 1,25-dihydroxycholecalciferol, include the increase of peripheral and hepatic glucose uptake, the improvement in synthesis and secretion of insulin, the protection of β-cells from cytokine induced apoptosis and attenuation of inflammation (47, 54–56). It has been confirmed that insulin resistance affects obesity-related pro-atherogenic changes in lipid profile (57, 58). Insulin resistance impairs the function of insulin-dependent hormone-sensitive lipase and lipoprotein lipase, which are involved in lipid metabolism, whereas insulin cannot act effectively due to obesity and dyslipidaemia (59, 60). The study by Wang et al. (61) including nearly three hundred prepubertal and pubertal, normal and overweight/obese children and adolescents, demonstrated significant association between HOMA-IR and BMI and serum 25(OH)D level based on the stepwise multiple linear regression analysis of age, sex, pubertal maturation, BMI, WHtR, TG, total cholesterol, HDL-C, LDL-C, 25(OH)D and HOMA-IR. Several studies showed also the association between lipid profile, CRP, uric acid and vitamin D deficit, but the serum 25(OH)D level, predictive for vitamin D deficit-dependent metabolic disorders, has not been strictly defined (11, 62–68). Reis et al. (69), based on cross-sectional analysis of a group of more than three thousand adolescents, reported that individuals with 25(OH)D levels lower than 15 ng/ml are more likely to have fasting hyperglycemia, low HDL-C, hypertriglyceridemia, hypertension and metabolic syndrome compared to adolescents with 25(OH)D levels above 26 ng/ml. Rusconi et al. (62), among a group of more than one hundred obese children with 25(OH)D levels above or below 20 ng/ml, found higher total cholesterol and LDL-C levels in the group with vitamin D deficit. It should be noted that the stage of puberty also affects the relationship between vitamin D and the components of metabolic syndrome in obese children (53, 70, 71). Pires et al. (53) reported that the significant increase in TG, fasting insulin and HOMA-IR, observed during puberty, was related to a decrease in 25(OH)D levels independent of sex, body mass and pubertal Tanner stage.

Metabolic effects of vitamin D supplementation seem to be dependent on the dose and time of intervention. Our analysis showed that six months of vitamin D supplementation between 2000 to 4000 IU/day led mainly to a significant decrease in CRP, total cholesterol and aminotransferases activity. We found also that the increase in 25(OH)D levels resulted in a decrease in fasting glucose and insulin secretion at 120' during the OGTT. Moreover, we noticed that after improvement of 25(OH)D level, a tendency for negative correlation between vitamin D status and chemerin appeared. Nader et al. (11) showed that twelve weeks of 2000 IU/day vitamin D supplementation in obese adolescents did not lead to detectable changes in fasting glucose, fasting insulin, HOMA-IR, lipids and highly sensitive CRP. On the other hand, the retrospective observational study by Pecoraro et al. (9), including overweight and obese children with 25(OH)D levels below 25 ng/ml, who underwent oral vitamin D supplementation (100 000 IU, one vial/month) for six months, indicated that vitamin D

10.3389/fendo.2023.1143755

TABLE 4 Comparison between biochemical parameters in the study group at baseline and after six months of vitamin D supplementation.

	BASELINE	SIX MONTHS	p value
25(OH)D (ng/ml)	16.0 (12.6 - 20.0)	27.1 (22.9 - 32.3)	< 0.001
Total cholesterol (mg/dl)	161.0 (142.0 - 181.0)	156.0 (139.0 - 171.0)	<0.05
Triglycerides (TG) (mg/dl)	108.0 (83.0 - 161.0)	112.0 (84.0 - 150.0)	ns
TG/HDL-C ratio	2.5 (2.0-4.2)	2.7 (1.9 - 3.7)	ns
LDL-C (mg/dl)	94.3 ± 27.1	88.8 ± 25.8	ns
HDL-C (mg/dl)	43.4 ± 11.1	44.3 ± 11.8	ns
CRP (mg/dl)	0.5 – (0.5-0.7)	0.5 (0.5 - 0.5),	<0.01
Glucose fasting (mg/dl)	86.0 (82.0 - 91.0)	85.2 (82.0 - 88.3)	ns
Glucose 30' OGTT (mg/dl)	139.0 (127.0 - 156.3)	135.5 (122.2 - 155.8)	ns
Glucose 60' OGTT (mg/dl)	120.0 (106.0 - 150.5)	120.6 (103.7 - 141.4)	ns
Glucose 90' OGTT (mg/dl)	116.0 (102.9 - 136.8)	112.0 (99.0 - 127.0)	ns
Glucose 120' OGTT (mg/dl)	110.0 (99.4 - 125.4)	105.8 (92.6 - 120.0)	ns
Glucose 150' OGTT (mg/dl)	91.0 (79.0 - 106.2)	85.4 (76.0 - 104.0)	<0.05
Glucose 180' OGTT (mg/dl)	75.9 (67.5 - 93.5)	75.0 (67.0 - 85.0)	ns
Insulin fasting (uIU/ml)	14.7 (9.1 - 25.6)	14.6 (9.9 - 23.6)	ns
Insulin 30' OGTT (uIU/ml)	93.7 (66.1 - 152.0)	91.8 (70.4 - 178.0)	ns
Insulin 60' OGTT (uIU/ml)	92.2 (62.8 - 143.0)	96.9 (67.8 - 145.0)	ns
Insulin 90' OGTT (uIU/ml)	89.8 (58.3 - 135.0)	81.7 (53.8 - 124.0)	ns
Insulin 120' OGTT (uIU/ml)	76.3 (54.6 - 121.0)	72.0 (49.5 - 122.0)	ns
Insulin 150' OGTT (uIU/ml)	61.3 (34.3 - 108.0)	45.9 (33.1 - 85.8)	ns
Insulin 180' OGTT (uIU/ml)	35.0 (20.3 - 56.3)	25.3 (17.6 - 45.3)	ns
HOMA-IR	3.0 (1.9 - 5.4)	3.4 (2.0 - 5.0)	ns
QUICKI	0.3 (0.3 - 0.4)	0.3 (0.3 - 0.3)	ns
HbAle (%)	5.2 (5.0 - 5.4)	5.2 (5.0 - 5.5)	ns
chemerin (pg/ml)	212.0 (177.5 - 238.3)	206.8 (180.5 - 236.0)	ns
AST (U/I)	28.0 (23.0 - 35.0)	25.0 (23.0 - 30.0)	<0.01
ALT (U/I)	27.0 (21.0 - 36.0)	25.5 (20.0 - 35.0)	< 0.01
Uric acid (mg/dl)	5.85 (5.3 - 6.8)	5.9 (5.3 - 6.9)	ns

Data are presented as means ± standard deviations score or as median with interquartile range, as appropriate. 25(OH)D, 25-hydroxyvitamin D; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CRP, C-reactive protein; HOMA-IR, Homeostasis Model Assessment- Insulin Resistance, QUICKI, Quantitative Insulin Sensitivity Check Index; HbA1c, Glycated Hemoglobin, AST, aspartate aminotransferase; ALT, alanine aminotransferase; ns, not significant.

supplementation was associated with a significant decrease in total cholesterol, LDL-C and ALT serum levels and an increase in HDL-C.

In our study we also aimed to investigate the role of chemerin as a factor which could link vitamin D, insulin resistance and dyslipidaemia in overweight and obese children and adolescents. Those dependencies are not widely described, but some experimental studies suggest that protective effects of vitamin D supplementation could be exerted by reduction in chemerin levels (13, 14, 72). The number of clinical studies in that field is scarce. The cross-sectional study by Reyman et al. (12) revealed that vitamin D deficient obese children have significantly lower insulin sensitivity coexisted with higher chemerin, cathepsin S and soluble vascular adhesion molecule, which are known as pro-inflammatory, pro-diabetic and pro-atherogenic factors.

The mechanisms explaining the relationship between vitamin D status and chemerin are still not fully understood. Our multivariable backward linear regression models revealed WHtR and ALT activity as factors significantly positively associated with chemerin levels, both baseline and after six months of vitamin D supplementation. Based on our results we could presume that the relation between chemerin and body fat and hepatic steatosis is stronger than its association with

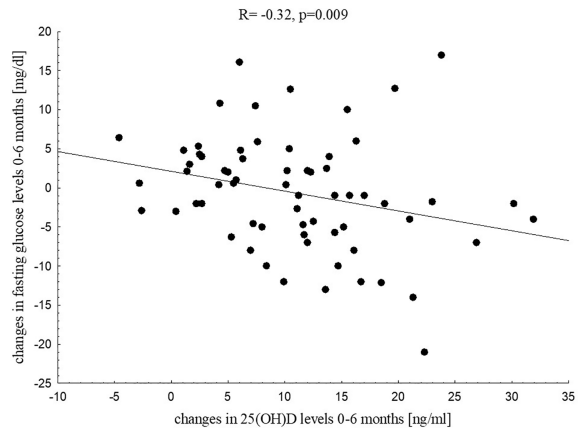


FIGURE 3
Correlation between changes in 25(OH)D levels and changes in fasting glucose in the study group after six months of vitamin D supplementation.

vitamin D, especially in vitamin D deficient obese children (median 25(OH)D 16 ng/ml). Improvement of vitamin D status in our study group (median 25(OH)D 27.1 ng/ml) revealed a tendency for negative relationship between 25(OH)D and chemerin. In our control group with normal body weight and median 25(OH)D 25.7 ng/ml the significant negative correlation between 25(OH)D and chemerin levels was observed.

The study by Niklowitz et al. (25), including nearly one hundred obese children, confirmed that weight loss was associated with a decrease of chemerin levels and improvement of metabolic syndrome parameters such as insulin, HDL-C and TG. The associations between chemerin and weight loss were reported in several studies in obese pediatric and adult population (73–77). A study by Liu et al. (75) conducted among obese female adolescents showed that chemerin reduction achieved as a result of lifestyle changes correlated positively with fasting glucose, fasting insulin, HOMA-IR, TG and total cholesterol.

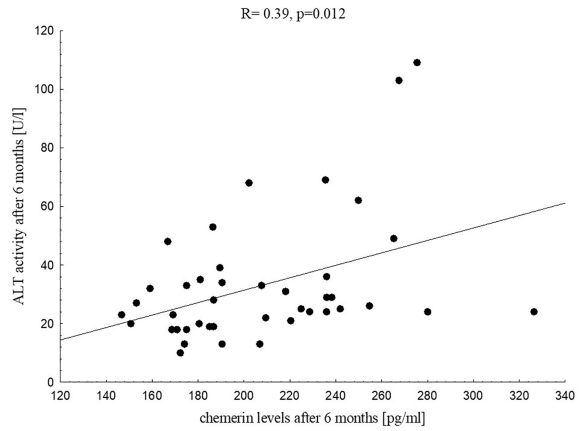


FIGURE 4
Correlation between chemerin levels and ALT activity in the study group after six months of vitamin D supplementation.

Gad et al. (78) revealed higher chemerin serum levels in obese children with metabolic syndrome. In his study chemerin positively correlated with fasting blood glucose and negatively with HDL-C. Other studies confirmed positive relationship between chemerin and insulin and HOMA-IR (25–27). In our study chemerin was related to insulin levels secreted in the response to OGTT, this relationship was present both before and after six months of vitamin D supplementation. Our study showed also positive correlation between chemerin and TG and TG/HDL-C ratio after the intervention.

Our study revealed positive correlation between chemerin levels and ALT activity. Elevated liver enzymes are the markers of non-alcoholic fatty liver disease (NAFLD), which is considered a liver manifestation of metabolic syndrome (79). Hamza et al. (44) among a group of fifty obese children also found a positive correlation between chemerin and liver enzymes, moreover he noticed that the increase in chemerin levels correlated positively with NAFLD severity.

Our study showed that vitamin D and chemerin are involved in metabolic processes. Taking into account that both of them affect adipose tissue and bone tissue, there may be some considerable overlap between their action, but it requires further investigation. We intend to continue our study on larger population and in longer follow-up.

Conclusion

Our study confirmed that vitamin D has positive effect on metabolic profile in overweight and obese children. The relationship between vitamin D and chemerin is not clear, nevertheless we have observed a tendency to decrease chemerin concentrations after improving vitamin D status, even without a significant reduction in body fat mass. Taking into account the role of chemerin as early indicator of obesity-related diseases, the studies in this field seem to be valuable.

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.

Ethics statement

The studies involving human participants were reviewed and approved by Bioethics Committee at the Medical University of Warsaw (decision number KB/257/2013). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MK, EW-S and MR contributed to conception and design of the study. MK and MR organized the database. MK and EW-S prepared the tables. AM performed anthropometric measurements. MK and

AS-E took measurements of serum chemerin levels. MK, EW-S and MS performed statistical analysis. MK and EW-S wrote the first draft of the manuscript. BP, AK supervised the work. MK and EW-S wrote the final version of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The article was not funded by a grant.

Acknowledgments

The authors would like to thank the nurse Małgorzata Przybylska for her contribution in blood sample collection.

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

References

1. Thomas MM, Zaki ME, Youness E, Hamed K, Khedr AA, Abd El-Massieh PM, et al. Measurement of serum chemerin, oxidized LDL, and vitamin d levels in prader-willi syndrome: a cross-sectional study in pediatric Egyptian patients. *J Child Sci* (2020) 10:187–95. doi: 10.1055/s-0040-1718896

2. Blüher M, Mantzoros CS. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. *Metabolism* (2015) 64 (1):131–45. doi: 10.1016/j.metabol.2014.10.016

3. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. *Arch Med Sci* (2013) 9(2):191–200. doi: 10.5114/aoms.2013.33181

4. Booth A, Magnuson A, Fouts J, Foster M. Adipose tissue: an endocrine organ playing a role in metabolic regulation. *Horm Mol Biol Clin Investig* (2016) 26(1):25–42. doi: 10.1515/hmbci-2015-0073

5. Hui HX, Feng T. Adipose tissue as an endocrine organ. *London: IntechOpen* (2018). doi: 10.5772/intechopen.76220

6. 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 (Lausanne)* (2019) 1:103(10). doi: 10.3389/fendo.2019.00103

7. Gharib AF, El Askary A, Almehmadi M, Elsawy WE, Allam HH, Elsayyad LK, et al. Association of vitamin d deficiency and inflammatory cytokines with the clinicopathological features of breast cancer in female Saudi patients. *Eur J Inflammation* (2022) 20:1–23. doi: 10.1177/1721727X221106507

8. Szymczak-Pajor I, Miazek K, Selmi A, Balcerczyk A, Śliwińska A. The action of vitamin d in adipose tissue: is there the link between vitamin d deficiency and adipose tissue-related metabolic disorders? *Int J Mol Sci* (2022) 23(2):956. doi: 10.3390/ijms23020956

9. Pecoraro L, Nisi F, Serafin A, Antoniazzi F, Dalle Carbonare L, Piacentini G, et al. Vitamin d supplementation in the assessment of cardiovascular risk factors in overweight and obese children. *Med Sci (Basel)* (2022) 10(3):49. doi: 10.3390/medsci10030049

10. Kelishadi R, Salek S, Salek M, Hashemipour M, Movahedian M. Effects of vitamin d supplementation on insulin resistance and cardiometabolic risk factors in children with metabolic syndrome: a triple-masked controlled trial. *J Pediatr (Rio J)* (2014) 90(1):28–34. doi: 10.1016/j.jped.2013.06.006

11. Nader NS, Aguirre Castaneda R, Wallace J, Singh R, Weaver A, Kumar S. Effect of vitamin D3 supplementation on serum 25(OH)D, lipids and markers of insulin resistance in obese adolescents: a prospective, randomized, placebo-controlled pilot trial. *Horm Res Paediatr* (2014) 82(2):107–12. doi: 10.1159/000362449

12. Reyman M, Verrijn Stuart AN, van Summeren M, Rakhshandehroo M, Nuboer R, de Boer FK, et al. Vitamin d deficiency in childhood obesity is associated with high levels of circulating inflammatory mediators, and low insulin sensitivity. *Int J Obes (Lond)* (2014) 38(1):46–52. doi: 10.1038/ijo.2013.75

13. Nassar SZ, Badae NM. Protective effect of vitamin d supplementation in a rat modal of preeclampsia: a possible implication of chemerin. *Hypertens Pregnancy* (2019) 38(3):149–56. doi: 10.1080/10641955.2019.1597108

14. Said AA, Moursi SMM. Vitamin d supplementation reduces serum chemerin level in gestational diabetes mellitus rat model. *Med J Cairo Univ* (2019) 87(5):3069–80. doi: 10.21608/mjcu.2019.59509

15. Buechler C, Feder S, Haberl EM, Aslanidis C. Chemerin isoforms and activity in obesity. *Int J Mol Sci* (2019) 20(5):1128. doi: 10.3390/ijms20051128

16. Yu M, Yang Y, Huang C, Ge L, Xue L, Xiao Z, et al. Chemerin: a functional adipokine in reproductive health and diseases. *Biomedicines* (2022) 10(8):1910. doi: 10.3390/biomedicines10081910

17. Muruganandan S, Sinal CJ. The impact of bone marrow adipocytes on osteoblast and osteoclast differentiation. *IUBMB Life* (2014) 66:147–55. doi: 10.1002/iub.1254

18. Zdanowicz K, Bobrus-Chociej A, Lebensztejn DM. Chemerin as potential biomarker in pediatric diseases: a PRISMA-compliant study. *Biomedicines* (2022) 10 (3):591. doi: 10.3390/biomedicines10030591

19. Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD, et al. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. *J Biol Chem* (2007) 282(38):28175–188. doi: 10.1074/jbc.M700793200

20. Zabel BA, Kwitniewski M, Banas M, Zabieglo K, Murzyn K, Cichy J. Chemerin regulation and role in host defense. *Am J Clin Exp Immunol* (2014) 3(1):1–19.

21. Acewicz M, Kasacka I. Chemerin activity in selected pathological states of human body - a systematic review. *Adv Med Sci* (2021) 66(2):270–8. doi: 10.1016/j.advms.2021.05.002

22. Su X, Cheng Y, Zhang G, Wang B. Chemerin in inflammatory diseases. *Clin Chim Acta* (2021) 517:41–7. doi: 10.1016/j.cca.2021.02.010

23. Treeck O, Buechler C, Ortmann O. Chemerin and cancer. *Int J Mol Sci* (2019) 20 (15):3750. doi: 10.3390/ijms20153750

24. Shin WJ, Zabel BA, Pachynski RK. Mechanisms and functions of chemerin in cancer: potential roles in therapeutic intervention. *Front Immunol* (2018) 9:2772. doi: 10.3389/fimmu.2018.02772

25. Niklowitz P, Rothermel J, Lass N, Barth A, Reinehr T. Link between chemerin, central obesity, and parameters of the metabolic syndrome: findings from a longitudinal study in obese children participating in a lifestyle intervention. *Int J Obes (Lond)* (2018) 42(10):1743–52. doi: 10.1038/s41366-018-0157-3

26. Ba HJ, Xu LL, Qin YZ, Chen HS. Serum chemerin levels correlate with determinants of metabolic syndrome in obese children and adolescents. *Clin Med Insights Pediatr* (2019) 5(13):1179556519853780. doi: 10.1177/1179556519853780

27. Sledzińska M, Szlagatys-Sidorkiewicz A, Brzezinski M, Kaźmierska K, Sledziński T, Kamińska B. Serum chemerin in children with excess body weight may be associated with ongoing metabolic complications - a pilot study. *Adv Med Sci* (2017) 62(2):383–6. doi: 10.1016/j.advms.2017.04.002

28. Rourke JL, Dranse HJ, Sinal CJ. Towards an integrative approach to understanding the role of chemerin in human health and disease. *Obes Rev* (2013) 14(3):245–62. doi: 10.1111/obr.12009

29. Maghsoudi Z, Kelishadi R, Hosseinzadeh-Attar MJ. Association of chemerin levels with anthropometric indexes and c-reactive protein in obese and non-obese adolescents. *ARYA Atheroscler* (2015) 11(Suppl 1):102–8.

30. Menzel J, Biemann R, Aleksandrova K, Schulze MB, Boeing H, Isermann B, et al. The cross-sectional association between chemerin and bone health in peri/pre and postmenopausal women: results from the EPIC-potsdam study. *Menopause* (2018) 25 (5):574–8. doi: 10.1097/GME.0000000000001041

31. Muruganandan S, Roman AA, Sinal CJ. Role of chemerin/CMKLR1 signaling in adipogenesis and osteoblastogenesis of bone marrow stem cells. *J Bone Miner Res* (2010) 25:222–34. doi: 10.1359/jbmr.091106

32. Muruganandan S, Dranse HJ, Rourke JL, McMullen NM, Sinal CJ. Chemerin neutralization blocks hematopoietic stem cell osteoclastogenesis. *Stem Cells* (2013) 31:2172–82. doi: 10.1002/stem.1450

33. Shi L, Mao C, Wang X, Liu R, Li L, Mou X, et al. Association of chemerin levels and bone mineral density in Chinese obese postmenopausal women. *Med (Baltimore)* (2016) 95(35):e4583. doi: 10.1097/MD.0000000000004583

34. Jiang XY, Wang Q, Zhang Y, Chen Y, Wu LF. Association of high serum chemerin with bone mineral density loss and osteoporotic fracture in elderly Chinese women. *Int J Womens Health* (2022) 2(14):107–18. doi: 10.2147/IJWH.S337985

35. Ostrowska Z, Morawiecka-Pietrzak M, Pluskiewicz W, Świętochowska E, Strzelczyk J, Gołąbek K, et al. The relationship between chemerin, bone metabolism, the RANKL/RANK/OPG system, and bone mineral density in girls with anorexia nervosa. *Endokrynol Pol* (2022) 73(1):26–34. doi: 10.5603/EP.a2021.0103

36. Rusińska A, Płudowski P, Walczak M, Borszewska-Kornacka MK, Bossowski A, Chlebna-Sokoił D, et al. Vitamin d supplementation guidelines for general population and groups at risk of vitamin d deficiency in Poland - recommendations of the polish society of pediatric endocrinology and diabetes and the expert panel with participation of national specialist consultants and representatives of scientific societies-2018 update. *Front Endocrinol (Lausanne)* (2018) 9:246. doi: 10.3389/fendo.2018.00246

37. Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, et al. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol* (1988) 60(5):709–23.

38. Kułaga Z, Litwin M, Tkaczyk M, Palczewska I, Zajączkowska M, Zwolińska D, et al. Polish 2010 growth references for school-aged children and adolescents. *Eur J Pediatr* (2011) 170(5):599–609. doi: 10.1007/s00431-010-1329-x

39. Cole TJ. The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* (1990) 44(1):45–60.

40. Physical status: the use and interpretation of anthropometry. report of a WHO expert committee. *World Health Organ Tech Rep Ser* (1995) 854:1–452.

41. Szapary PO, Bloedon LT, Samaha FF, Duffy D, Wolfe ML, Soffer D, et al. Effects of pioglitazone on lipoproteins, inflammatory markers, and adipokines in nondiabetic patients with metabolic syndrome. *Arterioscler Thromb Vasc Biol* (2006) 26(1):182–8. doi: 10.1161/01.ATV.0000195790.24531.4f

42. Kwiterovich POJr. Recognition and management of dyslipidemia in children and adolescents. *J Clin Endocrinol Metab* (2008) 93(11):4200–9. doi: 10.1210/jc.2008-1270

43. Fiamenghi VI, Mello ED. Vitamin d deficiency in children and adolescents with obesity: a meta-analysis. *J Pediatr (Rio J)* (2021) 97(3):273–9. doi: 10.1016/j.jped.2020.08.006

44. Hamza RT, Elkabbany ZA, Shedid AM, Hamed AI, Ebrahim AO. Serum chemerin in obese children and adolescents before and after l-carnitine therapy: relation to nonalcoholic fatty liver disease and other features of metabolic syndrome. *Arch Med Res* (2016) 47(7):541–9. doi: 10.1016/j.arcmed.2016.11.010

45. Rowicka G, Dyląg H, Chełchowska M, Weker H, Ambroszkiewicz J. Serum calprotectin and chemerin concentrations as markers of low-grade inflammation in prepubertal children with obesity. *Int J Environ Res Public Health* (2020) 17(20):7575. doi: 10.3390/ijerph17207575

46. Nassar MF, Emam EK, Allam MF. Is there a benefit of vitamin d supplementation in deficient children and adolescents suffering from obesity? a meta-analysis. *Glob Pediatr Health* (2021) 8:2333794X211018352. doi: 10.1177/2333794X211018352

47. Peterson CA. Vitamin d deficiency and childhood obesity: interactions, implications, and recommendations. *Nutr Diet Suppl* (2015) 7:29–39. doi: 10.2147/NDS.S52024

48. Delvin E, Lambert M, Levy E, O'Loughlin J, Mark S, Gray-Donald K, et al. Vitamin d status is modestly associated with glycemia and indicators of lipid metabolism in French-Canadian children and adolescents. *J Nutr* (2010) 140(5):987–91. doi: 10.3945/jn.109.112250

49. Buyukinan M, Ozen S, Kokkun S, Saz EU. The relation of vitamin d deficiency with puberty and insulin resistance in obese children and adolescents. *J Pediatr Endocrinol Metab* (2012) 25(1-2):83–7. doi: 10.1515/jpem-2011-0426

50. Khadgawat R, Thomas T, Gahlot M, Tandon N, Tangpricha V, Khandelwal D, et al. The effect of puberty on interaction between vitamin d status and insulin resistance in obese Asian-Indian children. *Int J Endocrinol* (2012) 2012:173581. doi: 10.1155/2012/173581

51. Olson ML, Maalouf NM, Oden JD, White PC, Hutchison MR. Vitamin d deficiency in obese children and its relationship to glucose homeostasis. *J Clin Endocrinol Metab* (2012) 97(1):279–85. doi: 10.1210/jc.2011-1507

52. Corica D, Zusi C, Olivieri F, Marigliano M, Piona C, Fornari E, et al. Vitamin d affects insulin sensitivity and β-cell function in obese non-diabetic youths. *Eur J Endocrinol* (2019) 181(4):439–40. doi: 10.1530/EJE-19-0369

53. Pires LV, González-Gil EM, Anguita-Ruiz A, Bueno G, Gil-Campos M, Vázquez-Cobela R, et al. The vitamin d decrease in children with obesity is associated with the development of insulin resistance during puberty: the PUBMEP study. *Nutrients* (2021) 13(12):4488. doi: 10.3390/nu13124488

54. Peterson CA, Tosh AK, Belenchia AM. Vitamin d insufficiency and insulin resistance in obese adolescents. *Ther Adv Endocrinol Metab* (2014) 5(6):166–89. doi: 10.1177/2042018814547205

55. Bornstedt ME, Gjerlaugsen N, Pepaj M, Bredahl MKL, Thorsby PM. Vitamin d increases glucose stimulated insulin secretion from insulin producing beta cells (INS1E). *Int J Endocrinol Metab* (2019) 17(1):e74255. doi: 10.5812/ijem.74255

56. Szymczak-Pajor I, Drzewoski J, Śliwińska A. The molecular mechanisms by which vitamin d prevents insulin resistance and associated disorders. *Int J Mol Sci* (2020) 21(18):6644. doi: 10.3390/ijms21186644

57. Jago R, Harrell JS, McMurray RG, Edelstein S, El Ghormli L, Bassin S. Prevalence of abnormal lipid and blood pressure values among an ethnically diverse population of eighth-grade adolescents and screening implications. *Pediatrics* (2006) 117(6):2065–73. doi: 10.1542/peds.2005-1716

58. Chung ST, Katz LEL, Stettler-Davis N, Shults J, Sherman A, Ha J, et al. The relationship between lipoproteins and insulin sensitivity in youth with obesity and abnormal glucose tolerance. *J Clin Endocrinol Metab* (2022) 107(6):1541–51. doi: 10.1210/clinem/dgac113

59. Holland WL, Bikman BT, Wang LP, Yuguang G, Sargent KM, Bulchand S, et al. Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. *J Clin Invest* (2011) 121(5):1858–70. doi: 10.1172/JCI43378

60. Erol M, Bostan Gayret Ö, Hamilçıkan Ş, Can E, Yiğit ÖL Vitamin d deficiency and insulin resistance as risk factors for dyslipidemia in obese children. *Arch Argent Pediatr* (2017) 115(2):133–9. doi: 10.5546/aap.2017.eng.133

61. Wang L, Wang H, Wen H, Tao H, Zhao X. Relationship between HOMA-IR and serum vitamin d in Chinese children and adolescents. *J Pediatr Endocrinol Metab* (2016) 29(7):777–81. doi: 10.1515/jpem-2015-0422

62. Rusconi RE, De Cosmi V, Gianluca G, Giavoli C, Agostoni C. Vitamin d insufficiency in obese children and relation with lipid profile. *Int J Food Sci Nutr* (2015) 66(2):132–4. doi: 10.3109/09637486.2014.959902

63. Kim MR, Jeong SJ. Relationship between vitamin d level and lipid profile in non-obese children. *Metabolites* (2019) 9(7):125. doi: 10.3390/metabo9070125

64. Rodríguez-Rodríguez E, Aparicio A, Andrés P, Ortega RM. Moderate vitamin d deficiency and inflammation related markers in overweight/obese schoolchildren. *Int J Vitam Nutr Res* (2014) 84(1-2):98–107. doi: 10.1024/0300-9831/a000197

65. Krajewska M, Witkowska-Sędek E, Rumińska M, Stelmaszczyk-Emmel A, Sobol M, Majcher A, et al. Vitamin d effects on selected anti-inflammatory and pro-inflammatory markers of obesity-related chronic inflammation. *Front Endocrinol (Lausanne)* (2022) 13:920340. doi: 10.3389/fendo.2022.920340

66. Bellia A, Garcovich C, D'Adamo M, Lombardo M, Tesauro M, Donadel G, et al. Serum 25-hydroxyvitamin d levels are inversely associated with systemic inflammation in severe obese subjects. *Intern Emerg Med* (2013) 8(1):33–40. doi: 10.1007/s11739-011-0559-x

67. Isnuwardana R, Bijukchhe S, Thadanipon K, Ingsathit A, Thakkinstian A. Association between vitamin d and uric acid in adults: a systematic review and meta-analysis. *Horm Metab Res* (2020) 52(10):732–41. doi: 10.1055/a-1240-5850

68. Wojcik M, Janus D, Kalicka-Kasperczyk A, Sztefko K, Starzyk JB. The potential impact of the hypovitaminosis d on metabolic complications in obese adolescents - preliminary results. *Ann Agric Environ Med* (2017) 24(4):636–9. doi: 10.5604/12321966.1230676

69. Reis JP, von Mühlen D, Miller ERIII, Michos ED, Appel LJ. Vitamin d status and cardiometabolic risk factors in the united states adolescent population. *Pediatrics* (2009) 124(3):e371–9. doi: 10.1542/peds.2009-0213

70. Cediel G, Corvalán C, López de Romaña D, Mericq V, Uauy R. Prepubertal adiposity, vitamin d status, and insulin resistance. *Pediatrics* (2016) 138(1):e20160076. doi: 10.1542/peds.2016-0076

71. Saneifard H, Shakiba M, Sheikhy A, Baniadam L, Abdollah Gorji F, Fallahzadeh A. Vitamin d deficiency in children and adolescents: role of puberty and obesity on vitamin d status. *Nutr Metab Insights* (2021) 14:11786388211018726. doi: 10.1177/11786388211018726

72. Albanesi C, Scarponi C, Pallotta S, Daniele R, Bosisio D, Madonna S, et al. Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment. *J Exp Med* (2009) 206(1):249–58. doi: 10.1084/jem.20080129

73. Marti A, Martínez I, Ojeda-Rodríguez A, Azcona-Sanjulian MC. Higher lipopolysaccharide binding protein and chemerin concentrations were associated with metabolic syndrome features in pediatric subjects with abdominal obesity during a lifestyle intervention. *Nutrients* (2021) 13(2):289. doi: 10.3390/nu13020289

74. Chakaroun R, Raschpichler M, Klöting N, Oberbach A, Flehming G, Kern M, et al. Effects of weight loss and exercise on chemerin serum concentrations and adipose tissue expression in human obesity. *Metabolism* (2012) 61(5):706–14. doi: 10.1016/j.metabol.2011.10.008

75. Liu M, Lin X, Wang X. Decrease in serum chemerin through aerobic exercise plus dieting and its association with mitigation of cardio-metabolic risk in obese female adolescents. *J Pediatr Endocrinol Metab* (2018) 31(2):127–35. doi: 10.1515/jpem-2017-0431

76. Ress C, Tschoner A, Engl J, Klaus A, Tilg H, Ebenbichler CF, et al. Effect of bariatric surgery on circulating chemerin levels. *Eur J Clin Invest.* (2010) 40(3):277–80. doi: 10.1111/j.1365-2362.2010.02255.x

77. Saremi A, Shavandi N, Parastesh M, Daneshmand H. Twelve-week aerobic training decreases chemerin level and improves cardiometabolic risk factors in overweight and obese men. *Asian J Sports Med* (2010) 1(3):151–8. doi: 10.5812/asjsm.34860

78. Gad SS, Shora HA, Abdelwahab A, Abdou RM, Abdel Raouf BM, Elmikaty HA, et al. Chemerin, IL-18 and IL-1 beta as biomarkers of metabolic syndrome in Egyptian obese children. *ASMS* (2022) 6(7):66–7. doi: 10.31080/ASMS.2022.06.1310

79. Weihe P, Weihrauch-Blüher S. Metabolic syndrome in children and adolescents: diagnostic criteria, therapeutic options and perspectives. *Curr Obes Rep* (2019) 8 (4):472–9. doi: 10.1007/s13679-019-00357-x

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 10 May 2023
DOI 10.3389/fendo.2023.1124019

OPEN ACCESS

EDITED BY
Sally Radovick,
Rutgers, The State University of New Jersey, United States

REVIEWED BY
Tetyana Chaychenko,
Kharkiv National Medical University, Ukraine
Barbara Glowinska-Olszewska,
Medical University of Bialystok, Poland

*CORRESPONDENCE
Viktoriya Furdela
furdela_v@tdmu.edu.ua

RECEIVED 14 December 2022
ACCEPTED 19 April 2023
PUBLISHED 10 May 2023

CITATION
Furdela V, Pavlyshyn H, Shulhai A-M, Kozak K and Furdela M (2023) Triglyceride glucose index, pediatric NAFLD fibrosis index, and triglyceride-to-high-density lipoprotein cholesterol ratio are the most predictive markers of the metabolically unhealthy phenotype in overweight/obese adolescent boys.
Front. Endocrinol. 14:1124019.
doi: 10.3389/fendo.2023.1124019

Triglyceride glucose index, pediatric NAFLD fibrosis index, and triglyceride-to-high-density lipoprotein cholesterol ratio are the most predictive markers of the metabolically unhealthy phenotype in overweight/obese adolescent boys

Viktoriya Furdela[1*], Halyna Pavlyshyn[1], Anna-Mariia Shulhai[1], Kateryna Kozak[1] and Mykhailo Furdela[2]

[1]Department of Pediatrics No2, I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine, [2]Department of Pathologic Anatomy, Autopsy Course and Forensic Pathology, I.Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine

Introduction: The prevalence of obesity constantly increases worldwide and definitely increases the risk of premature death in early adulthood. While there is no treatment yet with proven efficacy for the metabolic clamp such as arterial hypertension, dyslipidemia, insulin resistance, diabetes type 2, and fatty liver disease, it is imperative to find a way to decrease cardiometabolic complications. Early prevention strategies beginning in childhood are the most logical step to reduce future cardiovascular morbidity and mortality. Therefore, the aim of the current study is to determine the most sensitive and specific predictive markers of the metabolically unhealthy phenotype with high cardiometabolic risk in overweight/obese adolescent boys.

Methods: This study was carried out at the Ternopil Regional Children's hospital (Western Ukraine) and involved 254 randomly chosen adolescent overweight or obese boys [median age was 16.0 (15.0,16.1) years]. A control group of 30 healthy children with proportional body weight comparable in gender and age to the main group was presented. A list of anthropometrical markers with biochemical values of carbohydrate and lipid metabolism with hepatic enzymes was determined. All overweight/obese boys were divided into three groups: 51.2% of the boys with metabolic syndrome (MetS) based on the IDF criteria; 19.7% of the boys were metabolically healthy obese (MHO) without hypertension, dyslipidemia, and hyperglycemia; and the rest of the boys (29.1%) were classified as metabolically unhealthy obese (MUO) with only one criterion (hypertension, dyslipidemia, or hyperglycemia).

10.3389/fendo.2023.1124019

Results: Based on multiple logistic regression analysis that included all anthropometric and biochemical values and calculated indexes in boys from the MHO group and MetS, it was revealed that the maximum likelihood in the prediction of MetS makes the combination of triglyceride glucose index, pediatric nonalcoholic fatty liver disease fibrosis index (PNFI), and triglyceride-to-high-density lipoprotein cholesterol ratio (R^2 =0.713, p<0.000). By tracing the receiver operating characteristic curve, the model is confirmed as a good predictor of MetS (AUC=0.898, odds ratio=27.111 percentage correct=86.03%) in overweight and obese boys.

Conclusion: Triglyceride glucose index, pediatric NAFLD fibrosis index, and triglyceride-to-high-density lipoprotein cholesterol ratio are a valuable combination of predictive markers of the metabolically unhealthy phenotype in Ukrainian overweight/obese boys.

KEYWORDS

MetS, TyG index, PNFI, predictive markers, obesity

1 Introduction

In the previous 20 years, the global childhood prevalence of obesity increased from 0.70% in 1975 to 5.60% in 2016 in girls and even higher in boys from 0.90% in 1975 to 7.80% in 2016 (1). In Ukraine, the prevalence of obesity in children was much lower, but a significant rise over the last decades has been seen from 0.08% among 0–18 years old in 2003 to 1.34% in 2016 (2), and in school-age children (6.0–18.9 years), the prevalence of obesity was 4.20% in 2018 by the World Health Organization (WHO) growth standard criteria (3). Obesity and metabolic syndrome (MetS), in particular, are associated with a higher risk of comorbidities such as arterial hypertension (AH), cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), liver disease, renal disease, psychological effects, and even cancer. Moreover, childhood obesity tracks well into adulthood and is tendentious to premature mortality in the case of MetS (4, 5). Obese children have a 3 times higher risk of mortality in early adulthood compared with the general population (6) and an 18 times higher risk of developing T2DM in young adulthood (7). The definition of MetS in childhood was provided by the International Diabetic Federation (IDF) in 2007 and is still widely used (8). Nevertheless, MetS remains a controversial topic in pediatrics for diagnostic criteria and treatment strategy. The attention of modern studies shifted from strictly MetS criteria in children to the differentiation between metabolically healthy and unhealthy phenotypes in overweight and obese children for the detection of early signs of CVD risk and prevention of progression (9, 10), and further studies are much needed.

Acknowledging the potential implications for public health, screening for well-known cardiometabolic comorbidities such as abdominal obesity, hyperglycemia, dyslipidemia, and fatty liver disease in overweight children is now recommended by the American Academy of Pediatrics (AAP) (9, 11), the European Society of Hypertension (12), and the Endocrine Society (13). However, in the last decade, multiple research studies have been done to find early and sensitive markers of the basic pathogenic component of MetS such as insulin resistance (IR) and impaired hepatic metabolism of lipids and carbohydrates in childhood, for instance, the homeostatic model assessment for insulin resistance (HOMA-IR) (14), triglyceride (TG)-to-high-density lipoprotein cholesterol (HDL-c) ratio, total cholesterol (TC)-to-HDL-c ratio (15–17), triglyceride glucose index (18–20), and markers of inflammation such as uric acid (10, 15, 21), alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and gamma-glutamyltransferase (GGT) (22–24). Most of the markers are sensitive and predictable in adults (21) but are still controversial in children of different ages.

Fatty liver disease is among the most common comorbidities in children with obesity (approximately 30%–70%) (25–27). Owing to the coexistence of abdominal obesity, dyslipidemia, and IR, nonalcoholic fatty liver disease (NAFLD) is considered to be the hepatic manifestation of MetS but it was not included in the IDF criteria for children despite evidence of close association (27, 28). Recently, the new definition and diagnostic criteria of metabolic dysfunction-associated fatty liver disease (MAFLD), formerly named NAFLD, were established in adult patients (29) and applied in the pediatric population (30). Based on this consensus, two criteria are sufficient for the diagnosis of MAFLD: the appearance of hepatic steatosis (detected by imaging techniques, blood biomarkers/scores, or liver histology) and overweight or obesity in person.

It is well-known that most of the non-communicable disorders in adulthood such as lipid and glycemic abnormalities start in childhood; however, little is known about dyslipidemia and dysglycemia among Ukrainian adolescents.

At the same time, up to 30% of obese people do not display the "typical" metabolic obesity-associated comorbidities and may be classified as metabolically healthy obese (MHO) (10, 30–33). This

phenotype, frequently defined by the absence of MetS components, was first described during the early 1980s; a consensus-based definition of pediatric MHO was introduced in 2018 by an international panel of 46 experts in a four-round Delphi study (31). The overall estimated prevalence of metabolically healthy phenotype in children of all weight statuses varied from 7% to 21%, whereas the prevalence of MHO among overweight and obese children varied from 3% to 87% (34). We noticed that little attention is paid in the literature to overweight/obese patients who cannot be classified MHO, as experience one of the metabolic abnormalities and at the same time do not meet enough criteria to be classified as MetS. In our opinion, they need a more careful examination and study as a group with potential manifestations of cardiometabolic complications in adolescence and youth.

In the context of the childhood obesity pandemic, taking into account the aforementioned, the detection of diagnostic criteria for a particular subgroup of children with metabolically unhealthy obesity (MUO), more prone to the development of CVD, is crucial for practice even if the child is overweight now and not experiencing all criteria of MetS yet.

Therefore, the aim of the current study is to determine the most sensitive and specific predictive markers of the metabolically unhealthy phenotype with high cardiometabolic risk in overweight/obese adolescent boys.

2 Materials and methods

The research was conducted at the Department of Pediatrics № 2, I. Horbachevsky Ternopil National Medical University, and was based on the Ternopil Regional Children's Clinical Hospital according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2008 (5), as well as national law. The patient safety rules and the ethical standards and procedures for research on human beings (2000) were followed in carrying out the work. The Ethics Committee of the I. Horbachevsky Ternopil National Medical University approved the study (protocol number 58, 29 April 2020).

In 2017, the new guideline of the Endocrine Society concerning the assessment, treatment, and prevention of overweight and obesity in the pediatric population was adopted (13). We accepted this Clinical Practice Guideline in our clinic and collected a cohort of patients during this period. In the examination algorithm, we strongly followed the recommendation for screening for related comorbidities (prediabetes, DM, dyslipidemia, hypertension, and NAFLD) in overweight and obese children.

Initially, 305 boys agreed to participate in the study. In all cases, informed consent was obtained from patients and their parents. The inclusion criteria for the study were the following: age 12–17 years and body mass index (BMI) above the 85th percentile (>1 SD) according to WHO age–sex nomograms that were accepted as national.

The exclusion criteria were as follows: obesity due to endocrine diseases (hypercortisolism, hypopituitarism, hypothyroidism, and hypothalamic–pituitary injury), chronic somatic illness (bronchial asthma, chronic renal failure, oncologic disease, liver disease, etc.), patients receiving medications that might impact body weight (glucocorticoids, antidiabetic, psychiatric drugs, or anticonvulsants), and patients with hereditary and congenital disorders, or diabetes mellitus, previously diagnosed.

Finally, 254 overweight or obese male adolescents were involved and included in the research. Their median age was 16.0 (15.0,16.1) years. Also, a control group of 30 healthy children with normal body weight (BMI<85th percentile), who were of comparable gender and age to comparison groups, was presented.

2.1 Measurements of anthropometric parameters and blood pressure

Anthropometric measurements were made and included the following: body weight using electronic scales (with an accuracy of within 0.1 kg), height using a stadiometer (within the accuracy of 0.1 cm), and waist circumference (WC) and hip circumference (HC) using a flexible measuring tape (within the accuracy of 0.1 cm). WC was measured with the tape measure at the point midway between the iliac crest and the costal margin (lower rib). HC was measured at the level of the greatest protrusion of the buttocks. Next, the waist-to-hip ratio (WHR) was calculated as WC divided by HC. The index was accessed by WHO recommendation, and the cutoff point of abdominal obesity was applied above 0.9 in men (35). The waist-to-height ratio (WHtR) was defined as WC divided by height. Because there is no national recommended threshold for abdominal obesity in children and adolescents, a cutoff of 0.5 was used to separate participants into having normal or elevated WHtR (36). BMI was calculated according to the formula (body weight (kg)/height^2 (m^2)). To assess the physical development of each child, values of body weight, height, height-SDS, BMI, and BMI-SDS were assessed according to gender and age charts based on WHO recommendations by AnthroPlus software (37).

Measurement of blood pressure (BP) was performed on both upper limbs three times in a sitting position. After sitting quietly for more than 10 min, BP was measured twice using a desktop mercury sphygmomanometer with a 2-min interval between measurements. The average systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded.

2.2 Determination of lipid metabolism and liver function test

Serum concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG) were measured by the enzymatic colorimetric method, using the Cholesterol Reagent and Cobas c111 automatic analyzer by Roche Diagnostics test systems (Rotkreuz, Switzerland) and assessed by the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines, 2018 (13, 38). We calculated some indexes and

ratios that were previously declared as informative for the detection of MetS in obese children and compared their sensitivity, such as TC/HDL-c ratio, LDL-c/HDL-c ratio, TG/HDL-c ratio, triglyceride to glucose index [TyG index=Log10(TG/HDL-c)], the atherogenic index (TC-/-HDL-c/HDL-c), and the pediatric NAFLD fibrosis index (PNFI) (15, 17, 20, 39, 40) The pediatric NAFLD fibrosis index was calculated using the original formula proposed by Nobili et al. (40).

We have analyzed some of the biochemical markers of hepatic metabolism (ALT, AST, and ALT/AST ratio), which might be alternative predictors of an early stage of NAFLD/MAFLD (22, 29, 30, 41–44), but are not presented in the IDF definition of MetS. Chronic viral hepatitis was excluded by the determination of anti-HCV (ORTHO HCV 3.0, Elisa Test) and HBsAg (Hepanostika HBsAg Uni-Form II Lab Biomerieux) in all obese and overweight cases with elevated hepatic enzymes.

2.3 Determination of glucose metabolism

A standard oral glucose tolerance test (OGTT) (1.75 g of glucose/kg body weight up to 75 g with capillary blood samples taken at 0, 60, and 120 min) was performed to evaluate glucose metabolism in all patients. Altered glucose metabolism was defined according to the American Diabetes Association criteria: impaired fasting plasma glucose (IFG) was diagnosed if fasting plasma glucose was from 5.6 to 6.9 mmol/L, impaired glucose tolerance (IGT) was revealed if 2-h plasma glucose was from 7.8 to 11.0 mmol/L, and diabetes mellitus was confirmed if fasting plasma glucose levels > 7.0 mmol/L or 2-h post-load >11.1 mmol/L during OGTT (45).

2.4 Definition of overweight/obesity phenotype

In order to determine MetS in adolescents, IDF diagnostic criteria were used (8). Accordingly, only individuals with abdominal obesity based on large WC (≥90th percentile or ≥94 cm) as a mandatory condition and the presence of two or more other clinical features—TG ≥1.7 mmol/L (150 mg/dl), HDL-c <1.03 mmol/L (<40 mg/dl), SBP ≥130 mmHg or DBP ≥85 mmHg, fasting plasma glucose ≥5.6 mmol/L (>100 mg/dl)—are diagnosed with MetS.

Boys were defined as MHO by consensus-based definition in children, which was generated by an international panel of 46 experts in a four-round Delphi study in 2018 (31): the absence of AH, HDL-c > 40 mg/dl (or >1.03 mmol/L), TG ≤ 150 mg/dl (or ≤1.7 mmol/L), and fasting plasma glucose < 5.6 mmol/L (<100 mg/dl). It was applied because thresholds of hyperglycemia, hypertriglyceridemia, and decreased HDL-c are identical to the IDF criteria of MetS. Only AH is recommended to be confirmed by percentile tables (>90th percentile). In the IDF criteria of MetS, the threshold of AH for children from 10 years old and above is mentioned as ≥130/≥85 mm Hg, and we applied it in the study.

In our cohort, subjects who could not be classified as either MHO due to some metabolic abnormalities or MetS (due to lack of criteria) were gathered to form a separate group, MUO. This phenotype was defined as the presence of only one of the following criteria: SBP ≥130 mmHg or DBP ≥85 mmHg, fasting plasma glucose ≥5.6 mmol/L (>100 mg/dl), TG≥1.7 mmol/L (150 mg/dl), and HDL cholesterol <1.03 mmol/L (≤40 mg/dl).

2.5 Statistical analysis

Statistical analysis was conducted using the Statistica 12.0 software package (StatSoft Inc., USA) and table editor Microsoft Excel Version 2013. Normality of the distribution of features in the variation series was assessed according to the Kolmogorov–Smirnov criterion. Quantitative data were presented depending on the nature of the characteristic's distribution. In the case of the normal distribution of features, the data were presented as the mean (M) and standard deviation (SD). In cases of non-normal distribution, the median (Me), lower quartile (Lq), and upper quartile (Uq) were calculated. Comparisons between groups in continuous variables were examined using the Student's *t*-test for two independent samples with normal distributions, and comparisons between groups in continuous variables were examined using one-way ANOVA for independent samples with normal distributions or the Kruskal–Wallis test for independent groups of variables with skewed distribution. Statistical differences in qualitative features were determined using the chi-squared test (χ^2) with Fisher's exact test. Correlation analysis between quantitative variables was done using the Pearson correlation coefficient. Multiple Logistic Regression was used to predict which independent variables have a major effect on MetS manifestation in children. The significance of the differences between the values was considered significant at $p \leq 0.05$.

3 Results

In the general cohort of 254 boys, 81.50% were obese, 83.85% have abdominal obesity, 62.99% have AH, 24.80% have IFG, 4.55% have IGT, 26.38% have hypertriglyceridemia, and 34.64% have a pathologically low level of HDL-c. In addition, in 38.58% of overweight and obese subjects, ALAT was increased above >25 U/L, the PNFI was elevated in 57.87 % of boys.

All overweight and obese patients were divided into three groups based on their metabolic phenotype. Group 1 (MHO) consisted of overweight/obese boys without AH, dyslipidemia, and hyperglycemia (50/19.7%), group 3 consisted of 130 boys (51.2%) with MetS based on the IDF criteria, and the remaining 74 boys (29.1%) comprised group 2 (MUO). The comparison of parameters used as criteria to define metabolic phenotype in the cohort and by groups are presented in Table 1.

As can be seen from Table 2, all groups are comparable by age, but anthropometrical parameters of overweight/obese boys are significantly different from healthy samples. In general, boys with MetS are especially taller than boys in the control group. The

10.3389/fendo.2023.1124019

TABLE 1 Comparison of parameters used as criteria to define the metabolic phenotype in an observed cohort.

Parameters	Control group	All obese or overweight	Group 1 MHO	Group 2 MUO	Group 3 MetS	Pearson chi-square P-value in general
Number	30	254/100%	50/19.7%	74/29.1%	130/51.2%	
Age (years)	15.08 (15.01, 16.0)	16.00 (15.00, 16.01)	16.0 (15.0, 16.5)	16.0 (14.0, 16.1)	16.0 (15.1, 16.1)	>0.05
Waist > 90th perc	0	213/83.85%××	16/ 32.00%××	67/90.54%××/ **	130/100.00%××/**	**<0.001**
Arterial hypertension	0	160/62.99%××	0	54/72.97%××	106/81.54%××	**<0.001**
Fasting glucose, mmol/L	4.69 ± 0.54	5.13 ± 0.74×	4.76 ± 0.58	4.92 ± 0.59	5.39 ± 0.78××/ **/##	
IFG > 5.6 mmol/L (>100 mg/dl)	0	63/24.80%××	0	5/6.76%	58/44.62%××/**/##	**<0.001**
Triglycerides, mmol/L	0.91 ± 0.15	1.33 ± 0.57××	0.84 ± 0.21	1.15 ± 0.35**	1.62 ± 0.63××/ **/##	
Triglycerides > 1.7 mmol/L (>150 mg/ dl)	0	67/26.38%××	0	2/2.70%	65/50.00%××/**/##	**<0.001**
HDL-cholesterol mmol/L	1.30 ± 0.14	1.17 ± 0.26××	1.35 ± 0.18	1.25 ± 0.29	1.05 ± 0.21××/ **/##	
HDL-cholesterol < 1.03 mmol/L (<40 mg/dl)	0	88/34.64%××	0	14/18.92%×/*	74/56.92%××/**/##	**<0.05**

P-value with control <0.05 (×), <0.01 (××); p-value with MHO <0.05 (*), <0.01 (**); p-value with MUO <0.01 (##).
p values of Pearson test of significant levels in multiple comparisons are bold.

percentage of overweight boys was the highest in group 1 (36.0%), but also present in 13.5% and 14.5% in groups with MUO and MetS, respectively. BMI and BMI-SDS significantly increase in boys from groups 2 and 3 (<0.001). Anthropometrical indexes of metabolic obesity such as WC >90th percentile, WHR >0.9, and WHtR >0.5 were identified in the vast majority of cases with MUO and MetS; nevertheless, in the group of MHO, these indexes were positive in some boys.

The AH was registered with the same high frequency in groups 2 and 3 while absent in control and group 1 (<0.001).

Fasting glucose, on average, was significantly higher only in the group with MetS due to IFG in 44.6% of boys (<0.001) (Table 3). We recorded altered 1-h post-load glucose (>8.6 mmol/L) and 2-h post-load glucose from 6.6 to 7.8 mmol/L during OGTT, which seemed to be more accurate in the early biomarker of dysglycemia than the 2-h post-load glucose above 7.8 mmol/L (46–48) and needed further study. In comparison, 15.38% of boys from group 3 were identified to have markers of dysglycemia by OGTT (excessive 1-h post-load glucose excursions >8.6 mmol/L and slow 2-h post-load reduction 6.6–7.8 mmol/L), while in the same group, the IGT was three times less frequent (4.62%) and DM was not confirmed in any person.

Traditional markers of dyslipidemia by the IDF criteria (level of triglycerides and HDL-cholesterol) are most prominently increased

TABLE 2 Anthropometrical parameters of the study cohort by groups.

Parameters	Control group	All obese or overweight	Group 1 MHO	Group 2 MUO	Group 3 MetS	Pearson chi-square P-value in general
Height-SDS	0.24 ± 0.97	0.79 ± 1.07××	0.51 ± 0.83	0.78 ± 1.04×	0.92 ± 1.13×/*	
BMI	19.79 ± 1.68	30.89 ± 3.90××	28.73 ± 2.98××	31.05 ± 3.67××/**	31.64 ± 4.07××/**	
BMI-SDS	−0.11 ± 0.66	2.50 ± 0.63××	2.15 ± 0.49××	2.58 ± 0.66××/**	2.62 ± 0.75××/**	
Obesity	0	207/81.50%××	32/64.00%××	64/86.49%××/**	111/85.38%××/**	**<0.001**
WHR	0.81 ± 0.04	0.92 ± 0.05××	0.89 ± 0.05××	0.92 ± 0.05××/**	0.93 ± 0.05××/**	
WHR > 0.9	0	173/68.11%××	14/28.00%××	54/72.97%××/**	105/80.77%××/**	**0.001**
WHtR	0.44 ± 0.05	0.58 ± 0.06××	0.55 ± 0.04××	0.59 ± 0.05××/**	0.60 ± 0.06××/**	
WHtR > 0.5	0	212/83.46%××	14/28.00%××	68/91.89%××/**	130/100.00%××/**	**<0.001**

P-value with control <0.05 (×), <0.01 (××); p-value with MHO <0.05 (*), <0.01 (**).
p values of Pearson test of significant levels in multiple comparisons are bold.

10.3389/fendo.2023.1124019

TABLE 3 Biochemical markers of glycose metabolism of the study cohort by groups.

Parameters	Control group	All obese or overweight	Group 1 MHO	Group 2 MUO	Group 3 MetS	Pearson chi-square P-value in general
OGTT 1st hour		6.46 ± 1.30	6.46 ± 0.82	6.39 ± 1.40	6.49 ± 1.40	
1 h post-load glucose >8.6 mmol/L	0	26/10.24%	0	6/8.11%*	20/15.38%**	**<0.02**
GTT 2nd hour		5.23 ± 1.07	4.97 ± 0.82	5.00 ± 1.00	5.49 ± 1.15*/#	
2 h post-load 6.6–7.8 mmol/L	0	26/10.24%	1/2.00%	5/6.76%	20/15.38%*	**<0.05**
IGT = 2 h post-load >7.8 mmol/L	0	7/4.55%	0	1/1.35%	6/4.62%	>0.05
2 h post-load >11.1 mmol/L	0	0	0	0	0	

P-value with MHO <0.05 (*), <0.01 (**); p-value with MUO <0.05 (#).
p values of Pearson test of significant levels in multiple comparisons are bold.

in group 3; moreover, borderline high triglycerides and borderline low HDL-cholesterol (13) were found in approximately 50% of the boys in group 2 (MUO) and approximately 20% of boys with MHO (Table 4).

In addition, we have analyzed other markers and indexes of lipid metabolism and liver enzymes that were previously declared as informative for the detection of MetS in obese/overweight (15–20, 22, 23, 37, 38, 49, 50) children, which might be alternative sensitive predictors of cardiovascular comorbidities (Table 5).

We have found that TC was prominently high in boys with MetS (in 28.46%) and with MUO (in 13.51%) without some difference between these groups (<0.05); however, the borderline high level was registered even more often in these groups, in 35.14% and 51.54%, respectively (<0.001). On average, LDL-cholesterol levels slightly increased in overweight and obese boys compared to the control group but without differences in the rest of the groups (>0.05). A remarkably high level of LDL-c was identified in 23.85% of cases in group 3, which is significantly higher in contrast to other groups (<0.001). Nevertheless, borderline high level of LDL-cholesterol was found at 43.08% of cases in group 3 and 33.78% of cases in group 2, which is much more often than in the control or group 1 (<0.001) but with no difference in-between (>0.05).

It is essential to state that calculated indexes of metabolic disorders [TG/HDL-c ratio, TC/HDL-c ratio, Atherogenic index (TC-HDL-c/HDL-c), LDL-c/HDL-c ratio, and TyG index] are prominently increased in boys with MetS and in cases with MUO (<0.001) in comparison to the control group and the group with MHO where they are in the normal range.

TABLE 4 Biochemical markers of lipid metabolism in the study cohort by groups.

Parameters	Control group	All obese or overweight	Group 1 MHO	Group 2 MUO	Group 3 MetS	Pearson chi-square P-value in general
Total cholesterol mmol/L	3.77 ± 0.56	4.25 ± 0.89××	3.74 ± 0.57	4.19 ± 0.87	4.48 ± 0.93××/**	
Cholesterol > 5.1 mmol/L (>200 mg/dl)	0	47/18.50%××	0	10/13.51%**	37/28.46%**/#	**<0.05**
Cholesterol *Borderline high* > 4.3 mmol/L (>170 mg/dl)	0	94/37.00%	5/10.00%××	26/35.14%××/**	67/51.54%××/**/##	**<0.001**
Triglycerides *Borderline high* >1 mmol/L (>90 mg/dl)	3/10.00%	166/65.35%××	12/24.00%	47/63.51%××/**	107/82.30%××/**/##	**<0.001**
HDL-cholesterol *Borderline low* <1.20 mmol/L (<45 mg/dl)	0	140/55.12%××	9/18.00%×	32/43.24%××/*	99/76.15%××/**/#	**<0.001**
LDL-cholesterol mmol/L	2.04 ± 0.37	2.55 ± 0.80×	2.35 ± 0.74×	2.49 ± 0.75×	2.77 ± 0.83××	
LDL-cholesterol >3.4 mmol/L (>130 mg/dl)	0	39/15.35%×	0	8/10.81%*	31/23.85%××/**/##	**<0.001**
LDL-cholesterol *Borderline high* >2.8 mmol/L (>110 mg/dl)	2/6.66%	88/34.64%××	5/10.00%	25/33.78%×/*	56/43.08%××/**	**<0.001**

P-value with control <0.05 (×), <0.01 (××); p-value with MHO <0.05 (*), <0.01 (**); p-value with MUO <0.05 (#), <0.01 (##).
p values of Pearson test of significant levels in multiple comparisons are bold.

10.3389/fendo.2023.1124019

TABLE 5 Additional biochemical markers in the study cohort by groups.

Parameters	Control group	All obese or overweight	Group 1 MHO	Group 2 MUO	Group 3 MetS	Pearson chi-square P-value in general
TG/HDL-c ratio	1.72 ± 0.35	2.83 ± 1.61××	1.42 ± 0.45	2.28 ± 1.03×/*	3.69 ± 1.65××/**/##	
TC/HDL-c ratio	3.01 ± 0.50	3.80 ± 1.13××	2.77 ± 0.51	3.49 ± 0.97**	4.37 ± 1.04××/**/##	
Atherogenic index	2.01 ± 0.50	2.78 ± 1.11×	1.77 ± 0.51	2.45 ± 0.87**	3.37 ± 1.04××/**/##	
LDL-c/HDL-c ratio	1.72 ± 0.38	2.32 ± 0.96××	1.64 ± 0.50	2.07 ± 0.75××/**	2.76 ± 0.97××/**/##	
TyG index	8.08 ± 0.23	8.49 ± 0.46××	8.01 ± 0.27	8.35 ± 0.35××/**	8.77 ± 0.39××/**/##	
PNFI	0.44 ± 0.09	7.84 ± 2.60××	4.85 ± 2.88××	8.21 ± 2.17××/**	8.78 ± 1.74××/**	
ALAT U/L	16.37 ± 5.70	24.34 ± 12.22××	16.12 ± 4.67	25.04 ± 10.84××/**	27.11 ± 13.22××/**	
ALAT >25 U/L	0	98/38.58%××	3/6.00%	35/47.95%××/**	60/48.46%××/**	**<0.001**
ASAT U/L	22.35 ± 6.26	22.13 ± 10.29	18.65 ± 5.20	22.21 ± 10.07	23.42 ± 11.55	
ALAT/ASAT	0.74 ± 0.22	1.18 ± 0.56××	0.92 ± 0.38	1.21 ± 0.52××/*	1.27 ± 0.61××/**	
ALAT/ASAT >1.5	0	69/27.16%××	5/10.20%	19/26.03%××/*	45/34.62%××/**/#	**<0.005**

P-value with control <0.05 (×), <0.01 (××); p-value with MHO <0.05 (*), <0.01 (**); p-value with MUO <0.05 (#), <0.01 (##).
p values of Pearson test of significant levels in multiple comparisons are bold.

Regarding markers of MAFLD, we have identified the same trend. The average absolute level of ALAT was significantly higher in boys from groups 2 and 3 in comparison to control and group 1 (<0.001) but no significant difference in-between (>0.05), as well as in frequency (47.95% and 48.46%, respectively, p>0.05). We also found no significant difference between groups in the level of ASAT at all; at the same time, the ALAT/ASAT ratio increases in groups 2 and 3 in comparison to the control and the group with MHO (<0.05) but without difference in-between (>0.05).

Thereby, despite all boys in our cohort being adolescents and the fact that the impact of a temporary IR typical for puberty may be relevant, we recognized almost 20% of obese or overweight boys without evidence of metabolic comorbidities and cardiometabolic risk (group 1). At the same time, we identified no significant difference between groups 2 and 3 based on all biochemical markers. Thus, we can conclude that patients from group 2 (MUO) are very close to patients with MetS and they are also at high cardiometabolic risk.

Based on multiple logistic regression analysis, which includes all anthropometric and biochemical values and calculated indexes in boys from groups 1 and 3, it was assumed that the maximum likelihood in the prediction of MetS makes the combination of TyG index, PNFI, and TG/HDL ratio (R^2 =0.713, p<0.000). By tracing the receiver operating characteristic (ROC) curve, the model is confirmed as a good predictor of MetS (AUC=0.898, odds ratio=27.111, percentage correct=86.03%) (Figure 1).

4 Discussion

There are a lot of discussions about the existence of a metabolically healthy phenotype, associated with a lower cardiovascular risk, in obese adolescents. A recent publication suggests the existence of a subgroup of overweight individuals with a metabolically healthy phenotype and a low risk of CVD (50). Although this low cardiovascular risk group is observed in clinical practice and was recognized in the current study, the definition of MHO is not widely agreed upon by experts and practitioners.

The prevalence of MetS in our study is 51.2%, which is almost higher than that reported by previous pediatric studies in European countries (51) but similar to other regions in Ukraine (52). The reasons for this difference may be attributed to age, ethnicity, socioeconomic status, environment, and the interactions among these variables. The prevalence of MHO in our cohort of obese and overweight boys is 19.7%. In publications, these proportions vary widely from 3% to 87%, depending on definitions, obesity criteria, cutoff values, age of the participants, sample sizes, and characteristics (10, 31, 32, 34, 53, 54). In the research of

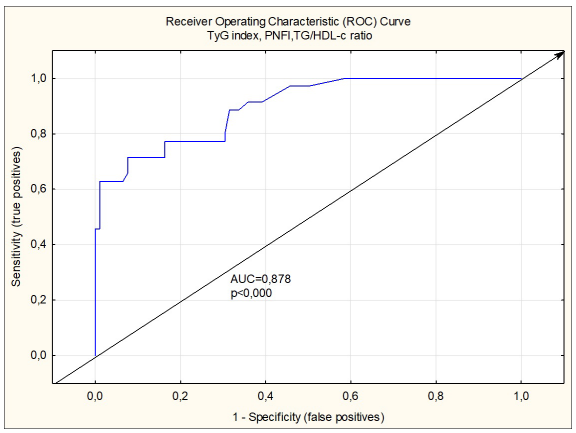


FIGURE 1
Receiver operating charactristic curve analysis of TyG index, PNFI and TG/HDL-c ratio for detection of metabolic syndrome in boys.

Jankowska et al., the prevalence of MetS based on IDF criteria in boys in neighboring Poland was 14.6%; MHO, 39.2%; and MUO, 46.2%, but the cohort included younger children (10–12 years) (55). In comparison, in the report from BIOSHARE-EU, the prevalence of MetS in obese men ranged from 43% to 78% and substantially exceeded MHO values (33).

To avoid conflicts concerning the definition of overweight and obesity, experts recommend using the WHO definition of overweight (one standard deviation BMI for age and sex and obesity; two standard deviations BMI for age and sex) in children and adolescents (42). However, BMI is considered insufficient to assess abdominal obesity in children and adolescents, as it does not provide information about the percentage of body fat or the distribution of fat in a body. It is important to note that in the IDF criteria of MetS, WC>90th percentile is used as a marker of abdominal obesity, not BMI (8). Therefore, alternative screening tests to assess obesity in childhood and adolescence have been suggested as being superior to BMI in predicting CVD risks such as waist-to-height ratio (WHtR) and waist-to-hip ratio (WHR) (10, 35, 36, 56). The WHR as a marker of abdominal obesity was approved by WHO for adults without age restrictions and with high sensitivity in young people (35). WHtR is a simple, quick, and sensitive indicator of obesity in general. The cutoff value, which is age and gender independent, is above 0.5 (36). The debate is still ongoing on whether WHR and WHtR are criteria for obesity in general or abdominal obesity as a separate type (56). The results of our study showed that WHtR and WHR are significantly higher in subjects with MUO and MetS than in those with MHO and the control group, which might indicate a higher risk of CVD as it was recognized in other studies (10, 53, 57).

In our cohort, boys with MUO and MetS have higher BMI and BMI-SDS, which may show a negative correlation with insulin sensitivity, as has been shown in large-cohort studies across various ethnic and age groups. At the same time, the pattern of stronger lipid deposition determines the IR in a body more than the degree of obesity (58); namely, the abdominal type of obesity (WC>90th percentile) was recognized in predominant cases of our patients with MUO and MetS. In addition, our results revealed that overweight boys may have also experienced metabolic comorbidities; 13.51% and 14.62% of patients were overweight in groups 2 and 3, respectively.

The results of last years studies convincingly show that overweight adolescents and obese ones may also experience AH, dyslipidemia, and dysglycemia and may be at risk of CVD (22, 32, 49, 50, 59–63). In the recent review, 142,142 children and adolescents from 76 eligible articles were included to compute the pooled prevalence of MetS and its components in low- and middle-income countries. MetS among the overweight and obese population was computed from 20 articles with the pooled prevalence of 24.09%, 36.5%, and 56.32% in IDF, ATP III, and de Ferranti criteria, respectively. Similarly, a total of 56 articles were eligible to compute the pooled prevalence of MetS in the general population of children and adolescents. Hence, MetS was found in 3.98% (IDF), 6.71% (ATP III), and 8.91% (de Ferranti) of study subjects (64).

Thereby, in our opinion, applying the criteria of MetS in overweight adolescents is reasonable, as the criteria help to recognize children with higher CVD risk earlier, as soon as they experience metabolic comorbidities.

It is a well-known fact that the underground of MetS is IR and MAFLD is the hepatic expression of MetS, though it is still controversial whether IR is a risk factor or a consequence of fat liver accumulation (21, 23, 28, 30). There is yet no treatment with proven efficacy for these conditions, but it is imperative to find a way to decrease cardiometabolic complications, and early detection in childhood is paramount (42). That is why studies of predictive biochemical markers of IR in children are actively established, most of which have different sensitivities and specificities, depending on age, gender, ethnicity, etc.

IFG may be a clinical sign of IR and a component of MetS. A large prospective cohort study in Sweden confirms that the pediatric obese population has a markedly higher prevalence of T2DM in early adulthood in comparison with a population-based group. In adults, IFG results in a cumulative incidence of T2DM over 6–9 years have been reported to range from 29% to 39%, but in children and adolescents, it is associated with lesser risk (7). In the current research, the prevalence of IFG in the whole cohort of overweight and obese boys was 24.08%, and 44.62% in patients with MetS in particular. It is much higher than that in Germany (3.9%) and in Sweden (17.1%) in children from 2 to 18 years old (65). Despite the high frequency of IFG in our cohort, the prevalence of IGT by OGTT was expectedly much less (4.55%), as was shown in other research (66, 67). These differences may occur as IFG is an unstable condition, which reacts to stress; therefore, repeated fasting glucose measurements might be provided to establish a more accurate rate.

OGTT is a validated diagnostic tool for early signs of dysglycemia detection (48, 68), not only for IGT. The results of our study revealed that increasing 1-h post-load glucose ≥8.6 mmol/L and 2-h post-load glucose between 6.6 and 7.8 mmol/L in boys with MetS occurs three times more often (15.38%) than IGT (4.62%). According to recently published data, these values are more sensitive predictors of the mid-term and long-term incidents of T2DM in adults and adolescents than IFG or IGT (46–48). However, in puberty, such dysglycemia may be transient due to physiological light IR. It was shown that 22% to 52% of children and adolescents with prediabetes return to normal glycemia or normal glucose tolerance levels without intervention over 6 months to 2 years (69) but the rest of adolescents' dysglycemia might persist in adulthood. Thus, the importance of screening for prediabetes in asymptomatic children and adolescents for health outcomes is still controversial.

At the same time, the prevalence of dyslipidemia (at least one of the lipid disorders) in our cohort of obese and overweight boys was 74.0%, which is close to the result reported by another Ukrainian center (70) and it is much higher than the dysglycemia rate. The obtained data revealed hypertriglyceridemia (TG>1.7 mmol/L) in 26.38%, hypercholesterolemia (TC>5.2 mmol/L) in 18.50%, HDL-cholesterol <1.03 mmol/L in 34.64%, and LDL-cholesterol >3.4 mmol/L in 15.35%. The study by Brzeziński et al. that included 1,948 overweight or obese Polish patients from 6 to 14 years old showed that at least one lipid disorder occurred in 40.51% of boys (59). The most common lipid disorders were decreased HDL-c levels (23.79% of the boys), elevated LDL-c (14.25%), and elevated TC (13.94%), which is close to our results, but the elevated TG was much

more frequent in our cohort. In comparison, in the United States, between 2011 and 2014, in individuals aged 6 to 19 years with obesity, the reported frequency of dyslipidemia was 43.3%, with decreased HDL-c levels in 33.2% and elevated LDL-c in 16.7% (71). In Turkish children, Elmaoğulları et al. have reported that 42.9% of obese patients (2–18 years) met the dyslipidemia criteria: 21.7% of the patients had hypertriglyceridemia, 19.7% had low levels of HDL-C, 18.6% had hypercholesterolemia, and 13.7% had high levels of LDL-C (72). In the majority of published studies, dyslipidemia (low HDL and/or high TG) was the most frequent risk factor of MetS, whereas high fasting glucose was the least frequent (10, 51).

The role of atherogenic dyslipidemia in CVD complications is well-established in adults and is the leading cause of morbidity and mortality worldwide. A recently published prospective study conducted among 1,779 adolescents who were 15 years old and followed up for 9 years until 24 years of age revealed that almost 1 in 5 adolescents had elevated lipids or dyslipidemia at age 15 years. The prevalence of elevated lipids and dyslipidemia increased to 1 in 4 young adults, 9 years later (73). Moreover, the researchers observed that lipid treatment intervention at 24 years failed to stop worsening atherosclerosis while lipid treatment intervention at the age of 17 effectively stopped and reversed atherosclerosis progression. Thus, early control of pediatric dyslipidemia is certainly necessary to slow down the progression of CVD in young adulthood.

The current study revealed quite a high percentage of borderline dyslipidemia in Ukrainian overweight adolescents based on cutoff values from the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines, 2018 (38).

As disturbance of lipid metabolism is found much more often in boys with MUO and MetS than dysglycemia, it is reasonable to suggest that cholesterol fraction levels have a higher sensitivity compared to blood glucose levels. In the last decades, some indexes were proposed and tested as markers of IR and NAFLD at first and, eventually, MetS in adolescents and adults, such as WHR, WtHR, LDL-c/HDL-c ratio, TyG index TG/HDL-c ratio, atherogenic index, ALAT/ASAT, and PNFI. In our research, we tested all markers simultaneously in the cohort of normal, overweight, and obese boys with MHO or MetS and, based on multiple logistic regression analysis, assumed the most sensitive and specific combination of three predictors (TyG index, PNFI, and TG/HDL ratio), which is one of the main strengths of our study.

Interestingly, the TyG index is widely tested in different populations and age groups and was confirmed superior to HOMA-IR in terms of detection of T2DM (18, 19, 74). In a recently published systematic review of eight cross-sectional studies with individuals aged ≥2 and ≤20 years old from the United States, Korea, Mexico, Brazil, and Iran, the authors concluded that the TyG index was positively associated with other IR prediction methods and appears to be advantageous in terms of predicting IR risk and other cardiometabolic risk factors in children and adolescents (20).

Also, the TG/HDL ratio was tested and proved to be a good predictor of IR and MetS in childhood (15, 16). A large-cohort Korean study that included data from 2,721 adolescents (1,436 boys and 1,285 girls) aged 10–18 years showed significant associations between TG/HDL-C ratio and MetS. Furthermore, in boys, unlike in girls, areas under the ROC curve to identify MetS were 0.947 for TG/HDL-C, which was higher than that of HOMA-IR (0.822) (17).

In recent studies, less attention has been paid to the PNFI, which was proposed and tested by Nobili et al. in 2009 (40). However, in a recently published study that enrolled 286 adolescents with biopsy-proven NAFLD, the data confirmed that the PNFI remains the best non-invasive score in pediatric age for NAFLD prediction (75).

Remarkably, all three indexes (TyG index, PNFI, and TG/HDL ratio) are based on TG levels, which might be more sensitive than glucose or transaminase levels alone and may help to recognize MUO even if the combination of classic MetS criteria is insufficient yet.

One of the limitations of this study is the lack of insulin detection and HOMA index for the detection of IR. Nevertheless, plenty of studies, neither in adults nor in adolescents, established convincing evidence that IR is the key and driving factor in the development of MetS (coexistence of abdominal obesity, dyslipidemia, dysglycemia, MAFLD, and AH) (9, 10, 15, 16, 18, 20, 23, 42). However, there is no well-defined cutoff point that differentiates normal from abnormal insulin sensitivity in youth and there is no universally accepted, clinically useful, numeric expression in the HOMA-index that defines insulin resistance. In pediatrics, the transient puberty-related insulin resistance that occurs with the completion of puberty further complicates this. In addition, our study is based on boys recruited from a tertiary care center, which may influence the true prevalence of metabolically healthy and unhealthy phenotypes in adolescents in our population, as patients are present in hospitals with some signs and symptoms.

The most interesting cohort in our study is group 2 (MUO) as, based on all other biochemical markers, these patients are very close to patients with MetS in contrast to the control group or boys with MHO. Thus, we can assume that they are also at high CVD risk and need further follow up and monitoring.

In conclusion, our study showed that the combination of the lipid indexes—TyG index, PNFI, and TG/HDL ratio—was a good predictor for cardiometabolic risk in overweight and obese adolescent boys and may be better than traditional lipid and glucose examinations. These ratios can provide a beneficial and significant value to assessing CVD risk in adolescence in the absence of certain blood biomarkers and in resource-limited settings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee of the I. Horbachevsky Ternopil National Medical University approved the study (protocol number 58, 04/29/2020). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

VF and HP conceptualized and designed the study, contributed to the discussion, and critically revised the manuscript. VF, A-MS and MF analyzed data, interpreted results, and drafted the manuscript. VF, A-MS and KK enrolled patients, collected data, and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The Ministry of Health of Ukraine supported the study for I. Horbachevsky Ternopil National Medical University grant number 0118U000361.

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

References

1. NCD Risk Factor Collaboration (NCD-RisC). 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(10113):2627–42. doi: 10.1016/S0140-6736(17)32129-3

2. Yakovenko V, Henn L, Bettendorf M, Zelinska N, Soloviova G, Hoffmann GF, et al. Risk factors for childhood overweight and obesity in Ukraine and Germany. *J Clin Res Pediatr Endocrinol* (2019) 11(3):247–52. doi: 10.4274/jcrpe.galenos.2019.2018.0157

3. Dereń K, Nyankovskyy S, Nyankovska O, Łuszczki E, Wyszyńska J, Sobolewski M, et al. The prevalence of underweight, overweight and obesity in children and adolescents from Ukraine. *Sci Rep* (2018) 8(1):3625. doi: 10.1038/s41598-018-21773-4

4. Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. *Obes Rev* (2016) 17 (2):95–107. doi: 10.1111/obr.12334

5. Agirbasli M, Tanrikulu AM, Berenson GS. Metabolic syndrome: bridging the gap from childhood to adulthood. *Cardiovasc Ther* (2016) 34(1):30–6. doi: 10.1111/1755-5922.12165

6. Lindberg L, Danielsson P, Persson M, Marcus C, Hagman E. Association of childhood obesity with risk of early all-cause and cause-specific mortality: a Swedish prospective cohort study. *PloS Med* (2020) 17(3):e1003078. doi: 10.1371/journal.pmed.1003078

7. Hagman E, Danielsson P, Brandt L, Ekbom A, Marcus C. Association between impaired fasting glycaemia in pediatric obesity and type 2 diabetes in young adulthood. *Nutr Diabetes* (2016) 6(8):e227. doi: 10.1038/nutd.2016.34

8. Zimmet P, Alberti KG, Kaufman F, Tajima N, Silink MIDF Consensus Group. The metabolic syndrome in children and adolescents - an IDF consensus report. *Pediatr Diabetes.* (2007) 8(5):299–306. doi: 10.1111/j.1399-5448.2007.00271

9. Magge SN, Goodman E, Armstrong SCCommittee on nutrition, Section on endocrinology and Section on obesity. The metabolic syndrome in children and adolescents: shifting the focus to cardiometabolic risk factor clustering. *Pediatrics.* (2017) 140(2):e20171603. doi: 10.1542/peds.2017-1603

10. Genovesi S, Antolini L, Orlando A, Gilardini L, Bertoli S, Giussani M, et al. Cardiovascular risk factors associated with the metabolically healthy obese (MHO) phenotype compared to the metabolically unhealthy obese (MUO) phenotype in children. *Front Endocrinol (Lausanne).* (2020) 11:27. doi: 10.3389/fendo.2020.00027

11. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AESubcommittee on screening and management of high blood pressure in children. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics.* (2017) 140(3):e20171904. doi: 10.1542/peds.2017-1904

12. Lurbe I Ferrer E. Guías europeas para el manejo de la hipertensión arterial en niños y adolescentes: nuevos conceptos para un viejo problema [2016 - European society of hypertension guidelines for the management of high blood pressure in children and adolescents. *Pediatr (Barc)* (2016) 85(4):167–9. doi: 10.1016/j.anpedi.2016.08.001

13. Styne DM, Arslanian SA, Connor EL, Farooqi IS, Murad MH, Silverstein JH, et al. Pediatric obesity-assessment, treatment, and prevention: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2017) 102(3):709–57. doi: 10.1210/jc.2016-2573

14. Andrade MI, Oliveira JS, Leal VS, Lima NM, Costa EC, Nathalia Barbosa de Aquino, et al. Identificação dos pontos de corte do índice homeostatic model assessment for insulin resistance em adolescentes: revisão sistemática [Identification of cutoff points for homeostatic model assessment for insulin resistance index in adolescents: systematic review]. *Rev Paul Pediatr* (2016) 34(2):234–42. doi: 10.1016/j.rpped.2015.08.006

15. Katsa ME, Ioannidis A, Sachlas A, Dimopoulos I, Chatzipanagiotou S, Rojas Gil AP. The roles of triglyceride/high-density lipoprotein cholesterol ratio and uric acid as predisposing factors for metabolic syndrome in healthy children. *Ann Pediatr Endocrinol Metab* (2019) 24(3):172–9. doi: 10.6065/apem.2019.24.3.172

16. Park JM, Lee JY, Dong JJ, Lee DC, Lee YJ. Association between the triglyceride to high-density lipoprotein cholesterol ratio and insulin resistance in Korean adolescents: a nationwide population-based study. *J Pediatr Endocrinol Metab* (2016) 29(11):1259–65. doi: 10.1515/jpem-2016-0244

17. Chu SY, Jung JH, Park MJ, Kim SH. Risk assessment of metabolic syndrome in adolescents using the triglyceride/high-density lipoprotein cholesterol ratio and the total cholesterol/high-density lipoprotein cholesterol ratio. *Ann Pediatr Endocrinol Metab* (2019) 24(1):41–8. doi: 10.6065/apem.2019.24.1.41

18. Mohd Nor NS, Lee S, Bacha F, Tfayli H, Arslanian S. Triglyceride glucose index as a surrogate measure of insulin sensitivity in obese adolescents with normoglycemia, prediabetes, and type 2 diabetes mellitus: comparison with the hyperinsulinemic-euglycemic clamp. *Pediatr Diabetes.* (2016) 17(6):458–65. doi: 10.1111/pedi.12303

19. Angoorani P, Heshmat R, Ejtahed HS, Motlagh ME, Ziaodini H, Taheri M, et al. Validity of triglyceride-glucose index as an indicator for metabolic syndrome in children and adolescents: the CASPIAN-V study. *Eat Weight Disord* (2018) 23 (6):877–83. doi: 10.1007/s40519-018-0488-z

20. Brito ADM, Hermsdorff HHM, Filgueiras MS, Suhett LG, Vieira-Ribeiro SA, Franceschini SDCC, et al. Predictive capacity of triglyceride-glucose (TyG) index for insulin resistance and cardiometabolic risk in children and adolescents: a systematic review. *Crit Rev Food Sci Nutr* (2021) 61(16):2783–92. doi: 10.1080/10408398.2020.1788501

21. Srikanthan K, Feyh A, Visweshwar H, Shapiro JI, Sodhi K. Systematic review of metabolic syndrome biomarkers: a panel for early detection, management, and risk stratification in the West Virginian population. *Int J Med Sci* (2016) 13(1):25–38. doi: 10.7150/ijms.13800

22. Johansen MJ, Gade J, Stender S, Frithioff-Bøjsøe C, Lund MAV, Chabanova E, et al. The effect of overweight and obesity on liver biochemical markers in children and adolescents. *J Clin Endocrinol Metab* (2020) 105(2):dgz010. doi: 10.1210/clinem/dgz010

23. Pelin A-M, Balan G, Stefanescu C, Rosca S, Busila C. New criteria in defining the metabolic syndrome in children – an analysis of the relationship between the hepatic enzymes and the insulin resistance, HOMA-IR, glucose tolerance test in the obese children. *Progr Nutr* (2022) 23(4):e2021316. doi: 10.23751/pn.v23i4.11589

24. Draijer LG, Feddouli S, Bohte AE, Vd Baan Slootweg O, Pels Rijcken TH, Benninga MA, et al. Comparison of diagnostic accuracy of screening tests ALT and ultrasound for pediatric non-alcoholic fatty liver disease. *Eur J Pediatr* (2019) 178 (6):863–70. doi: 10.1007/s00431-019-03362-3

25. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *PloS One* (Geneva, Switzerland: WHO Document Production Services) (2015) 10 (10):e0140908. doi: 10.1371/journal.pone.0140908

26. Clemente MG, Mandato C, Poeta M, Vajro P. Pediatric non-alcoholic fatty liver disease: recent solutions, unresolved issues, and future research directions. *World J Gastroenterol* (2016) 22(36):8078–93. doi: 10.3748/wjg.v22.i36.8078

27. Bussler S, Penke M, Flemming G, Elhassan YS, Kratzsch J, Sergeyev E, et al. Novel insights in the metabolic syndrome in childhood and adolescence. *Horm Res Paediatr* (2017) 88(3-4):181–93. doi: 10.1159/000479510

28. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. *Dig Liver Dis* (2015) 47(3):181–90. doi: 10.1016/j.dld.2014.09.020

29. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* (2020) 73(1):202–9. doi: 10.1016/j.jhep.2020.03.039

30. Flisiak-Jackiewicz M, Bobrus-Chociej A, Wasilewska N, Lebensztejn DM. From nonalcoholic fatty liver disease (NAFLD) to metabolic dysfunction-associated fatty liver disease (MAFLD)-new terminology in pediatric patients as a step in good scientific direction? *J Clin Med* (2021) 10(5):924. doi: 10.3390/jcm10050924

31. Damanhoury S, Newton AS, Rashid M, Hartling L, Byrne JLS, Ball GDC, et al. Defining metabolically healthy obesity in children: a scoping review. *Obes Rev* (2018) 19 (11):1476–91. doi: 10.1111/obr.12721

32. Margolis-Gil M, Yackobovitz-Gavan M, Phillip M, Shalitin S. Which predictors differentiate between obese children and adolescents with cardiometabolic complications and those with metabolically healthy obesity? *Pediatr Diabetes* (2018) 19(7):1147–55. doi: 10.1111/pedi.12694

33. van Vliet-Ostaptchouk JV, Nuotio ML, Slagter SN, Doiron D, Fischer K, Foco L, et al. The prevalence of metabolic syndrome and metabolically healthy obesity in Europe: a collaborative analysis of ten large cohort studies. *BMC Endocr Disord* (2014) 14:9. doi: 10.1186/1472-6823-14-9

34. Vukovic R, Dos Santos TJ, Ybarra M, Atar M. Children with metabolically healthy obesity: a review. *Front Endocrinol (Lausanne).* (2019) 10:865. doi: 10.3389/fendo.2019.00865

35. WHO. *Waist circumference and waist-hip ratio: report of a WHO expert consultation*. Geneva (2011). Available at: http://www.who.int/entity/nutrition/publications/obesity/WHO_report_waistcircumference_and_ waisthip_ratio/en/.

36. Ashwell M, Gibson S. A proposal for a primary screening tool: 'Keep your waist circumference to less than half your height'. *BMC Med* (2014) 12:207. doi: 10.1186/s12916-014-0207-1

37. WHO child growth standards. *Body mass index-for-age (BMI-for-age)* (2007). Available at: https://www.who.int/toolkits/child-growth-standards/standards/body-mass-index-for-age-bmi-for-age.

38. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American college of Cardiology/American heart association task force on clinical practice guidelines. *Circulation* (2019) 139(25):e1082–143. doi: 10.1161/CIR.0000000000000625

39. Simental-Mendía LE, Gamboa-Gómez CI, Aradillas-García C, Rodríguez-Morán M, Guerrero-Romero F. The triglyceride and glucose index is a useful biomarker to recognize glucose disorders in apparently healthy children and adolescents. *Eur J Pediatr* (2020) 179(6):953–8. doi: 10.1007/s00431-020-03570-2

40. Nobili V, Alisi A, Vania A, Tiribelli C, Pietrobattista A, Bedogni G. The pediatric NAFLD fibrosis index: a predictor of liver fibrosis in children with non-alcoholic fatty liver disease. *BMC Med* (2009) 7:21. doi: 10.1186/1741-7015-7-21

41. Draijer LG, van Oosterhout JPM, Vali Y, Zwetsloot S, van der Lee JH, van Etten-Jamaludin FS, et al. Diagnostic accuracy of fibrosis tests in children with non-alcoholic fatty liver disease: a systematic review. *Liver Int* (2021) 41(9):2087–100. doi: 10.1111/liv.14908

42. Christian Flemming GM, Bussler S, Körner A, Kiess W. Definition and early diagnosis of metabolic syndrome in children. *J Pediatr Endocrinol Metab* (2020) J33 (7):821–33. doi: 10.1515/jpem-2019-0552

43. Hecht L, Weiss R. Nonalcoholic fatty liver disease and type 2 diabetes in obese children. *Curr Diabetes Rep* (2014) 14(1):448. doi: 10.1007/s11892-013-0448-y

44. D'Adamo E, Castorani V, Nobili V. The liver in children with metabolic syndrome. *Front Endocrinol (Lausanne).* (2019) 10:514. doi: 10.3389/fendo.2019.00514

45. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, et al. ISPAD clinical practice consensus guidelines 2018: definition, epidemiology, and classification of diabetes in children and adolescents. *Pediatr Diabetes.* (2018) 19 Suppl 27(Suppl 27):7–19. doi: 10.1111/pedi.12773

46. Park SK, Ryoo JH, Oh CM, Choi JM, Jung JY. 1-hour and 2-hour postload glucose level on oral glucose tolerance test and the risk of incident metabolic syndrome. *J Clin Endocrinol Metab* (2019) 104(2):539–49. doi: 10.1210/jc.2018-01102

47. Serbis A, Giapros V, Challa A, Chaliasos N, Siomou E. Elevated 1-hour post-load plasma glucose identifies obese youth with abnormal glucose metabolism and an unfavourable inflammatory profile. *Clin Endocrinol (Oxf).* (2018) 89(6):757–64. doi: 10.1111/cen.13859

48. Bergman M, Abdul-Ghani M, DeFronzo RA, Manco M, Sesti G, Fiorentino TV, et al. Review of methods for detecting glycemic disorders. *Diabetes Res Clin Pract* (2020) 165:108233. doi: 10.1016/j.diabres.2020.108233

49. Di Bonito P, Pacifico L, Licenziati MR, Maffeis C, Morandi ACARITALY Study on the behalf of the Childhood Obesity Study Group of the Italian Society of Pediatric Endocrinology and Diabetology. Elevated blood pressure, cardiometabolic risk and target organ damage in youth with overweight and obesity. *Nutr Metab Cardiovasc Dis* (2020) 30(10):1840–7. doi: 10.1016/j.numecd.2020.05.024

50. Videira-Silva A, Freira S, Fonseca H. Metabolically healthy overweight adolescents: definition and components. *Ann Pediatr Endocrinol Metab* (2020) 25 (4):256–64. doi: 10.6065/apem.2040052.026

51. Reisinger C, Nkeh-Chungag BN, Fredriksen PM, Goswami N. The prevalence of pediatric metabolic syndrome-a critical look on the discrepancies between definitions and its clinical importance. *Int J Obes (Lond).* (2021) 45(1):12–24. doi: 10.1038/s41366-020-00713-1

52. Strashok LA, Buznytska OV, Meshkova OM. Indicators of lipid metabolism disorders in the blood serum of adolescents with metabolic syndrome. *Ukr.Biochem.J.* (2020) 92(6):137–42. doi: 10.15407/ubj92.06.137

53. Khokhar A, Chin V, Perez-Colon S, Farook T, Bansal S, Kochummen E, et al. Differences between metabolically healthy vs unhealthy obese children and adolescents. *J Natl Med Assoc* (2017) 109(3):203–10. doi: 10.1016/j.jnma.2017.02.008

54. Rocha EPAA, Vogel M, Stanik J, Pietzner D, Willenberg A, Körner A, et al. Serum uric acid levels as an indicator for metabolically unhealthy obesity in children and adolescents. *Horm Res Paediatr* (2018) 90(1):19–27. doi: 10.1159/000490113

55. Jankowska A, Brzeziński M, Romanowicz-Sołtyszewska A, Szlagatys Sidorkiewicz A. Metabolic syndrome in obese children-clinical prevalence and risk factors. *Int J Environ Res Public Health* (2021) 18(3):1060. doi: 10.3390/ijerph18031060

56. Chabowska G, Czyżewski M, Barg E. Juvenile obesity in terms of various evaluation methods. *Pediatr Endocrinol Diabetes Metab* (2022) 28(2):132–40. doi: 10.5114/pedm.2022.116111

57. Jiang Y, Dou Y, Chen H, Zhang Y, Chen X, Wang Y, et al. Performance of waist-to-height ratio as a screening tool for identifying cardiometabolic risk in children: a meta-analysis. *Diabetol Metab Syndr* (2021) 13(1):66. doi: 10.1186/s13098-021-00688-7

58. Gepstein V, Weiss R. Obesity as the main risk factor for metabolic syndrome in children. *Front Endocrinol (Lausanne).* (2019) 10:568. doi: 10.3389/fendo.2019.00568

59. Brzeziński M, Metelska P, Myśliwiec M, Szlagatys-Sidorkiewicz A. Lipid disorders in children living with overweight and obesity- large cohort study from Poland. *Lipids Health Dis* (2020) 19(1):47. doi: 10.1186/s12944-020-01218-6

60. Deeb A, Attia S, Mahmoud S, Elhaj G, Elfatih A. Dyslipidemia and fatty liver disease in overweight and obese children. *J Obes* (2018) 2018:8626818. doi: 10.1155/2018/8626818

61. Oliveira RG, Guedes DP. Performance of different diagnostic criteria of overweight and obesity as predictors of metabolic syndrome in adolescents. *J Pediatr (Rio J).* (2017) 93(5):525–31. doi: 10.1016/j.jped.2016.11.014

62. Gregory JW. Prevention of obesity and metabolic syndrome in children. *Front Endocrinol (Lausanne).* (2019) 10:669. doi: 10.3389/fendo.2019.00669

63. Choukem SP, Tochie JN, Sibetcheu AT, Nansseu JR, Hamilton-Shield JP. Overweight/obesity and associated cardiovascular risk factors in sub-Saharan African children and adolescents: a scoping review. *Int J Pediatr Endocrinol* (2020) 2020:6. doi: 10.1186/s13633-020-0076-7

64. Bitew ZW, Alemu A, Ayele EG, Tenaw Z, Alebel A, Worku T. Metabolic syndrome among children and adolescents in low and middle-income countries: a systematic review and meta-analysis. *Diabetol Metab Syndr* (2020) 12:93. doi: 10.1186/s13098-020-00601-8

65. Hagman E, Reinehr T, Kowalski J, Ekbom A, Marcus C, Holl RW. Impaired fasting glucose prevalence in two nationwide cohorts of obese children and adolescents. *Int J Obes (Lond).* (2014) 38(1):40–5. doi: 10.1038/ijo.2013.124

66. Esquivel Zuniga R, DeBoer MD. Prediabetes in adolescents: prevalence, management and diabetes prevention strategies. *Diabetes Metab Syndr Obes* (2021) 14:4609–19. doi: 10.2147/DMSO.S284401

67. Chylińska-Frątczak A, Michalak A, Baranowska-Jaźwiecka A, Mianowska B, Szadkowska A. Incidence of hyperglycaemic disorders in children and adolescents with obesity. *Pediatr Endocrinol Diabetes Metab* (2022) 28(4):274–80. doi: 10.5114/pedm.2022.121369

68. Brar PC. Update on the current modalities used to screen high risk youth for prediabetes and/or type 2 diabetes mellitus. *Ann Pediatr Endocrinol Metab* (2019) 24 (2):71–7. doi: 10.6065/apem.2019.24.2.71

69. US Preventive Services Task Force, Mangione CM, Barry MJ, Nicholson WK, Cabana M, Chelmow D, et al. Screening for prediabetes and type 2 diabetes in children and adolescents: US preventive services task force recommendation statement. *JAMA.* (2022) 328(10):963–7. doi: 10.1001/jama.2022.14543

70. Chaychenko T, Kharkova M, Rybka O. Variants of hyperlipidemia in children with insulin resistance. *Inter Collegas.* (2020) 7(1):23–5. doi: 10.35339/ic.7.1.23-25

71. Nguyen D, Kit B, Carroll M. Abnormal cholesterol among children and adolescents in the united states, 2011-2014. *NCHS Data Brief.* (2015) 228):1–8.

72. Elmaoğulları S, Tepe D, Uçaktürk SA, Karaca Kara F, Demirel F. Prevalence of dyslipidemia and associated factors in obese children and adolescents. *J Clin Res Pediatr Endocrinol* (2015) 7(3):228–34. doi: 10.4274/jcrpe.1867

73. Agbaje AO, Lloyd-Jones DM, Magnussen CG, Tuomainen TP. Cumulative dyslipidemia with arterial stiffness and carotid IMT progression in asymptomatic adolescents: a simulated intervention longitudinal study using temporal inverse allocation model. *Atherosclerosis.* (2023) 364:39–48. doi: 10.1016/j.atherosclerosis.2022.11.011

74. Yoon JS, Lee HJ, Jeong HR, Shim YS, Kang MJ, Hwang IT. Triglyceride glucose index is superior biomarker for predicting type 2 diabetes mellitus in children and adolescents. *Endocr J* (2022) 69(5):559–65. doi: 10.1507/endocrj.EJ21-0560

75. Mosca A, Della Volpe L, Alisi A, Veraldi S, Maggiore G. Non-invasive diagnostic test for advanced fibrosis in adolescents with non-alcoholic fatty liver disease. *Front Pediatr* (2022) 10:885576. doi: 10.3389/fped.2022.885576

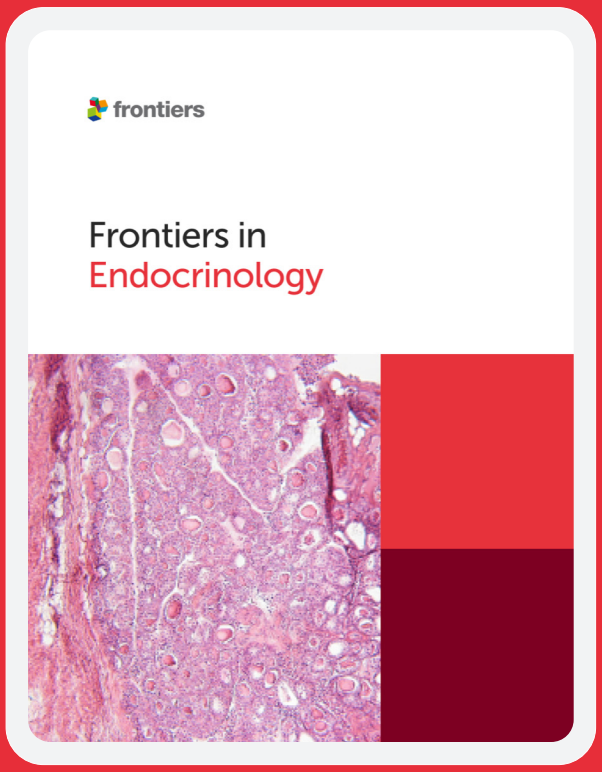
Frontiers in Endocrinology

Explores the endocrine system to find new therapies for key health issues

The second most-cited endocrinology and metabolism journal, which advances our understanding of the endocrine system. It uncovers new therapies for prevalent health issues such as obesity, diabetes, reproduction, and aging.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact