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Micronutrients and metabolic diseases, volume II

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Micronutrients and metabolic diseases, volume II

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Editorial: Micronutrients and metabolic diseases - volume II

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Editorial on the Research Topic
Micronutrients and metabolic diseases - volume II

Healthy dietary patterns are recommended as preventive or therapeutic approaches for metabolic diseases (1). Healthy dietary patterns include abundant vegetables, fruits, and whole grains, and are rich in micronutrients such as antioxidant vitamins and minerals (2). Suboptimal intake of these micronutrients adversely affects glucose and lipid metabolism, increasing the risk of metabolic diseases, such as cardiovascular disease (CVD) and type 2 diabetes mellitus (3). Targeted dietary supplementation with certain micronutrients has been shown to improve cardiometabolic risk factors and health outcomes in at-risk populations (4).

The previous Research Topic, "*Micronutrients and metabolic diseases – Volume I*," consisted of a series of articles focusing on the mechanisms of micronutrient metabolism and the relationship between micronutrients and human health (5). The current "*Micronutrients and metabolic diseases – Volume II*" extends this scope with of 14 new studies that investigate the influence of dietary patterns rich in micronutrients and the status of specific vitamins and minerals on metabolic diseases across diverse populations.

Adequate intake of B vitamins is critical for the maintenance of cardiometabolic health, especially the supplementation of B vitamins involved in homocysteine metabolism, which has been proposed as an effective strategy for the prevention of atherosclerotic cardiovascular diseases (6). In the current issue, three articles focus on the relationship between B vitamins and metabolic disease. Li H. et al. reported that dietary vitamin B1 intake was inversely associated with the risk of severe abdominal aortic calcification in a dose-response manner. Overton et al. reviewed the role of vitamin B_1 in human health and the underlying mechanisms behind the therapeutic efficacy of thiamine in gastrointestinal diseases. Jiang et al. performed a Two-Sample Mendelian Randomization screening to reveal the causal relationship between micronutrients/serum metabolites and intervertebral disk degeneration. Among 15 micronutrients and 1,091 blood metabolites, they found that vitamin B_{12} displayed a negative correlation with the incidence of intervertebral disk degeneration, and 4-acetaminophen sulfate may act as a potential mediator of the protective effect of vitamin B_{12}.

Metabolic dysfunction-associated fatty liver disease (MAFLD) affects approximately one-third of the global population. Individuals with MAFLD exhibit an elevated risk of other metabolic diseases (7). Three articles investigated the associations of diet patterns or a specific dietary micronutrient on the incidence of MAFLD (Yue et al., Lu et al., Tao et al.). Yue et al. observed that dietary antioxidant micronutrient vitamin E was negatively

associated with the incidence of MAFLD, suggesting a contributing role of inflammation in the development of MAFLD. Tao et al. and Lu et al. additionally explored the influence of dietary or tissue inflammatory index on MAFLD.

Dysregulated glucose and lipid metabolism is the underlying pathological mechanism for most metabolic diseases. The triglyceride-glucose index has emerged as a novel tool in evaluating insulin resistance and metabolic diseases. Based on the United State National Health and Nutrition Examination Survey (NHANES) data, two studies, respectively, reported that vitamin D or zinc status correlated with the triglyceride-glucose index, indicating that nutrient status could aid in assessing insulin resistance (Lai et al.) or insulin resistance-related mortality risk (Zhang and Li). A systematic review by Laurindo et al. concluded that intake of polyunsaturated fatty acids-enriched seed oils had a positive impact on lipid profiles, blood glucose, and oxidative stress markers in patients with diabetes and dyslipidemia. These findings support the integrating of these nutrients into personalized nutrition strategies for the management of metabolic health.

In addition to studies focusing on specific nutrients, several articles in current issue examined the impact of dietary patterns on cardiometabolic health. Oxidative balance score (OBS) indicates the equilibrium between prooxidants and antioxidants in an individual's diet or lifestyle. Jin et al. reported that higher dietary or lifestyle OBS was associated with a reduced atherosclerotic cardiovascular disease risk, suggesting that a dietary pattern or lifestyle promoting more prooxidants may help mitigate the development of cardiovascular disease. Li S. et al. found that body mass index was involved in the preventative effect of the DASH diet on metabolic-related diseases. LiYa et al. reported that nutritional status affected gut microbiota, influencing disease progression and survival outcomes in esophageal cancer patients, and that low intake of carbohydrates and fiber was closely associated with malnutrition in these patients.

By spanning diverse populations and adopting rigorous methods, these articles advance our knowledge of the association between micronutrients and multiple domains of human health. As metabolic diseases continue to pose a worldwide health challenge, these findings provide practical insights to improve personalized nutrition strategies, ultimately aiding long-term efforts to lower the incidence of metabolic issues diseases.

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JL: Writing – review & editing, Writing – original draft. AY: Writing – review & editing, Writing – original draft. YL: Writing – original draft, Writing – review & editing. PA: Writing – original draft, Writing – review & editing.

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References

1. Lichtenstein AH, Appel LJ, Vadiveloo M, Hu FB, Kris-Etherton PM, Rebholz CM, et al. 2021 Dietary Guidance to Improve Cardiovascular Health: A Scientific Statement From the American Heart Association. *Circulation.* (2021) 144:e472–87. doi: 10.1161/CIR.0000000000001031

2. Belardo D, Michos ED, Blankstein R, Blumenthal RS, Ferdinand KC, Hall K, et al. Practical, Evidence-Based Approaches to Nutritional Modifications to Reduce Atherosclerotic Cardiovascular Disease: An American Society For Preventive Cardiology Clinical Practice Statement. *Am J Prev Cardiol.* (2022) 10:100323. doi: 10.1016/j.ajpc.2022.100323

3. Berger MM, Shenkin A, Schweinlin A, Amrein K, Augsburger M, Biesalski H-K, et al. ESPEN micronutrient guideline. *Clin Nutr.* (2022) 41:1357–424. doi: 10.1016/j.clnu.2022.02.015

4. An P, Wan S, Luo Y, Luo J, Zhang X, Zhou S, et al. Micronutrient Supplementation to Reduce Cardiovascular Risk. *J Am Coll Cardiol.* (2022) 80:2269–85. doi: 10.1016/j.jacc.2022.09.048

5. An P, Yang A, Li J, Luo Y. Micronutrients and Metabolic Diseases. *Front Res Top* (2024) doi: 10.3389/978-2-8325-4671-0

6. Liu S, An P. Untangling the uncertainty in B vitamins for stroke prevention: folic acid fortification, dosage, and their interaction? *Am J Clin Nutr.* (2024) 119:593–4. doi: 10.1016/j.ajcnut.2024.01.005

7. Chan W-K, Chuah K-H, Rajaram RB, Lim L-L, Ratnasingam J, Vethakkan SR. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): A State-of-the-Art Review. *J Obes Metab Syndr.* (2023) 32:197–213. doi: 10.7570/jomes23052

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A cross-sectional survey study on the correlation analysis of nutritional status and intestinal flora in patients with esophageal cancer

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Objective: This study aims to examine the nutritional status of individuals diagnosed with esophageal cancer and compare the nutritional indicators and intestinal flora between malnourished and non-malnourished patients. The findings aim to contribute to the early prevention of malnutrition and the development of interventions targeting the intestinal flora to treat esophageal cancer.

Methods: An 80-patient sample of hospitalized individuals with esophageal cancer was selected from the radiotherapy department of our hospital between July 2021 and July 2022 to evaluate NRS2002 scores and PG-SGA scores. This cross-sectional analysis aimed to examine the disparities in dietary nutrient intake, blood indicators, body composition, and fecal intestinal flora between malnourished and non-malnourished patients with esophageal cancer. Additionally, we randomly selected 40 cases to predict and analyze the relationship between intestinal flora and malnutrition.

Results: The incidence of nutritional risk and malnutrition in patients with esophageal cancer was 62.5% and 60%, respectively. The low intake of carbohydrates and dietary fiber in the malnutrition group was statistically significant compared to those in the non-malnutrition group ($P < 0.05$). The albumin (ALB) level was lower in the malnutrition group than in the non-malnutrition group, while the C-reactive protein (CRP) level was higher; these differences were also statistically significant ($P < 0.05$). The basal metabolic rate, phase angle, body cell mass, muscle mass, skeletal muscle index, and fat-free mass index in the malnutrition group all decreased compared to the non-malnutrition group. The extracellular water/total body water was higher than that in the non-malnutrition group, which was also statistically significant ($P < 0.05$). As shown by 16S rDNA sequencing of fecal intestinal flora, there was no significant difference in α and β diversity between the malnutrition and non-malnutrition groups; at the genus

level, significant differences were observed for Selimonas, Clostridioides, Dielma, Lactobacillus, and [Eubacterium]_siraeum_group. However, Dielma, Sellimonas, and Clostridioides were significantly lower in the malnutrition group than in the non-malnutrition group, while Anaerococcus, Atopobium, Eubacterium_siraeum_group, and Lactobacillus were significantly higher in the malnutrition group. Correlation analysis between different genera and clinical indicators showed that Lactobacillus was positively correlated with ALB, dietary energy, intracellular water/total body water (ICW/TBW), phase angle (PA), muscle mass (MM), skeletal muscle mass (SMM), body cell mass (BCM), basal metabolic rate (BMR), appendicular skeletal muscle mass (ASMM), total body water (TBW), fat-free mass index (FFMI), skeletal muscle index (SMI), fat-free mass (FFM), Weight, body mass index (BMI) ($r > 0$, $P < 0.05$), but negatively correlated with PG-SGA score, NRS2002 score, and extracellular water/total body water (ECW/TBW) ($r < 0$, $P < 0.05$). Based on PG-SGA, there was only a low accuracy for identifying nutrient deficiency (most areas under curve (AUC) values fell within 0.5 to 0.7, or even lower), with Lachnoclostridium's AUC being 0.688 (CI = 0.518–0.858) and Lactobacillus_salivarius_g_Lactobacillus's AUC being 0.257 (CI = 0.098–0.416). A KEGG functional analysis based on 16S data indicated potential differences affecting glucose metabolism pathways and the synthesis or division of DNA, influencing the onset, development, and prognosis of esophageal cancer patients.

Conclusion: Esophageal cancer patients are more likely to be malnourished. The nutritional status of these patients is closely linked to the intake of carbohydrates and fiber, albumin levels, inflammation levels, and lean body mass. Furthermore, the patient's intestinal flora composition plays a significant role in their nutritional well-being. Consequently, modulating the intestinal flora holds promise as a potential therapeutic approach for addressing malnutrition in esophageal cancer patients.

Clinical trial registration: ChiCTR2100048141

KEYWORDS

esophageal cancer, malnutrition, PG-SGA, NRS2002, intestinal flora

1 Introduction

Esophageal cancer is globally recognized as a prevalent malignant tumor. According to the International Agency for Research on cancer's (IARC) statistical report in 2020 it is the eighth most common cancer, with 604,000 new cases, and the sixth leading cause of cancer-related deaths, with 544,000 deaths (1). This signifies that esophageal cancer will subsequently impose substantial economic and health burdens on China and the rest of the world over the next several decades.

Chronic esophageal cancer patients grapple with serious nutritional problems due to local tumor obstruction and destruction, systemic reactions caused by abnormal metabolism of tumor cells, and complications arising from antineoplastic therapies. It is considered to be have the highest incidence of nutritional risk, the rate of which, as reported globally, ranges between 67.5% and 78.9% (2, 3). Studies reveal that a significant proportion (60%−85%) of these patients are malnourished, the most common form of which is across all cancers (4, 5). Malnutrition in esophageal cancer impairs organ function, amplifies surgical risks, augments complications, and diminishes both short-term and long-term treatment outcomes (6). Furthermore, malnutrition reduces radiosensitivity and chemotherapy sensitivity (4, 7), decreases patient quality of life (8, 9), extends hospital stays (10), and precipitates readmission within a brief period (11). Optimal nutritional status significantly impacts the survival outcome of patients with cancer (2, 3, 12, 13), benefiting their prognosis and mitigating adverse reactions during treatment to enhance their quality of life. The European Society of Parenteral and Enteral Nutrition clearly pointed out the important role of nutritional support therapy in the comprehensive treatment of cancer patients (14). Currently, numerous national and international studies robustly validate nutrition intervention's positive influence on the nutritional status of esophageal cancer patients, its ability to decrease blood system toxicity and gastrointestinal responses, and improve therapeutic tolerance and immunity (8, 15–19). Providing nutritional support to these patients can maintain or restore their nutritional status, augment their tolerance to treatment, lessen the risk of complications, hasten recovery, and curtail hospital stays, potentially saving lives (20).

Despite optimal nutrition interventions, esophageal cancer patients may still experience poor treatment outcomes due to tumor characteristics. The Human Microbiome Project (HMP), initiated in 2007, ignited research on microbiomes. Intestinal flora is closely associated with prognosis in patients with esophageal cancer, such as promoting cancer susceptibility (21), enhanced

inflammatory response, and shortened survival time (22). However, most efforts have focused on differences between esophageal cancer and healthy people, overlooking dissimilarities among malnutrition and non-malnutrition patient microbiota. This study wants to explore whether intestinal flora is beneficial to patients with esophageal cancer and then support patients with intestinal flora to improve their prognosis.

To better detect malnourished patients with esophageal cancer as soon as possible, provide reasonable nutritional support programs. This research aims to identify clinical and anthropometric indicators and gut microbiota changes in esophageal cancer patients with malnutrition for effective nutritional support.

2 Research objective and methodology

2.1 Research object

We selected esophageal cancer patients admitted to the Radiation Therapy Department of General Hospital, PLA, from July 2021 to July 2022 for our study. Written informed consent was obtained as per hospital protocol. This research had ethics approval with registration number ChiCTR2100048141 at the China Clinical Trial Registration Center. The content presented in this paper was part of the study, which intended to provide nutritional support. However, due to missing data, the study design was modified to analyze cross-sectional data from the participants who agreed to participate.

2.1.1 Inclusion criteria

(1) Age > 18 years;
(2) diagnosed esophageal cancer pathologically;
(3) capacity to respond accurately to questionnaires; and
(4) well-informed about the diagnosis and willing to participate.
(5) At present, there is no drug treatment for esophageal cancer.

2.1.2 Exclusion criteria

(1) Critically ill cardiac, pulmonary, liver, or kidney patients;
(2) presence of fever or infection;
(3) organs transplantation or concurrent primary tumor;
(4) cognitive deficit; and
(5) psychological/psychiatric conditions requiring immediate attention.

3 Data collection

3.1 General data

The radiation oncologist strictly selected the trial participants according to inclusion and exclusion criteria, notifying our nutritionist to evaluate them. Our department's nutritionist completed the patient evaluation within 24 h after admission. Our nutritionist provided a concise overview of the trial's purpose and executed informed consent with those willing to participate. Simultaneously, the nutritionist collected general information using anthropometric measurements and an electronic questionnaire, including name, age, height, weight, BMI, occupation, education level, pre-existing conditions, tumor stage, marital status, contact details, etc. [Weight and height are recorded rounded off to the nearest tenth of a kilogram and centimeter, respectively; BMI = weight (kg)/height $(m)^2$].

3.2 Dietary survey

The patient's food intake (including food type and quantity) for the past 3 days is reviewed, with nutritionists accurately calculating total energy and nutrient intakes.

3.3 NRS2002 nutritional risk screening

This screening tool (23), endorsed by both the European Society for Parenteral and Enteral Nutrition and the Chinese Association for Parenteral and Enteral Nutrition, is administered within 24 h after admission by nurses. It captures nutritional scores, disease burden, and elderly status (1 point if aged over 70 years, zero otherwise). A maximum score of 3 indicates risk; below that, there is no risk.

3.4 PG-SGA nutrition evaluation (patient classification)

The PG-SGA evaluation (24) was designed for the nutritional status assessment of cancer patients, which was used to identify malnourished esophageal cancer patients in this study and was collected by nutritionists simultaneously when collecting general patient data. It consists of four parts: general condition (weight loss in the last 2 weeks, reduction in diet in the last week, gastrointestinal reactions, mobility), disease state and age (cancer, AIDS, pulmonary or cardiac cachexia, bedsore, open wound or sputum, and the age of trauma > 65 years), metabolic stress state (stress level, fever existence, duration, and hormone use per day), and physical examination (triceps skinfold thickness, grip strength, calf circumference, and ankle edema). In this study, we used a PG-SGA score of 4 as a cut-off value. Patients scoring <4 falls into the non-malnutrition group, while those scoring ≥4 forms the malnutrition group.

3.5 Body composition analysis

Multi-frequency BIA analysis via the portable body composition analyzer (NUTRILAB 003) considers total body water, muscle mass, fat-free mass, percentage of fat mass, and protein content. Measurements were conducted on the morning of the 2^{nd} day following patient enrollment, with instructions to fast for 2 h and abstain from water for 1 h.

3.6 Tumor patient quality of life scoring

Symptoms such as appetite, mental condition, sleep quality, fatigue levels, pain, family understanding, co-worker empathy, cancer self-awareness, treatment perceptions, daily activities, treatment side effects, and facial expressions are scored on a scale of 1 to 5. At present, life quality ratings range from <20 for poor, 21–30 for moderate, 31–40 for good, 41–50 for excellent, and 51–60 for great (25). Face expression scoring was subjectively conducted by a nutritionist based on the facial expression pain rating scale for patients with cancer pain.

3.7 Blood indicators

The fasting (8-h) venous blood samples are collected by nurses in our hospital. Complete blood cell tests utilized an ABX-MICROS-60 Automated Hematology Analyzer and its accompanying reagents, including hemoglobin (HB) (g/L), red blood count (RBC) (10^{12}/L), white blood count (WBC) (10^{9}/L), neutrophil (%), lymphocyte (%), monocyte, eosinophilic, platelet count (PLT) (10^{9}/L), and CRP (mg/dL)-median (P25-P75). Biochemical assays were run on a HITACHI-7100 Full Auto Biochemistry Analyzer, using the corresponding kits, including alanine aminotransferase (ALT) (U/L), aspartate aminotransferase (AST) (U/L), total protein (TP) (g/L), ALB (g/L), blood urea nitrogen (BUN) (umol/L), serum creatinine (Scr) (umol/L), uric acid (UA) (umol/L), calcium (mmol/L), and phosphorus (mmol/L). The levels of immunoglobulin A (IgA) (mg/dL), immunoglobulin G (IgG) (mg/dL), and immunoglobulin M (IgM) (mg/dL) were determined according to kit instructions (Beijing Orco Biotechnology Co., Ltd., Beijing, China) by a microplate reader (Multiskan FC, ThermoFisher, Beijing, China). The superoxide dismutase (SOD) (U/mL) index level was determined with an enzyme label detector with commercial enzyme-linked immunosorbent assay kits (Nanjing, Jiangsu, China).

3.8 Gut microbiota analysis

Inform the patient of the precautions, and the patient retained the stool sample. Considering that patients with esophageal cancer have limited eating and may not defecate in the morning, the sample is selected for the patient's feces before treatment, not limited to time. Provided the patient with our department's contact information. Once the specimen is retained, our nutritionist will collect it. The specific precautions for fecal specimen collection are as follows: Scoop a pea-sized stool and place it into the tube containing the stool preservation solution. To avoid contamination, the scoop could not touch any extraneous area, including urine or other body fluids. The microbiological diversity of these samples was analyzed by Fanxing Boao (Beijing) Technology Co., Ltd. using the 16S method.

Total genomic DNA was extracted using a DNA extraction kit following the manufacturer's instructions. The concentration of DNA was verified with NanoDrop and an agarose gel. The genome DNA was used as a template for PCR amplification with the barcoded primers and Tks Gflex DNA Polymerase (Takara). Amplicon quality was visualized using gel electrophoresis, purified with AMPure XP beads (Agencourt), and amplified for another round of PCR. After being purified with the AMPure XP beads again, the final amplicon was quantified using a Qubit dsDNA assay kit. Equal amounts of purified amplicon were pooled for subsequent sequencing.

Alpha diversity analysis includes the ACE index, the Chao1 index, the Shannon index, and the Simpson index. The former two represent the richness of intestinal flora, and the latter two represent the diversity of intestinal flora. The higher the value, the higher the species richness or diversity.

Beta diversity analysis is a comparative analysis of the composition of the two groups of samples. In this study, PCoA map analysis was used to compare the differences in the composition of intestinal flora between malnutrition and non-malnutrition groups. PCoA analysis provides results based on multiple distance matrices. Through PCoA, the differences between individuals or groups can be observed. Each point in the figure represents a sample, and the same color is the same group. The closer the sample distance of the same group is, and there is a significant distance from other groups, indicating that the grouping effect is good.

Linear discriminant analysis effect size (LEfSe) analysis can show the species with significant differences in abundance in different groups and is used to analyze the effect of each species' abundance on the difference effect. In this study, species with an impact value >4 were set as biomarkers.

Correlation analysis between genus-level differential bacteria and clinical indicators.

ROC curve analysis of differential bacteria based on PG-SGA malnutrition genus level.

Further, we analyzed the effect of classification differences between malnutrition and non-malnutrition groups on function. The PICRUSt program was used to predict the differentially expressed functions and metabolic pathways between the two groups based on the KEGG function of 16S.

4 Statistical analysis

Excel was used to establish a database and double-entry survey data. SPSS 26.0 was used for data analysis. Statistical software G * Power (developed by the University of Düsseldorf, Germany, specifically for the calculation of statistical power and sample size statistics) was used to estimate the sample size required 34 cases, effect size = 0.5, $\alpha = 0.05$, and power (1-β) = 0.9. Count data were described by frequency and percentage; the measurement data conforming to normality were described by mean ± standard deviation, and the measurement data not conforming to normality were expressed by median (25%, 75%). Frequencies were compared using the $\chi 2$ test. Means and SD values were compared with the Student's *t*-test. Correlation analysis was performed using Spearman. ROC analysis used sensitivity as the ordinate and (1-specificity) as the abscissa to construct a curve, and the cut-off point with the largest Youden index was used to determine the critical value of malnutrition. $P < 0.05$ indicated that the difference was statistically significant.

TABLE 1 Demographic and clinical characteristics of patients with esophageal cancer ($n = 80$).

Features	Esophageal cancer
Sex (male/female)	64/16
Age (years)	62.10 ± 7.56
Weight (kg)	66.10 ± 12.12
Height (cm)	167.43 ± 8.47
Body mass index (kg/m^2)	23.47 ± 3.19
Educational status	
Illiterate	6 (7.5%)
Primary school	10 (12.5%)
Middle school	46 (57.5%)
University and above	18 (22.5%)
Socioeconomic status (annual income, RMB)	
< ¥ 50,000	18(22.5%)
¥ 50,000–¥ 100,000	40(50%)
¥ 100,000–¥ 150,000	16(20%)
¥ 150,000 +	6(7.5%)
Drinking status	
No	22 (27.5%)
Occasionally	10 (12.5%)
Often	10 (12.5%)
Alcoholism	38 (47.5%)
Smoking status	
Don't smoke or quit	44 (55%)
Quit smoking <12 months	26 (32.5%)
Smoking	10 (12.5%)
Exercise	
No	30 (37.5%)
Occasionally	30 (37.5%)
Often	20 (25%)
History of diabetes	8 (10%)
History of hypertension	38 (47.5%)
History of CHD	4 (5%)
Family history of cancer	64 (80%)
Cancer staging	
I	6 (7.5%)
II	2 (2.5%)
III	2 (2.5%)
IV	70 (87.5%)
NRS2002 score	2.80 ± 1.40
<3	30 (37.5%)
≥3	50 (62.5%)
PG-SGA score	4.75 ± 3.16
<4	32 (40%)
≥4	48 (60%)
QOL score	53.53 ± 6.26

CHD, coronary heart disease. Exercise: No: no active activity; Often: at least 5 days of moderate-intensity physical activity per week, for more than 150 min; Occasionally: between no and often.

5 Experimental result

5.1 Demographic profile of 80 esophageal cancer patients

As shown in Table 1, a total of 80 patients, comprising 16 F and 64 M with an average age of 62.1 years, were included in the study. This study suggests that the average BMI of patients is at a normal level. Eighty percentage had a family history of cancer, and 87.5% presented as Stage IV esophageal carcinoma. A staggering total of 62.5% had nutritional risks, and 60% suffered from malnutrition, a majority share overall. The mean quality of life score was categorized as great.

5.2 Demographic profile of 80 esophageal cancer patients classified by PG-SGA

As shown in Table 2, the NRS2002 score was significantly higher in the malnutrition group than in the non-malnutrition group ($P < 0.05$), and the QOL score was significantly lower in the malnutrition group than in the non-malnutrition group ($P < 0.05$). The malnutrition group also reported drinking significantly more than the non-malnutrition group ($P < 0.05$).

5.3 Based on PG-SGA, the dietary intake and blood biochemical indexes of the malnutrition group and the non-malnutrition group were evaluated

As shown in Table 3, the intake of carbohydrates and dietary fiber was significantly lower in the malnutrition group than in the non-malnutrition group ($P < 0.05$). The inflammatory index CRP in the non-malnutrition group was significantly lower than that in the malnutrition group, and the ALB level was significantly higher ($P < 0.05$).

TABLE 2 Demographic and clinical characteristics of patients with esophageal cancer between the non-malnutrition and malnutrition groups ($n = 80$).

Features	Total (n = 80)	PG-SGA		*P*
		Non-malnutrition group (<4) $n = 32$	Malnutrition group(≥4) $n = 48$	
Age (y)	62.10 ± 7.56	59.38 ± 6.90	63.92 ± 7.58	0.062
Height (cm)	167.43 ±8.47	169.12 ± 8.23	166.29 ± 8.61	0.306
Weight (kg)	66.10 ±12.12	69.88 ± 10.78	63.58 ± 12.52	0.109
BMI (kg/m^2)	23.47 ± 3.19	24.30 ± 2.17	22.91 ± 3.66	0.142
NRS2002 score	2.80 ± 1.40	1.69 ± 1.20	3.54 ± 0.98	<0.001
QOL score	53.52 ± 6.26	57.81 ± 2.20	50.67 ± 6.48	<0.001
History of diabetes	8 (10.0%)	0 (0.0%)	8 (100.0%)	0.237
History of hypertension	4 (5.0%)	2 (50.0%)	2 (50.0%)	1.000
History of CHD	38 (47.5%)	10 (26.3%)	28 (73.7%)	0.093
Family history of cancer	64 (80.0%)	28 (43.75%)	36 (56.25%)	0.572
Sex				0.572
Male	64 (80.0%)	28 (43.8%)	36 (56.2%)	
Female	16 (20.0%)	4 (25.0%)	12 (75.0%)	
Educational status				0.238
Illiterate	6 (7.5%)	2 (33.3%)	4 (66.7%)	
Primary school	10 (12.5%)	0 (0.0%)	10 (100.0%)	
Middle school	46 (57.5%)	20 (43.5%)	26 (56.5%)	
University and above	18 (22.5%)	10 (55.6%)	8 (44.4%)	
Socioeconomic status (annual income, RMB)				0.051
< ¥ 50,000	18 (22.5%)	12 (66.7%)	6 (33.3%)	
¥ 50,000–¥ 100,000	40 (50.0%)	8 (20.0%)	32 (80.0%)	
¥ 100,000–¥ 150,000	16 (20.0%)	8 (50.0%)	8 (50.0%)	
¥ 150,000+	6 (7.5%)	4 (66.7%)	2 (33.3%)	
Drinking status				0.039
No	22 (27.5%)	4 (18.2%)	18 (81.8%)	
Occasionally	14 (17.5%)	12 (85.7%)	2 (14.3%)	
Often	6 (7.5%)	2 (33.3%)	4 (66.7%)	
Alcoholism	38 (47.5%)	14 (36.8%)	24 (61.2%)	
Smoking status				0.481
Don't smoke or quit	44 (55.0%)	14 (31.8%)	30 (68.2%)	
Quit smoking <12 months	26 (32.5%)	14 (53.8%)	12 (46.2%)	
Smoking	10 (12.5%)	4 (40.0%)	6 (60.0%)	
Exercise				0.108
No	30 (37.5%)	14 (46.7%)	16 (53.3%)	
Occasionally	30 (37.5%)	6 (20.0%)	24 (80.0%)	
Often	20 (25.0%)	12 (60.0%)	8 (40.0%)	
Cancer staging				0.513
I	6 (7.5%)	2 (33.3%)	4 (66.7%)	
II	2 (2.5%)	2 (100.0%)	0 (0.0%)	
III	2 (2.5%)	2 (100.0%)	0 (0.0%)	
IV	70 (87.5%)	26 (37.1%)	44 (62.9%)	

TABLE 3 Comparison of nutrient intake and blood biochemical indexes between non-malnutrition and malnutrition groups ($n = 80$).

Indicators		PG-SGA		*P*
		Non-malnutrition group (<4) $n = 32$	Malnutrition group (≥4) $n = 48$	
Nutrient intake				
	Energy (Kcal/d)	2,170.93 ± 349.77	1,964.92 ± 339.72	0.071
	Protein (g/d)	88.22 ± 22.04	96.31 ± 16.26	0.189
	Fat (g/d)	73.97 ± 15.51	73.32 ± 14.97	0.896
	Carbohydrates (g/d)	279.07 ± 75.66	221.45 ± 50.00	0.006
	Fiber (g/d)	12.64 ± 6.19	9.01 ± 3.72	0.026
Blood biochemical indexes				
	HB (g/L)	129.81 ± 15.13	124.42 ± 24.52	0.438
	RBC (10^{12}/L)	4.23 ± 0.48	4.07 ± 0.63	0.416
	WBC (10^9/L)	5.77 ± 1.99	6.12 ± 1.51	0.539
	Neutrophil (%)	64.96 ± 10.02	63.11 ± 10.39	0.580
	Lymphocyte (%)	24.49 ± 7.48	26.56 ± 9.30	0.462
	Monocyte	6.98 ± 2.67	7.80 ± 2.52	0.325
	Eosinophilic	3.19 ± 5.01	2.05 ± 2.16	0.330
	PLT (10^9/L)	222.25 ± 76.88	243.25 ± 76.88	0.407
	CRP (mg/dL)-median (P_{25}-P_{75})	0.25 (0.10–0.50)	0.88 (0.22–1.60)	0.033
	ALT (U/L)	19.44 ± 11.82	17.31 ± 8.63	0.513
	AST (U/L)	20.71 ± 12.78	17.33 ± 5.18	0.250
	TP (g/L)	68.62 ± 3.89	68.62 ± 4.89	0.673
	ALB (g/L)	40.74 ± 2.51	37.94 ± 2.58	0.002
	BUN (umol/L)	4.58 ± 1.66	5.13 ± 1.30	0.244
	Scr (umol/L)	72.86 ± 12.89	77.47 ± 22.94	0.471
	UA (umol/L)	340.84 ± 91.88	321.10 ± 96.98	0.524
	Calcium (mmol/L)	2.26 ± 0.09	2.30 ± 0.13	0.224
	Phosphorus (mmol/L)	1.11 ± 0.17	1.13 ± 0.21	0.836
	IgA (mg/dL)	261.81 ± 97.94	258.88 ± 92.08	0.924
	IgG (mg/dL)	1,098.25 ± 197.24	1,111.33 ± 331.86	0.888
	IgM (mg/dL)	62.06 ± 23.43	65.09 ± 28.07	0.723
	SOD (U/mL)	141.92 ± 13.72	137.15 ± 16.85	0.353

HB, Hemoglobin; RBC, Red blood count; WBC, White blood count; PLT, Platelet count; CRP, C-reactive protein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TP, Total protein; ALB, Albumin; BUN, Blood urea nitrogen; Scr, Serum creatinine; UA, Uric acid; Ca, Calcium; P, Phosphorus; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M; SOD, Superoxide dismutase.

5.4 Body composition variations in the malnutrition group and the non-malnutrition group

As shown in Table 4, the basal metabolic rate, phase angle, total body water, intracellular water/total body water, total body cell mass, muscle mass, skeletal muscle index, limb skeletal muscle mass, and fat-free body mass index of patients with good nutrition were significantly higher than those in the malnutrition group ($P < 0.05$). ECW/TBW (%) extracellular water/total body water was significantly lower in the non-malnutrition group than in the malnourished group ($P < 0.05$).

5.5 Analysis of intestinal flora diversity in the malnutrition group and non-malnutrition group

As shown in Figure 1, there was no significant difference in the Alpha diversity ACE index, Chao1 index, Shannon index, or

TABLE 4 Comparison of body composition analysis between non-malnutrition and malnutrition groups ($n = 80$).

Indicators	PG-SGA		*P*
	Non-malnutrition group (<4) $n = 32$	Malnutrition group(≥4) $n = 48$	
Height (cm)	169.44 ± 8.64	166.38 ± 8.59	0.277
Weight (kg)	69.38 ± 10.74	63.88 ± 12.05	0.148
BMI (kg/m^2)	23.97 ± 1.92	22.81 ± 3.30	0.214
BMR (kcal/d)	1,806.18 ± 320.73	1,592.93 ± 174.20	0.010
PA (°)	7.18 ± 2.07	5.76 ± 0.68	0.003
FFM (kg)	62.09 ± 12.10	55.28 ± 9.23	0.051
FFM/BW (%)	89.15 ± 7.24	87.19 ± 7.89	0.430
TBW (kg)	46.07 ± 8.79	41.08 ± 6.51	0.046
TBW/BW (%)	66.36 ± 7.50	65.01 ± 7.33	0.575
ECW (kg)	18.93 ± 2.63	19.25 ± 2.73	0.719
ECW/TBW (%)	42.00 ± 6.82	47.10 ± 3.53	0.004
ICW/TBW (%)	58.00 ± 6.82	52.90 ± 3.53	0.004
BCM (kg)	36.43 ± 11.06	29.06 ± 6.01	0.010
FM (kg)	7.29 ± 4.70	8.60 ± 5.69	0.451
FM/BW (%)	10.85 ± 7.24	12.81 ± 7.89	0.430
MM (kg)	32.90 ± 8.01	28.45 ± 5.47	0.043
MM/BW (%)	47.23 ± 8.69	45.05 ± 7.94	0.417
SMI	11.39 ± 2.14	10.10 ± 1.43	0.028
SMM (kg)	32.90 ± 8.01	28.45 ± 5.47	0.043
ASMM (kg)	24.58 ± 6.12	20.60 ± 3.75	0.015
FMI	2.54 ± 1.78	3.08 ± 2.10	0.406
FFMI	21.43 ± 2.54	19.73 ± 2.25	0.033

BMI, Body mass index; BMR, Basal metabolic rate; PA, Phase angle; FFM, Fat free mass; BW, Body mass; TBW, Total body water; ECW, Extracellular water; ICW, Intracellular water; BCM, Body cell mass; FM, Fat mass; MM, Muscle mass; SMI, Skeletal muscle index; SMM, Skeletal muscle mass; ASMM, Appendicular skeletal muscle mass; FMI, Fat mass index; FFMI, Fat-free mass index.

Simpson index of intestinal flora between the two groups ($P < 0.05$), but there were slight differences in the richness and diversity of the two groups.

According to the results of PCoA analysis, as shown in Figure 2, the two groups of samples were relatively clustered, but some of the flora between the two groups was also relatively discrete, indicating that some of the flora of the two groups of samples were significantly different.

5.5.1 LEfSe analysis results of two groups at the genus level

As shown in the following Figure 3, the results of LEfSe analysis showed that at the genus level, the genera with significantly increased relative abundance in the non-malnutrition group were Dielma, Sellimonas, and Clostridioides, respectively. The genera with significantly reduced relative abundance were Anaerococcus, Atopobium, Eubacterium _ siraeum _ group, and Lactobacillus, respectively.

5.5.2 Correlation analysis between genus-level differential bacteria and clinical indicators

As shown in the following Figure 4. The results showed that Sellimonas was positively correlated with Drinking ($r = 0.322$, $P = 0.042$) and negatively correlated with ALT ($r = -0.346$, $P = 0.029$) and AST ($r = -0.333\ 0$, $P = 0.036$). The genus Clostridioides was positively correlated with the QOL score ($r = 0.377$, $P = 0.016$). The genus Dielma was negatively correlated with Age ($r = -0.346$, $P = 0.026$). Lactobacillus was positively correlated with ALB ($r = 0.394$, $P = 0.012$), Dietary energy ($r = 0.370$, $P = 0.019$), ICWpct ($r = 0.315$, $P = 0.048$), PA ($r = 0.321$, $P = 0.043$), MM ($r = 0.469$, $P = 0.002$), SMM ($r = 0.469$, $P = 0.002$), BCM ($r = 0.484$, $P = 0.002$), ASMM ($r = 0.513$, $P = 0.001$), TBW ($r = 0.488$, $P = 0.001$), FFMI ($r = 0.446$, $P = 0.004$), SMI ($r = 0.367$, $P = 0.020$), Weight ($r = 0.456$, $P = 0.003$), and BMI ($r = 0.388$, $P = 0.013$). It was negatively correlated with PG-SGA score ($r = -0.424$, $P = 0.006$), NRS2002 score ($r = -0.334$, $P = 0.035$), and ECWpct ($r = -0.315$, $P = 0.048$). [Eubacterium] _ siraeum _ group was positively correlated with IgM ($r = 0.326$, $P = 0.040$), IgG ($r = 0.467$, $P = 0.002$), and monocyte ($r = 0.332$, $P = 0.036$), but negatively correlated with ALB ($r = -0.370$, $P = 0.019$) and UA ($r = -0.419$, $P = 0.007$).

5.5.3 ROC curve analysis of differential bacteria based on PG-SGA malnutrition genus level

As shown in Table 5 and Figure 5, the efficacy of [Eubacterium] _ siraeum _ group, Lactobacillus, Barnesiella, and Sellimonas genus levels in identifying malnutrition in patients with esophageal cancer only has low accuracy.

5.5.4 Function prediction of malnutrition group and non-malnutrition group

As shown in Figure 6, Cell division topological specificity factor, K06940; uncharacterized protein, pyrP, uraA; uracil permease, K07461; putative endonuclease, uxaC; glucuronate isomerase [EC: 5.3.1.12] pathway expression decreased, while RP-L36, MRPL36, rpmJ, and the expression of the large subunit ribosomal protein L36 pathway were increased.

6 Discussion

Esophageal cancer patients demonstrate high incidences of both nutritional risk and malnutrition, particularly among older people. Clinical screening should be performed promptly with these indicators as a basis for parenteral nutrition intervention (26). This study was a cross-sectional study. All patients were fed orally at the time of enrollment. Our research identified elevated nutritional risks and malnutrition prevalence in esophageal cancer hospitalized individuals; lower carbohydrate and dietary fiber intake was also noted in malnourished patients. Findings implicated nutrition deficiency facilitating low albumin or lean body weight, elevated

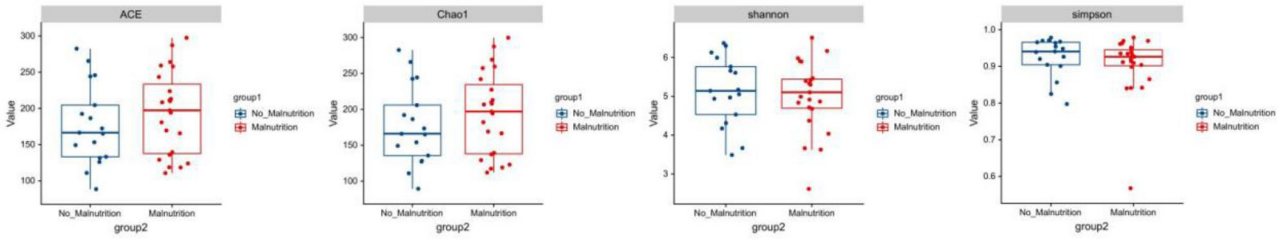

FIGURE 1
Analysis of α diversity of intestinal flora in two groups.

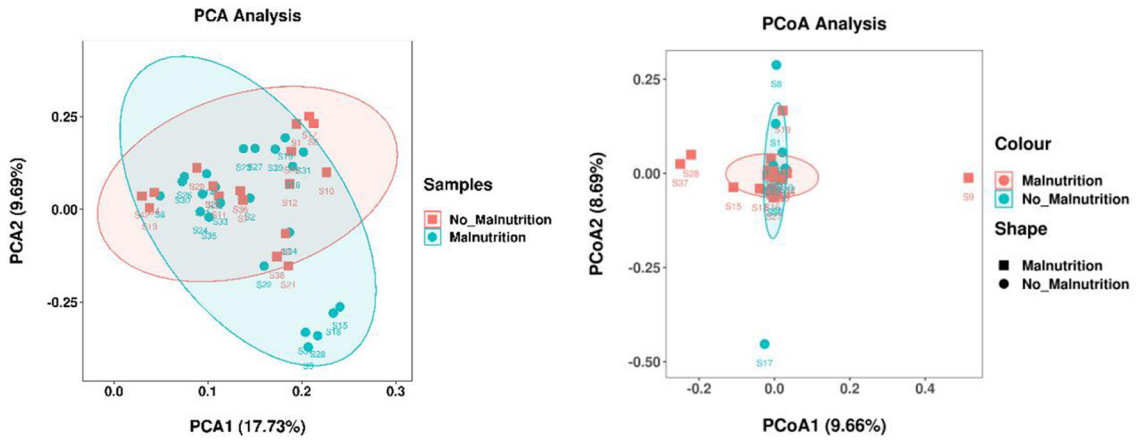

FIGURE 2
Analysis of β diversity of intestinal flora in two groups. The percentage in the figure is the interpretation rate of the principal component to the difference between the samples; a single sample is represented by a point, and the color of the same group is the same.

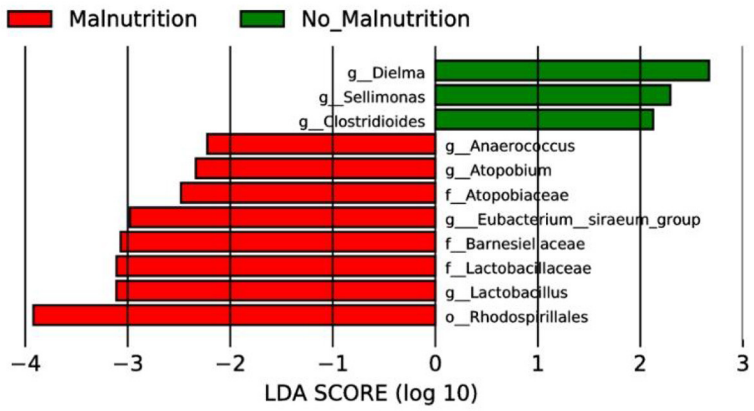

FIGURE 3
LEfSe analysis of two groups. In this study, species with an impact value greater than 4 were considered biomarkers.

inflammation markers, and a specific gut microbiota profile negatively correlated with PG-SGA and NRS2002 scores yet positively associated with ALB, PA, FFMI, SMI, FFM, weight, and BMI. Based on PG-SGA, there was only a low accuracy for identifying nutrient deficiency (most AUC values fell within 0.5 to 0.7, or even lower). Further exploration is warranted based on an expanded sample size to assess potential progressivity. We also uncovered that esophageal cancer patients' malnutrition impacts glucose metabolism and aspects of DNA synthesis/division metabolic pathways; these findings contribute to the understanding of esophageal cancer patients' malnutrition diagnosis, nutritional intervention, and clinical application of gut microbiome analysis.

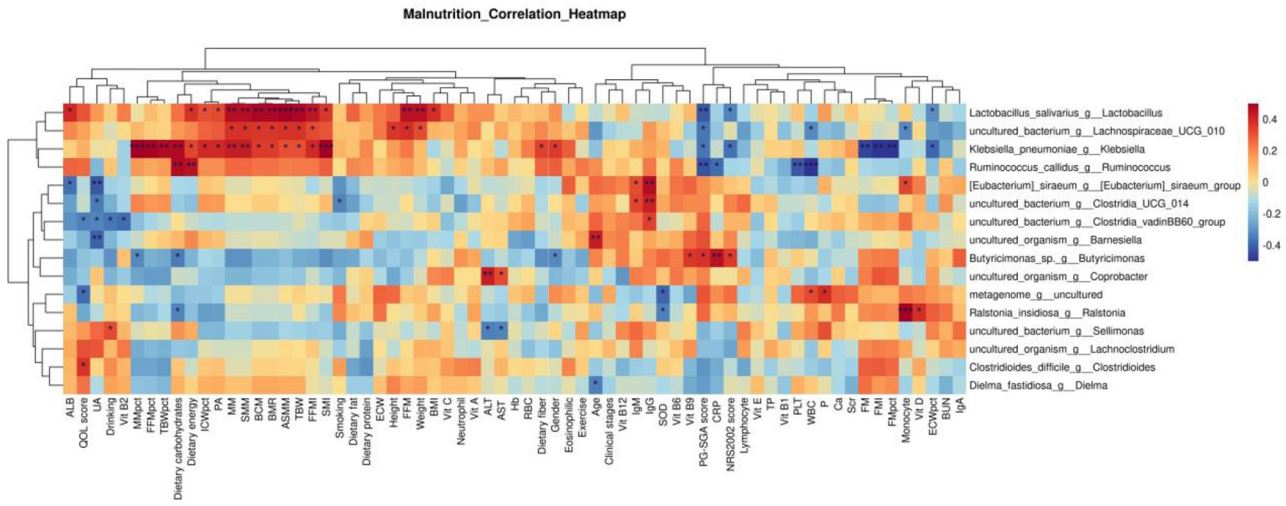

FIGURE 4
Correlation analysis between differential bacteria at the genus level and clinical indicators. $p \geq 0.05$ unmarked; $^{*}0.01<p<0.05$; $^{**}0.001<p<0.01$; $^{***}p \leq 0.001$.

TABLE 5 ROC curve was used to analyze the efficacy of differential bacteria at the genus level in identifying malnutrition in patients with esophageal cancer.

Bacteria	AUC	95% CI
Klebsiella_pneumoniae_g__Klebsiella	0.688	0.504–0.872
uncultured_organism_g__Lachnoclostridium	0.688	0.518–0.858
Ruminococcus_callidus_g__Ruminococcus	0.689	0.531–0.848
Butyricimonas_sp._g__Butyricimonas	0.678	0.564–0.791
[Eubacterium]_siraeum_g__[Eubacterium]_siraeum_group	0.664	0.535–0.793
uncultured_bacterium_g__Clostridia_UCG_014	0.609	0.523–0.695
Lactobacillus_salivarius_g__Lactobacillus	0.257	0.098–0.416
uncultured_organism_g__Barnesiella	0.650	0.536–0.763
Ralstonia_insidiosa_g__Ralstonia	0.665	0.508–0.822
uncultured_bacterium_g__Sellimonas	0.619	0.499–0.739
uncultured_bacterium_g__Clostridia_vadinBB60_group	0.652	0.556–0.748
metagenome_g__uncultured	0.609	0.523–0.695
uncultured_organism_g__Coprobacter	0.609	0.523–0.695
Clostridioides_difficile_g__Clostridioides	0.382	0.278–0.486
uncultured_bacterium_g__Lachnospiraceae_UCG_010	0.412	0.318–0.505
Dielma_fastidiosa_g__Dielma	0.412	0.318–0.505
uncultured_Kroppenstedtia_g__Kroppenstedtia	0.412	0.318–0.505

AUC, area under the curve.

The present study included patients with an 80% family history of cancer, most notably 87.5% with stage IV esophageal cancer, aligning well with the observed characteristics of esophageal cancer; that is, once found, they may be patients with advanced stage. Moreover, 62.5 % have nutritional risk, accounting for more than half. Concurrently, there was a significant prevalence of malnutrition at 60%, which constitutes more than half of the population. The QOL score was significantly lower in the malnutrition group. Drinking may be a risk factor for malnutrition in patients with esophageal cancer.

Tumorigenesis intertwines with environmental factors, of which dietary and lifestyle attributes rank as the most critical (27, 28). This study revealed that the non-malnutrition group consumed more carbohydrates and dietary fiber than the malnutrition group, conveying significance. However, other nutritional aspects were comparable. The results of this study showed that high

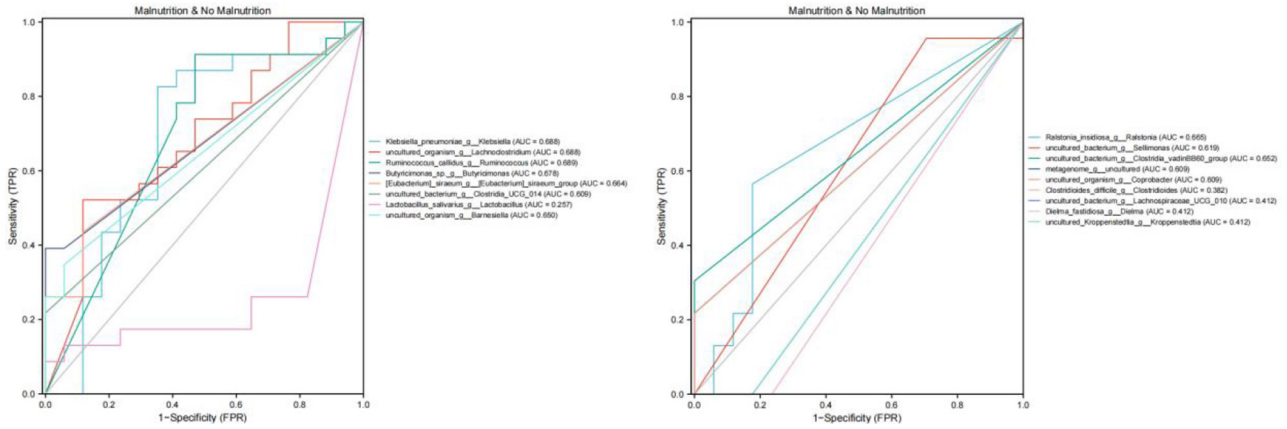

FIGURE 5
ROC curve analysis based on PG-SGA malnourished genus level differential bacteria. The area under the ROC curve is between 0.5 and 1. The closer the AUC is to 1, the better the diagnostic effect is. AUC has low accuracy when 0.5 ~ 0.7, AUC has certain accuracy when 0.7 ~ 0.9, and AUC has high accuracy when above 0.9.

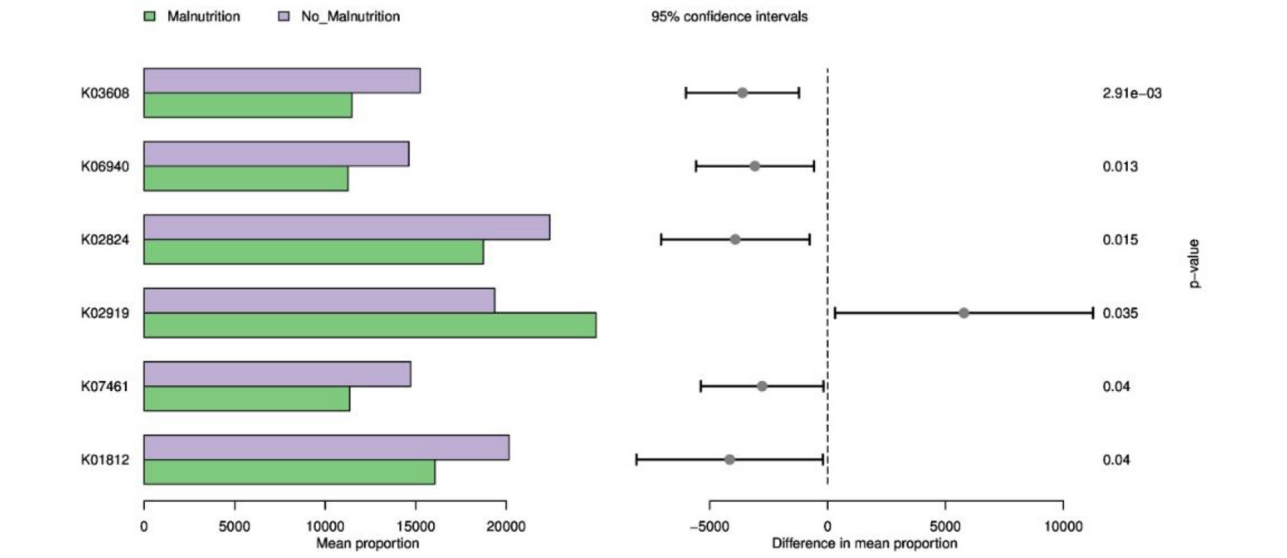

FIGURE 6
KEGG function prediction based on 16S. K03608: minE; cell division topological specificity factor; K06940: K06940; uncharacterized protein; K02824: pyrP, uraA; uracil permease; K02919: RP-L36, MRPL36, rpmJ; large subunit ribosomal protein L36; K07461: K07461; putative endonuclease; K01812: uxaC; glucuronate isomerase [EC:5.3.1.12].

carbohydrate intake might play an important role in the nutritional status of patients with esophageal cancer. However, so far, the range of carbohydrate intake and the role of carbohydrate quality (29, 30) in the occurrence and development of esophageal cancer still need to be further explored. Dietary fiber, derived from vegetables, fruits, grains, and soybeans, is indigestible by the human small intestine (31, 32) possessing anticancer properties (33, 34). In addition, some studies have shown that high-fiber diets can improve metabolic functions within the gut microbiome and suppress carcinogen production, thereby reducing the risk of esophageal cancer. This study showed that dietary fiber intake was negatively correlated with the occurrence of malnutrition in patients with esophageal cancer. Although dietary fiber intake did not reach the daily recommended intake of Chinese residents, statistical differences were still consistent with the results of related studies (34–36). Collectively, a high-fiber diet may provide a protective shield against esophageal cancer and protect against the occurrence of malnutrition in esophageal cancer patients.

Our discoveries showed that esophageal cancer patients without malnutrition had lower levels of the inflammatory marker CRP and higher levels of ALB than those with malnutrition. In recent years, the correlation between high CRP levels and malignancies has garnered significant attention. Numerous cancers display higher CRP, reflecting both systemic inflammatory response and tumor progression, both significantly linked to patient prognosis and survival (37). The 2018 GLIM standard also uses

inflammation indicators (including CRP) as the etiological criteria for the diagnosis of malnutrition. This study also confirmed malnutrition in patients with high CRP. Therefore, for patients with esophageal cancer with high CRP, whether nutritional support can be given in advance to improve the prognosis and survival of patients remains to be further explored. At the same time, most of the patients with esophageal cancer are the elderly, who experience a significant decline in vital organ functions due to multi-morbidity, extensive disease duration, elevated consumption, surgical trauma, and inadequate nutrient intake. The condition commonly results in negative nitrogen balance and low serum albumin levels (38). Also, it's worth noting that preoperative albumin levels have been found to correlate with patient outcomes. That is, patients with lower albumin levels tend to have less favorable prognoses (39–41), leading to increased postsurgical respiratory complications, wound infections, or anastomosis fistulae rates compared to those with normal albumin levels (42).

Furthermore, these patients experience longer hospital stays post-surgery and more frequent relapses, resulting in unfavorable outcomes and secondary surgeries (43). The possible mechanism is as follows: Hypoalbuminemia impairs host immunity, while muscle wasting and fat consumption can result in respiratory muscle weakness, undermining ventilation and gas exchange function; protein is crucial for wound healing. Insufficient protein intake delays wound healing and can contribute to local tissue edema, hindering wound repair and facilitating infection. Hence, monitoring albumin levels and implementing nutritional support can significantly reduce postoperative complications, simplify hospital and surveillance periods, and improve the prognosis. In summary, serum CRP and albumin levels act as reliable biomarkers, while high serum CRP and hypoalbuminemia are independent prognostic factors in esophageal cancer and can predict the survival of patients with esophageal cancer to a certain extent.

Body composition analysis is a scale to assess human components and functionality. It aids in diagnosing the nutritional state of cancer patients, monitoring their dynamics, evaluating interventions, and improving their quality of life. Emerging research (44, 45) indicates marked compositional changes in cancer patients throughout their illness. Numerous studies endorse an association between body composition and survival outcomes and a better understanding of how body composition is used to evaluate the prognosis of cancer patients. Our study found that non-malnourished patients displayed higher base metabolic rates (BMR), phase angles, muscle mass, skeletal myofiber index, limb lean tissue mass, and fat-free weight index than malnourished ones; these differences were statistically significant, $P < 0.05$, echoing previous findings (46–48). Recently, it was discovered that poor nutrition in cancer often manifests as severe muscle mass (MM) depletion at any stage, predicting poor physical function, lower quality of life, surgical complications, disease progression, and survival rate (46, 49–52). A high prevalence of low MM is observed in new cancer cases >50%, significantly surpassing healthy individuals around this age by approximately 65% (53). Reversing low MM could improve cancer treatment outcomes, morbidity, and, ultimately, mortality rate (53). Given the role of MM tissue in oncological outcomes, strategies to optimize body composition are an important part of successful cancer treatment, and nutrition is one such way to beneficially influence MM tissue. This can, in turn, improve general health and outcomes, including treatment and tolerance for survival (46, 47). The phase angle (PA) (54) is a highly sensitive marker for detecting patient malnutrition and predicting the outcomes of various diseases. It encapsulates bodily tissue attributes related to diseases, nutrition statuses, and hydration levels, allowing a holistic assessment of health and nutritional status. Compared with conventional nutritional evaluation tools, PA has unique advantages in nutritional assessment, efficacy monitoring, and prognosis prediction for patients with malignant tumors. It has broad application prospects in clinical practice. PA provides rapid measurements within 3 min, making it applicable even for individuals with abnormal shapes (54). Numerous studies show that (55–58) PA decreases with the aggravation of malnutrition. The larger the PA, the more complete the cell membrane is and the stronger the cell functions. Low levels of PA have been identified as poor prognostic factors affecting survival in diverse types of cancer patients across multiple body sites. Research indicates that lower PA correlates with elongated hospital stays, a significant decrease in survival time, a higher incidence of postoperative complications, and heightened mortality risks among tumor patients (55). The probable mechanism lies largely in the ECW/TBW ratio. Adjusting PA by this ratio could enhance prognosis and eventually improve palliative care in cancer cachexia patients, necessitating the determination of an optimal cut-off value for PA detection of malnutrition among cancer patients. Currently, minimal literature exists about the influence of body composition parameters, such as BMI, body fat percentage, SMI, and sarcopenia, on postsurgical morbidity and long-term survival in esophageal carcinoma patients. This study furnishes preliminary data on human body component parameter changes in esophageal carcinoma patients, facilitating prospective and extensive clinical trials based on body component analysis results for nutritional supplement dose standardization among cancer patients, thus achieving "precision" nutritional intervention.

Gut microbiota research has surged since the advent of next-generation sequencing, illuminating its role in health and disease. Evidence links it to various cancers (59, 60), potentially offering novel cancer therapies targeting the gut microbiome. However, research on esophageal cancer related to gut microbiota is primarily cross-sectional between patients and controls (61). Specifically, there remains a dearth of data exploring the differences in gut microbial composition between non-malnourished and malnourished patients with esophageal cancer. In this study, 16S rRNA high-throughput sequencing technology was used to analyze the composition and diversity of intestinal flora between non-malnourished and malnourished patients with esophageal cancer, and PICRUSt software was used to predict the differentially expressed functions and metabolic pathways, intending to provide novel therapeutic targets for early diagnosis and treatment of esophageal cancer. Our study divided esophageal cancer patients into non-malnutrition and malnutrition groups, conducted association analysis on bacterial communities, and observed significant changes. At the genus level, LEfSe analysis results showed that Dielma, Sellimonas, and Clostridioides were found to be significantly enriched among

the non-malnutrition group, while Anaerococcus, Atopobium, Eubacterium_siraeum_group, and Lactobacillus were depleted. Notably, Dielma abundance seems positively associated with a better prognosis, aligned with Jeffrey Gordon's team findings (62). An increase in Clostridioides appears linked to better quality of life, but no significant discrepancy was found here due to random sampling errors. It's worth noting that Clostridioides incites infections, causing substantial health and financial burdens globally (63). Therefore, whether we can find an intermediate value of Clostridioides to explain this opposite result still needs further study.

Eubacterium_siraeum_group (64) is a core gut bacteria facilitating nutrient digestion and maintaining gut balance. A significant negative correlation with serum albumin in our study indicates that reduced levels might prevent protein loss, leading to elevated albumin levels bolstering immunity against diseases. In 2022, Jiangsu Province Hospital lodged a patent (65) focusing on Eubacterium_siraeum_groupi as a predictive tool for chemotherapy-induced cachexia, aiding in timely intervention and reducing illness severity. Research reveals (66) that Lactobacillus reuteri, a well-studied probiotic strain, produces antimicrobial molecules and modulates the gut microbiome. Possibly, it supports the host immune system by reducing proinflammatory cytokines while enhancing regulatory T cells. It fortifies the gut barrier and prevents inflammatory conditions such as IBD. However, this study shows that the more Lactobacillus, the higher the potential nutritional risk may be, but it may contribute to increased physical albumin and lean tissue. Further, larger sample sizes are needed to ascertain its effects on esophageal cancer patients.

This research also utilized the PICRUSt pipeline to foresee variations in functional and metabolic pathways between two patient groups based on their respective 16S KEGG functions. Our results highlight that the gut microbiota may affect disease progression by influencing sugar metabolism, DNA synthesis, or division pathway alterations. The study observed that the cell division topological specificity factor K06940 plays a pivotal role in triggering precise regulation of cell division. The reduced expression of its genus in malnourished patients could result in inaccurate regulation and impede normal growth and genetic information transfer. Uncharacterized proteins, pyrP, uraA, and uracil permease-K07461, may affect the synthesis, transport, and metabolism of uracil. In this study, the expression of the pathway in the malnutrition group was decreased, which may affect the normal transcription of DNA. Putative endonuclease can repair damaged DNA and process accounting fragments during DNA replication. The expression of putative endonuclease in the malnutrition group is reduced, which may affect the repair of DNA damage in patients with esophageal cancer. In summary, the alterations in underfed patients' related factors might limit effective DNA repair following damage and may affect the precise regulation of cell division and then participate in the occurrence and development of tumors, aligning with studies conducted by Wilson's team (67) and Till's team (68). In this study, lower uxaC and glucuronate isomerase [EC:5.3.1.12] levels in underfed individuals, both key components involved in glucose metabolism, uphold the importance of these processes for energy production and immune regulation. Related studies (69, 70) also demonstrated evidence of disrupted glucose metabolism amongst tumors, leading to tumor growth, diminished immunity, and severe energy deficiency, ultimately fostering cachexia.

Additionally, increased RP-L36, MRPL36, rpmJ, and large subunit ribosomal protein L36 levels suggested their potential role as markers or therapeutic targets due to their central roles in promoting cell viability and protein synthesis. Some studies (71) have shown that the large subunit protein L36 may become a potential target for drug therapy, especially in the development of anti-tumor drugs, and may become a potential tumor marker and therapeutic target. Further study of a larger sample size may be required to reduce biases and reconcile conflicting findings. In general, the relationship between gut microbiota and tumorigenesis is a complex and multidimensional problem. The mechanism of the relationship between gut microbiota, human health, and cancer is still in its early stages, mainly revealing correlation rather than causality.

Further in-depth research is needed to explore the mechanism. Several studies have demonstrated that manipulating gut microflora structure and metabolic product production can potentially prevent/treat some cancers. Our research shows that the intake of carbohydrates and fibers in patients with malnutrition or esophageal cancer is lower. Whether this affects the state of intestinal flora and thus affects the nutritional status of patients warrants further research.

This research has several limitations: First, it involves a single center with limited participant and subgroup numbers. Second, insufficient follow-up time precludes adequate analysis of OS data. Finally, the small sample size limits additional sub-group analyses due to high levels of confounders. Future studies necessitate larger-scale samples and prospective ones to verify these findings.

In conclusion, esophageal cancer patients face substantial nutritional risks and malnutrition rates due to their unique anatomical features. Nutritional status is correlated with carbohydrate intake, dietary fiber intake, protein levels, inflammatory levels, and lean body mass; this also affects the gut microbiota, influencing disease progression, and outcomes. These patients require early screening and intervention based on patient-specific indicators of nutrition; this might enhance their clinical outcomes. Given the interest in noninvasive and nonpharmacological interventions for such patients (72), dietary therapy and gut microbiome manipulation require further evaluation as lower-risk treatment options.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

The study was approved by the Ethics Committee of the Chinese PLA General Hospital and registered in the Chinese Clinical Laboratory Registry under the registration number ChiCTR2100048141. The studies were conducted in accordance

with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The requirement of ethical approval was waived by the study was approved by the Ethics Committee of the Chinese PLA General Hospital and registered in the Chinese Clinical Laboratory Registry under the registration number ChiCTR2100048141 for the studies involving animals because the study has a good specification. The studies were conducted in accordance with the local legislation and institutional requirements.

Author contributions

LLi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. ZX: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HX: Project administration, Writing – original draft. LZ: Investigation, Writing – original draft. LLu: Investigation, Writing – original draft. LX: Project administration, Writing – original draft. LYe: Project administration, Writing – original draft. CJ: Project administration, Writing – original draft. ZK: Data curation, Writing – original draft. WH: Project administration, Writing – original draft. XJ: Data curation, Writing – original draft. CY: Project administration, Writing – original draft. CX: Data curation, Writing – original draft. LH: Data curation, Writing – original draft. YS: Data curation, Writing – original draft. LF: Supervision, Writing – review & editing. LYi: Supervision, Writing – review & editing.

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Conflict of interest

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References

1. Liu Z, Li Z, Zhang Y. Interpretation on the report of Global Cancer Statistics 2020. *J Multidiscipl Cancer Manage.* (2021) 7:1–14. doi: 10.12151/JMCM.2021.02-01

2. Huang C, Wu J, Xiao H. Influence factors of energy metabolism and nutritional risk screening in patients with newly diagnosed esophageal cancer. *Chin J Clin Nutr.* (2010) 18:5–8. doi: 10.3760/cma.j.issn.1674-635x.2010.01.002

3. Larrea J, Vega S, Martinez T, Torrent JM, Vega V, Núñez V. The nutritional status and immunological situation of cancer patients. *Nutr Hosp.* (1992) 7:178–84.

4. Bozzetti F, Mariani L, Vullo SL, Group SW, Amerio ML, Biffi R, et al. The nutritional risk in oncology: a study of 1,453 cancer outpatients. *Support Care Cancer.* (2012) 20:1919–28. doi: 10.1007/s00520-012-1387-x

5. Association CNCoCA-C, Branch CMAoPaEN, Nutrition and Supportive Therapy Group ROPB, Chinese Medical Doctor Association. Guidelines for nutritional treatment of esophageal cancer patients. *Chin J Clin Oncol.* (2020) 47:1–6. doi: 10.3969/j.issn.1000-8179.2020.01.430

6. Society CNMBoCN. *Chinese White Paper on Dietary Nutrition for Cancer Patients 2020–2021*. Beijing: Chinese Nutrition Society (2021).

7. Arends J, Bodoky G, Bozzetti F, Fearon K, Muscaritoli M, Selga G, et al. ESPEN Guidelines on Enteral Nutrition: Non-surgical oncology. *Clin Nutr.* (2006) 25:245–59. doi: 10.1016/j.clnu.2006.01.020

8. Langius JAE, Zandbergen MC, Eerenstein SEJ, van Tulder MW, Leemans CR, Kramer MHH, et al. Effect of nutritional interventions on nutritional status, quality of life and mortality in patients with head and neck cancer receiving (chemo)radiotherapy: a systematic review. *Clin Nutr.* (2013) 32:671–8. doi: 10.1016/j.clnu.2013.06.012

9. Capuano G, Gentile PC, Bianciardi F, Tosti M, Palladino A, Di Palma M. Prevalence and influence of malnutrition on quality of life and performance status in patients with locally advanced head and neck cancer before treatment. *Support Care Cancer.* (2010) 18:433–7. doi: 10.1007/s00520-009-0681-8

10. Pressoir M, Desné S, Berchery D, Rossignol G, Poiree B, Meslier M, et al. Prevalence, risk factors and clinical implications of malnutrition in French Comprehensive Cancer Centres. *Br J Cancer.* (2010) 102:966–71. doi: 10.1038/sj.bjc.6605578

11. Tan CSY, Read JA, Phan VH, Beale PJ, Peat JK, Clarke SJ. The relationship between nutritional status, inflammatory markers and survival in patients with advanced cancer: a prospective cohort study. *Support Care Cancer.* (2015) 23:385–91. doi: 10.1007/s00520-014-2385-y

12. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. *CA Cancer J Clin.* (2016) 66:271–89. doi: 10.3322/caac.21349

13. Li T, Li B, Lv J. Guidelines for nutritional treatment of esophageal cancer patients. *Electr J Metabol Nutr Cancer.* (2020) 7:32–42. doi: 10.16689/j.cnki.cn11-9349/r.2020.01.007

14. Muscaritoli M, Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, et al. ESPEN practical guideline: clinical nutrition in cancer. *Clin Nutr.* (2021) 40:2898–913. doi: 10.1016/j.clnu.2021.02.005

15. Cox S, Powell C, Carter B, Hurt C, Mukherjee S, Crosby TDL. Role of nutritional status and intervention in oesophageal cancer treated with definitive chemoradiotherapy: outcomes from SCOPE1. *Br J Cancer.* (2016) 115:172–7. doi: 10.1038/bjc.2016.129

16. Vasson M-P, Talvas J, Perche O, Dillies A-F, Bachmann P, Pezet D, et al. Immunonutrition improves functional capacities in head and neck and esophageal cancer patients undergoing radiochemotherapy: a randomized clinical trial. *Clin Nutr.* (2014) 33:204–10. doi: 10.1016/j.clnu.2013.06.008

17. Cong M-H, Li S-L, Cheng G-W, Liu J-Y, Song C-X, Deng Y-B, et al. An interdisciplinary nutrition support team improves clinical and hospitalized outcomes of esophageal cancer patients with concurrent chemoradiotherapy. *Chin Med J (Engl).* (2015) 128:3003–7. doi: 10.4103/0366-6999.168963

18. Talvas J, Garrait G, Goncalves-Mendes N, Rouanet J, Vergnaud-Gauduchon J, Kwiatkowski F, et al. Immunonutrition stimulates immune functions and antioxidant defense capacities of leukocytes in radiochemotherapy-treated head & neck and esophageal cancer patients: a double-blind randomized clinical trial. *Clin Nutr.* (2015) 34:810–7. doi: 10.1016/j.clnu.2014.12.002

19. Riccardi D, Allen K. Nutritional management of patients with esophageal and esophagogastric junction cancer. *Cancer Control.* (1999) 6:64–72. doi: 10.1177/107327489900600106

20. Qiu Y, You J, Wang K, Cao Y, Hu Y, Zhang H, et al. Effect of whole-course nutrition management on patients with esophageal cancer undergoing concurrent chemoradiotherapy: a randomized control trial. *Nutrition.* (2020) 69:110558. doi: 10.1016/j.nut.2019.110558

21. Meng C, Bai C, Brown TD, Hood LE, Tian Q. Human gut microbiota and gastrointestinal cancer. *Genom Proteom Bioinf.* (2018) 16:33–49. doi: 10.1016/j.gpb.2017.06.002

22. Wu C, Wang M, Zhou Q, Shi H. Associations of changes in intestinal flora and inflammatory factors with prognosis of patients with esophageal cancer. *J Healthc Eng.* (2022) 2022:2426301. doi: 10.1155/2022/2426301

23. Kondrup JE, Allison SP, Elia M, Vellas B, Plauth M. ESPEN guidelines for nutrition screening 2002. *Clin Nutr.* (2003) 22:415–421. doi: 10.1016/S0261-5614(03)00098-0

24. Bauer J, Capra S, Ferguson M. Use of the scored Patient-Generated Subjective Global Assessment (PG-SGA) as a nutrition assessment tool in patients with cancer. *Eur J Clin Nutr.* (2002) 56:779–85. doi: 10.1038/sj.ejcn.1601412

25. Zhang MY. *Psychiatric Rating Scale Manual.* Changsha: Hunan Science and Technology Press (1998).

26. Cha W, Gu X. Analysis of nutritional status and nutritional support in hospitalized patients with esophageal cancer. *Med Diet Health.* (2021) 19:12–4.

27. Xu M, Zhang C. Association between dietary fiber intake and cancer risk and prognosis. *Electr J Metabol Nutr Cancer.* (2015) 3:7. doi: 10.16689/j.cnki.cn11-9349/r.2015.03.016

28. Li S, Ye J, Lin Z, Lin Z, Tang X, Rao W, et al. Dietary inflammatory nutrients and esophageal squamous cell carcinoma risk: a case-control study. *Nutrients.* (2022) 14:5179. doi: 10.3390/nu14235179

29. Skeie G, Braaten T, Olsen A, Kyrø C, Tjønneland A, Landberg R, et al. Intake of whole grains and incidence of oesophageal cancer in the HELGA cohort. *Eur J Epidemiol.* (2016) 31:405–14. doi: 10.1007/s10654-015-0057-y

30. Petrick JL, Li N, McClain KM, Steck SE, Gammon MD. Dietary risk reduction factors for the barrett's esophagus-esophageal adenocarcinoma continuum: a review of the recent literature. *Curr Nutr Rep.* (2015) 4:47–65. doi: 10.1007/s13668-014-0108-0

31. Cummings JH, Mann JI, Nishida C, Vorster HH. Dietary fibre: an agreed definition. *Lancet.* (2009) 373:365–6. doi: 10.1016/S0140-6736(09)60117-3

32. Yuan K, Shi H. Progress in dietary fiber and colorectal tumor research. *Parenter Enteral Nutr.* (2011) 18:182–4. doi: 10.16151/j.1007-810x.2011.03.020

33. Feng X, Ge S. Sugar, dietary fiber and cancer. *Electr J Metabol Nutr Cancer.* (2014) 1:31–4. doi: 10.16689/j.cnki.cn11-9349/r.2014.03.012

34. Coleman HG, Murray LJ, Hicks B, Bhat SK, Kubo A, Corley DA, et al. Dietary fiber and the risk of precancerous lesions and cancer of the esophagus: a systematic review and meta-analysis. *Nutr Rev.* (2013) 71:474–82. doi: 10.1111/nure.12032

35. Sun L, Zhang Z, Xu J, Xu G, Liu X. Dietary fiber intake reduces risk for Barrett's esophagus and esophageal cancer. *Crit Rev Food Sci Nutr.* (2017) 57:2749–57. doi: 10.1080/10408398.2015.1067596

36. Zhao Z, Yin Z, Zhang C. Lifestyle interventions can reduce the risk of Barrett's esophagus: a systematic review and meta-analysis of 62 studies involving 250,157 participants. *Cancer Med.* (2021) 10:5297–320. doi: 10.1002/cam4.4061

37. Huang Y, Feng JF, Liu JS, Chen QX. Prognostic role of serum C-reactive protein in esophageal cancer: a systematic review and meta-analysis. *Ther Clin Risk Manag.* (2015) 11:89–94. doi: 10.2147/TCRM.S70954

38. Sun C, Zhou L, Shao X. The influence of hypoalbuminemia on postoperative complications of esophageal cancer. *Hebei Medicine.* (2015) 21:4. doi: 10.3969/j.issn.1006-6233.2015.04.014

39. Ku JH, Kim M, Choi WS, Kwak C, Kim HH. Preoperative serum albumin as a prognostic factor in patients with upper urinary tract urothelial carcinoma. *Int Braz J Urol.* (2014) 40:753–62. doi: 10.1590/S1677-5538.IBJU.2014.06.06

40. Ataseven B, du Bois A, Reinthaller A, Traut A, Heitz F, Aust S, et al. Preoperative serum albumin is associated with postoperative complication rate and overall survival in patients with epithelial ovarian cancer undergoing cytoreductive surgery. *Gynecol Oncol.* (2015) 138:560–5. doi: 10.1016/j.ygyno.2015.07.005

41. Geisler JP, Linnemeier GC, Thomas AJ, Manahan KJ. Nutritional assessment using prealbumin as an objective criterion to determine whom should not undergo primary radical cytoreductive surgery for ovarian cancer. *Gynecol Oncol.* (2007) 106:128–31. doi: 10.1016/j.ygyno.2007.03.008

42. Wang C-Y, Hsieh M-J, Chiu Y-C, Li S-H, Huang H-W, Fang F-M, et al. Higher serum C-reactive protein concentration and hypoalbuminemia are poor prognostic indicators in patients with esophageal cancer undergoing radiotherapy. *Radiother Oncol.* (2009) 92:270–5. doi: 10.1016/j.radonc.2009.01.002

43. Yin Y. Effect of Hypoproteinemia on prognosis of esophageal cancer and cardia cancer. *Health Everyone.* (2015) 21:48–9.

44. Tang PL, Wang HH, Lin HS, Liu WS, Chen LM, Chou FH. Body composition early identifies cancer patients with radiotherapy at risk for malnutrition. *J Pain Symptom Manage.* (2018) 55:864–71. doi: 10.1016/j.jpainsymman.2017.10.005

45. Baracos VE, Arribas L. Sarcopenic obesity: hidden muscle wasting and its impact for survival and complications of cancer therapy. *Ann Oncol.* (2018) 29:ii1–9. doi: 10.1093/annonc/mdx810

46. Prado CM, Purcell SA, Laviano A. Nutrition interventions to treat low muscle mass in cancer. *J Cachexia Sarcopenia Muscle.* (2020) 11:366–80. doi: 10.1002/jcsm.12525

47. Hagens ERC, Feenstra ML, van Egmond MA, van Laarhoven HWM, Hulshof MCCM, Boshier PR, et al. Influence of body composition and muscle strength on outcomes after multimodal oesophageal cancer treatment. *J Cachexia Sarcopenia Muscle.* (2020) 11:756–67. doi: 10.1002/jcsm.12540

48. Nakashima Y, Saeki H, Nakanishi R, Sugiyama M, Kurashige J, Oki E, et al. Assessment of sarcopenia as a predictor of poor outcomes after esophagectomy in elderly patients with esophageal cancer. *Ann Surg.* (2018) 267:1100–4. doi: 10.1097/SLA.0000000000002252

49. Prado CM. Body composition in chemotherapy: the promising role of CT scans. *Curr Opin Clin Nutr Metab Care.* (2013) 16:525–33. doi: 10.1097/MCO.0b013e328363bcfb

50. Prado CM, Lieffers JR, Bowthorpe L, Baracos VE, Mourtzakis M, McCargar LJ. Sarcopenia and physical function in overweight patients with advanced cancer. *Can J Diet Pract Res.* (2013) 74:69–74. doi: 10.3148/74.2.2013.69

51. Prado CMM, Baracos VE, McCargar LJ, Reiman T, Mourtzakis M, Tonkin K, et al. Sarcopenia as a determinant of chemotherapy toxicity and time to tumor progression in metastatic breast cancer patients receiving capecitabine treatment. *Clin Cancer Res.* (2009) 15:2920–6. doi: 10.1158/1078-0432.CCR-08-2242

52. Daly LE, Power DG, O'Reilly Á, Donnellan P, Cushen SJ, O'Sullivan K, et al. The impact of body composition parameters on ipilimumab toxicity and survival in patients with metastatic melanoma. *Br J Cancer.* (2017) 116:310–7. doi: 10.1038/bjc.2016.431

53. von Haehling S, Morley JE, Anker SD. An overview of sarcopenia: facts and numbers on prevalence and clinical impact. *J Cachexia Sarcopenia Muscle.* (2010) 1:129–33. doi: 10.1007/s13539-010-0014-2

54. Prado CM, Cushen SJ, Orsso CE, Ryan AM. Sarcopenia and cachexia in the era of obesity: clinical and nutritional impact. *Proc Nutr Soc.* (2016) 75:188–98. doi: 10.1017/S0029665115004279

55. Ma XL, Deng JH, Su XX, Wang H. Application of the phase angle in patients with malignant tumors. *J Nutr Oncol.* (2022) 7:161–74.

56. Zhang X, Zhang J, Du Y, Wu X, Chang Y, Li W, et al. The clinical application value of phase angle of six parts in nutritional evaluation of tumor patients. *Support Care Cancer.* (2022) 30:7983–9. doi: 10.1007/s00520-022-07240-x

57. Rinaldi S, Gilliland J, O'Connor C, Chesworth B, Madill J. Is phase angle an appropriate indicator of malnutrition in different disease states? A systematic review. *Clin Nutr ESPEN.* (2019) 29:1–14. doi: 10.1016/j.clnesp.2018.10.010

58. Arab A, Karimi E, Vingrys K, Shirani F. Is phase angle a valuable prognostic tool in cancer patients' survival? A systematic review and meta-analysis of available literature. *Clin Nutr.* (2021) 40:3182–90. doi: 10.1016/j.clnu.2021.01.027

59. Garrett WS. Cancer and the microbiota. *Science.* (2015) 348:80–6. doi: 10.1126/science.aaa4972

60. Hamm AK, Weir TL. Editorial on "Cancer and the microbiota" published in Science. *Ann Transl Med.* (2015) 3:175. doi: 10.3978/j.issn.2305-5839.2015.07.16

61. Ma S, Wang S, Zhu L. Esophageal microbiota and esophageal diseases. *World Chinese J Digestology.* (2019) 27:767–72. doi: 10.11569/wcjd.v27.i12.767

62. Chen RY, Ahmed T, Gordon JI. Duodenal microbiota in stunted undernourished children with enteropathy. *Reply N Engl J Med.* (2021) 384:1778. doi: 10.1056/NEJMc2030388

63. Sacks D, Baxter B, Campbell BC, Carpenter JS, Cognard C, Dippel D, et al. Multisociety consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke. *Int J Stroke.* (2018) 13:612–32. doi: 10.1177/1747493018778713

64. Chung WSF, Meijerink M, Zeuner B, Holck J, Louis P, Meyer AS, et al. Prebiotic potential of pectin and pectic oligosaccharides to promote anti-inflammatory commensal bacteria in the human colon. *FEMS Microbiol Ecol.* (2017) 93:fix127. doi: 10.1093/femsec/fix127

65. Sun Q, Wu J, Zhang X. *A Microbial Marker for Predicting Gastric Cancer Cachexia and Its Application.* Nanjing: Jiangsu Province Hospital of Chinese Medicine. CN115491422A [P/OL].

66. Mu Q, Tavella VJ, Luo XM. Role of lactobacillus reuteri in human health and diseases. *Front Microbiol.* (2018) 9:757. doi: 10.3389/fmicb.2018.00757

67. Wilson MR, Jiang Y, Villalta PW, Stornetta A, Boudreau PD, Carrá A, et al. The human gut bacterial genotoxin colibactin alkylates DNA. *Science.* (6428) 2019:363. doi: 10.1126/science.aar7785

68. Tili E, Michaille JJ, Wernicke D, Alder H, Costinean S, Volinia S, et al. Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer. *Proc Natl Acad Sci U S A.* (2011) 108:4908–13. doi: 10.1073/pnas.1101795108

69. Chang CH, Curtis JD, Maggi LB, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell.* (2013) 153:1239–51. doi: 10.1016/j.cell.2013.05.016

70. Li X, Wang M. Research progress of therapeutic targets and targeted medicine of aerobic glycolysis. *Electr J Metabol Nutr Cancer.* (2022) 9:253–8.

71. Hou W, Hou Y, Wu C. *Application of E.coli BL21 Ribosomal Protein Subunit L36 in the Preparation of Anticancer Drugs*. Nanchong: China West Normal University. CN102614495A [P/OL].

72. Newberry C, Lynch K. Can we use diet to effectively treat esophageal disease? A review of the current literature. *Curr Gastroenterol Rep.* (2017) 19:38. doi: 10.1007/s11894-017-0578-5

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Association between oxidative balance score and 10-year atherosclerotic cardiovascular disease risk: results from the NHANES database

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Introduction: The oxidative balance score (OBS) is a holistic measure that represents the overall equilibrium between prooxidants and antioxidants in one's diet and lifestyle. Little research has been conducted on the correlation between OBS and 10-year atherosclerotic cardiovascular disease risk (ASCVD). Therefore, the objective of this investigation was to examine the potential correlation between OBS and 10-year risk.

Methods: A total of 11,936 participants from the NHANES conducted between 2001 and 2016 were chosen for the study and their dietary and lifestyle factors were used to assess the OBS score. Logistic regression and restricted cubic splines (RCS) were employed in the cross-sectional study to evaluate the correlation between OBS and the 10-year ASCVD risk. The cohort study utilized Cox proportional hazards models and RCS to assess the correlation between OBS and all-causes and cardiovascular disease (CVD) mortality in individuals with high ASCVD risk.

Results: The cross-sectional study found that the OBS (OR=0.94, 95% CI=0.93–0.98), as well as the dietary OBS (OR=0.96, 95% CI=0.92–0.96) and lifestyle OBS (OR=0.74, 95% CI=0.69–0.79), were inversely associated with the 10-year ASCVD risk. A significant linear relationship was observed between OBS, dietary OBS, lifestyle OBS, and the 10-year ASCVD risk. The cohort study found that the OBS was inversely associated with all-cause (aHRs=0.97, 95% CI=0.96–0.99) and CVD (aHRs=0.95, 95% CI=0.93–0.98) mortality in individuals with high ASCVD risk. A significant linear correlation was observed between OBS, dietary OBS, lifestyle OBS, and all-cause and CVD mortality in participants with high ASCVD risk.

Conclusion: The findings indicate that OBS, OBS related to diet, and OBS related to lifestyle were significantly inversely correlated with the 10-year ASCVD risk. Adopting a healthy eating plan and making positive lifestyle choices that result in increased OBS levels can help lower the likelihood of all-cause and CVD mortality in individuals with high ASCVD risk.

KEYWORDS

oxidative balance score, 10-year ASCVD risk, NHANES, dietary, lifestyle

Background

Cardiovascular disease (CVD) is a condition affecting the heart and blood vessels and is recognized as the primary reason for global mortality (1). Hence, it is crucial to avert cardiovascular disease (CVD) and its related complexities while lessening the impact of the ailment and mortality rate. The accurate evaluation of CVD risk is crucial for formulating effective prevention and screening strategies. The recent most-utilized tool for evaluating the 10-year risk of atherosclerotic CVD (ASCVD), introduced by the American Heart Association (AHA) and the American College of Cardiology (ACC), is the 10-year ASCVD risk index (2).

It is widely recognized that detrimental habits, such as a lack of physical activity, an unhealthy eating pattern, smoking, and excessive alcohol consumption, have been linked to cardiovascular disease (CVD) occurrence and untimely fatalities (3, 4). Therefore, it is crucial to emphasize the potential for reducing CVD mortality by adopting healthier lifestyle choices. Maintaining a nutritious eating plan is a fundamental approach to proactively prevent cardiovascular diseases (5, 6). Observational research indicates that adopting a nutritious diet is associated with a decreased likelihood of experiencing cardiovascular events. Consequently, numerous individuals are urging for more robust public policies that encourage the consumption of healthy food options (7). Meanwhile, critics point out the lack of causal evidence linking a healthy diet with cardiovascular disease(CVD) prevention (8) and there are few studies looking at a healthy diet that addresses the primary prevention of CVD (9).

Various OBSs have been utilized in epidemiological research to consider both dietary and non-dietary lifestyle exposures (10). The OBS is a holistic measure that represents the overall equilibrium between prooxidants and antioxidants in one's diet and lifestyle. In general, a higher OBS suggests that there are more antioxidants than prooxidants. Many studies have reported the negative associations between OBS and type 2 diabetes (11), osteoarthritis (12), cardiovascular disease (13), and cancers (14, 15). However, several studies have also documented the link between OBS and its constituents with various illnesses, including ASCVD mortality, and arrived at a contrasting outcome (16–19). This could be attributed to the intricate connections and associations among numerous prooxidant and antioxidant elements (10, 20), making it challenging to determine the individual impacts of these factors on disease susceptibility.

We consider that it is difficult to determine the roles played by exposure to dietary and non-dietary lifestyles and it is necessary to consider them as a complete whole. To summarize, there is insufficient evidence to determine the expected reduction in 10-year ASCVD risk resulting from established lifestyle and dietary interventions. Therefore, to gain a deeper comprehension of the impact of OBS, this study utilizes information from the NHANES 2001–2016, a comprehensive and representative survey of the American populace. It aims to explore the potential correlation between OBS and the risk of ASCVD over a span of 10 years. The results of our study could provide an important understanding of the involvement of OBS in the progression of ASCVD, thereby offering vital knowledge for the global prevention of ASCVD.

Materials and methods

Study design and participants

The data from the National Health and Nutrition Examination Survey data set of the United States (NHANES) were employed in this study. The Centers for Disease Control and Prevention (CDC) conducted a comprehensive cross-sectional study to assess the general health and nutritional well-being of the American population. This survey utilized a meticulously chosen representative sample of the United States populace. The NHANES dataset consists of five main parts, including information on demographics, diet, physical exams, lab results, and questionnaire answers. To collect pertinent data, the survey includes comprehensive interviews and extensive physical evaluations. Collecting data is essential for shaping public health policies and programs, assessing the effectiveness of health and nutrition initiatives, and monitoring the prevalence of different diseases and conditions. The National Center for Health Statistics Research Ethics Review Board duly approved all NHANES protocols, and all study participants provided informed consent prior to their involvement. Furthermore, all studies carried out strictly followed the applicable protocols and rules. To obtain extensive information about the collection of NHANES data, individuals who were interested could refer to the published materials accessible at https://www.cdc.gov/nchs/nhanes.htm.

In NHANES 2001–2016, a total of 82,097 individuals were initially registered. Exclusion criteria for individuals included: (1) loss of data for any of the OBS components ($n = 9,034$) or 10-year ASCVD risk ($n = 59,522$); (2) diagnosis of ASCVD in participants ($n = 1,456$); (3) missing other data such as education ($n = 5$), marital status ($n = 8$), white blood cell count (WBC) ($n = 27$), chronic kidney disease (CKD) ($n = 65$), alanine transaminase (ALT) ($n = 38$), and lymphocyte count (LYM) ($n = 23$). In the end, a total of 11,936 participants were included in the observational study (Figure 1). In the cohort study, we initially enrolled 4,897 individuals with high ASCVD risk. Five participants were excluded because missed mortality data. A total of 4,892 participants were included finally.

Variable definition

The OBS, which is a holistic measure indicating the equilibrium between prooxidants and antioxidants in one's diet and lifestyle, was determined by evaluating 16 nutrients and four lifestyle factors. This evaluation included five pro-oxidants and 15 antioxidants, using existing knowledge of the association between OBS and these factors (21). The nutrient intake data were obtained from the initial dietary review interview. As per the methodology employed by Zhang et al. (21). To determine OBS, alcohol intake was divided into three categories: heavy drinkers (15 g/day for females and 30 g/day for males), non-heavy drinkers (0–15 g/day for females and 0–30 g/day for males), and abstainers. Each group was assigned scores of 0, 1, and 2, respectively (21). Following this, the remaining elements were initially categorized into two sets according to gender and then further divided into three sets based on their tertile. In groups 1–3, pro-oxidants were assigned scores ranging from 2 to 0, while antioxidants were assigned scores ranging from 0 to 2. In general, a higher OBS suggests a prevalence of antioxidants compared to prooxidants (21).

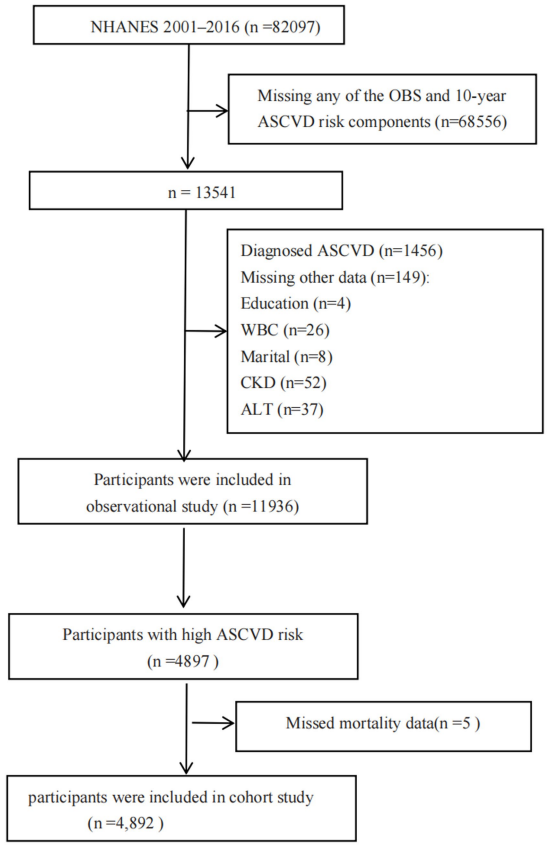

FIGURE 1
Flow chart of the study population.

The American College of Cardiology (ACC)/American Heart Association (AHA) guidelines for ASCVD 10-year risk assessment consider nine factors: race, sex, age, total cholesterol, HDL cholesterol, BP, diabetes, hypertension treatment, and smoking, we were able to categorize the study samples into two distinct groups. Individuals who have 10-year ASCVD risk <7.5% were categorized as having a "low ASCVD risk" and 2, those with 10-year ASCVD risk ≥7.5% were defined "high ASCVD risk" (22).

Assessment of covariates

In this study, the covariates were certain factors previously shown or hypothesized to be associated with ASCVD or OBS, including demographic factors, blood examination, lifestyle, and concomitant disease. The demographic factors included the following: age, gender (male/female), ethnicity (black, white, other), level of education (less than high school, high school, college), Body Mass Index (BMI), and marital status. The blood test results included WBC, LYM, ALT, total cholesterol level (TC), high-density lipoprotein level (HDL), creatinine level/glomerular filtration rate (GFR), albumin level, hemoglobin level, levels of trace elements in the blood, dietary fiber level, carotene level, riboflavin level, niacin level, total folate level, cotinine level, and vitamin levels. Lifestyle includes of healthy eating index, alcohol use, smoking, total energy intake, and physical activity. Accompanying conditions included: diabetes mellitus (DM), hypertension, hyperlipidemia, and anemia.

Statistical analyses

Our study considered the NHANES analytic guidelines, and considered complex sampling designs and sampling weights. Mobile examination centers (MECs) weights were utilized for all analyses. Continuous variables are presented as means and standard errors (SEs), while categorical variables are presented as proportions. The OBS were divided into four groups based on quartiles. Student's *t*-test was used to compare continuous variables among the quartiles of the OBS groups, while chi-square test was used for categorical variables.

Three models were built to provide statistical inference. The first model solely consisted of OBS. Model 2 consisted of Model 1, sex, age, ethnicity, and educational background. The inclusion of Model 2 in Model 3 comprised creatinine, LYM, WBC, ALT, alcohol user, DM, hypertension, hyperlipidemia, anemia, and total energy intake.

A logistic regression analysis was conducted in the cross-sectional study to determine the adjusted odds ratio (aOR) and 95% CIs for the correlation between OBS and the risk of ASCVD over a period of 10 years. To flexibly model the relationship between OBS and 10-year ASCVD risk, we employed restricted cubic spline (RCS) regression.

The cohort study utilized Cox proportional hazards models to calculate adjusted hazard ratios (aHRs) and 95% CI, assessing the correlation between OBS and all-causes, CVD mortality in individuals with a high predicted 10-year ASCVD risk. The association of OBS with all-cause mortality and CVD mortality was modeled using Kaplan–Meier survival analysis in a flexible manner.

R Studio 4.2.0 was utilized for statistical analyses. A significance level of $p < 0.05$ was established for the statistical analysis.

Results

The cross-sectional study

Basic characteristics

This study utilized a NHANES dataset comprising 11,936 participants. The basic features of the 11,936 participants are displayed in Table 1. Among the total sample, 6,072 (49.146%) were male. OBS were categorized into quartiles: Q1 (3–15), Q2 (16–21), Q3 (22–26), and Q4 (27–37). Significant differences were observed between OBS quartiles and various variables, such as age, BMI, race, education, marital status, smoking, SBP, TC, HDL, dietary fiber, carotene, alpha-carotene, beta-carotene, riboflavin, niacin, total folate, vitamin B12, vitamin B6, vitamin C, vitamin E, calcium, magnesium, zinc, copper, total fat, iron, physical activity, cotinine, anemia, CKD, hypertension, hyperlipidemia, DM, 10-year ASCVD risk, and high ASCVD risk (all $p < 0.0001$). In addition, there were significant differences in hemoglobin, physical activity, and alcohol across the OBS quartiles ($p < 0.005$). It was important to note that the participants with high ASCVD risk also showed a significant trend from the lower OBS quartiles to the high OBS quartiles (p value <0.001).

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TABLE 1 Baseline characteristics of study participants based on the OSB quartiles.

Variable	Total	Q1	Q2	Q3	Q4	*p*-value
Age (year)	54.765 (0.149)	54.689 (0.235)	55.003 (0.235)	55.005 (0.245)	54.284 (0.280)	0.145
Sex						0.054
Female	5,864 (50.854)	1,355 (47.834)	1,526 (50.597)	1,700 (51.372)	1,283 (53.253)	
Male	6,072 (49.146)	1,683 (52.166)	1,570 (49.403)	1,660 (48.628)	1,159 (46.747)	
BMI (kg/m^2)	28.645 (0.095)	29.476 (0.147)	29.211 (0.149)	28.586 (0.154)	27.358 (0.165)	<0.0001*
Race						<0.0001*
White	6,075 (77.909)	1,326 (71.562)	1,542 (77.016)	1,821 (80.199)	1,386 (81.830)	
Black	2,312 (8.344)	883 (14.411)	624 (8.973)	514 (6.391)	291 (4.539)	
Other	3,549 (13.747)	829 (14.027)	930 (14.011)	1,025 (13.410)	765 (13.631)	
Education						<0.0001*
<high	2,471 (11.609)	876 (18.049)	709 (13.047)	570 (9.640)	316 (6.626)	
High	2,717 (22.363)	828 (28.695)	731 (25.728)	743 (20.814)	415 (14.917)	
College	6,748 (66.027)	1,334 (53.256)	1,656 (61.224)	2,047 (69.546)	1,711 (78.457)	
Marital						<0.0001*
Yes	7,559 (67.651)	1,761 (61.122)	1,938 (66.371)	2,210 (69.643)	1,650 (72.522)	
No	4,377 (32.349)	1,277 (38.878)	1,158 (33.629)	1,150 (30.357)	792 (27.478)	
Alcohol.user						<0.0001*
Never	1,458 (9.639)	374 (11.045)	413 (10.829)	400 (9.085)	271 (7.779)	
Former	2,345 (16.430)	718 (20.246)	598 (15.727)	595 (15.032)	434 (15.413)	
Mild	4,524 (41.547)	953 (32.427)	1,144 (39.335)	1,335 (44.014)	1,092 (49.189)	
Moderate	1,821 (17.440)	470 (17.247)	457 (16.704)	532 (18.258)	362 (17.377)	
Heavy	1,788 (14.944)	523 (19.035)	484 (17.405)	498 (13.611)	283 (10.243)	
Alcohol (g/day)	10.513 (0.358)	10.330 (0.672)	11.115 (0.751)	11.185 (0.563)	9.208 (0.581)	0.057
Smoke						<0.0001*
Yes	2,203 (17.426)	886 (30.661)	590 (19.126)	525 (14.084)	202 (7.635)	
No	9,733 (82.574)	2,152 (69.339)	2,506 (80.874)	2,835 (85.916)	2,240 (92.365)	
Physical activity (MET-minute/week)	2876.530 (68.968)	2623.927 (127.134)	2846.104 (121.182)	2947.550 (108.621)	3052.215 (114.543)	0.034*
SBP	124.378 (0.267)	126.592 (0.487)	125.737 (0.449)	123.882 (0.405)	121.524 (0.435)	<0.0001*
Total energy intake, Mean (S.E)	2142.145 (11.832)	1574.933 (17.543)	1972.425 (17.782)	2291.644 (20.394)	2656.452 (31.031)	<0.0001*
TC	206.450 (0.685)	208.433 (1.289)	206.546 (1.051)	206.130 (1.126)	204.928 (1.173)	0.203
HDL	55.115 (0.260)	53.522 (0.487)	53.739 (0.468)	55.680 (0.455)	57.329 (0.534)	<0.0001*
Alt	25.951 (0.216)	26.124 (0.459)	26.660 (0.565)	25.617 (0.286)	25.456 (0.466)	0.317
Blood urea nitrogen (mg/dL)	13.771 (0.084)	13.001 (0.140)	13.696 (0.120)	13.900 (0.117)	14.398 (0.142)	<0.0001*
Creatinine (mg/dL)	0.895 (0.003)	0.924 (0.008)	0.897 (0.005)	0.889 (0.005)	0.874 (0.005)	<0.0001*
Albumin (g/L)	42.985 (0.051)	42.569 (0.097)	42.918 (0.083)	43.077 (0.074)	43.325 (0.075)	<0.0001*
Wbc_1000cells (/μL)	7.043 (0.030)	7.363 (0.062)	7.172 (0.053)	7.015 (0.059)	6.648 (0.053)	<0.0001*
Lym	30.006 (0.104)	30.181 (0.190)	29.917 (0.189)	29.759 (0.194)	30.245 (0.186)	0.164
Neu	58.433 (0.111)	58.438 (0.222)	58.448 (0.227)	58.664 (0.211)	58.127 (0.221)	0.394
Hemoglobin (g/dL)	14.394 (0.032)	14.469 (0.046)	14.455 (0.046)	14.348 (0.046)	14.320 (0.041)	0.007*
Healthy eating index	53.210 (0.253)	46.330 (0.339)	51.057 (0.304)	54.584 (0.361)	60.096 (0.476)	<0.0001*
Dietary fiber (g/day)	17.807 (0.145)	9.876 (0.109)	14.562 (0.128)	19.245 (0.174)	26.735 (0.298)	<0.0001*
Carotene (RE/day)	238.507 (6.335)	103.602 (4.937)	179.929 (6.857)	243.795 (7.380)	417.803 (22.479)	<0.0001*
Alpha carotene (mcg/day)	495.118 (21.045)	495.118 (21.045)	495.118 (21.045)	495.118 (21.045)	495.118 (21.045)	<0.0001*
Beta carotene (mcg/day)	2614.526 (67.059)	1133.500 (53.827)	1968.792 (73.378)	2680.789 (80.458)	4575.477 (233.678)	<0.0001*
Riboflavin (mg/day)	2.269 (0.016)	1.445 (0.015)	1.973 (0.018)	2.450 (0.023)	3.112 (0.033)	<0.0001*

(Continued)

TABLE 1 (Continued)

Variable	Total	Q1	Q2	Q3	Q4	*p*-value
Niacin (mg/day)	25.045 (0.158)	16.327 (0.173)	22.115 (0.252)	27.193 (0.230)	33.477 (0.380)	<0.0001*
Total folate (mcg/day)	417.050 (3.336)	242.641 (2.103)	339.682 (3.286)	451.642 (3.933)	616.029 (7.580)	<0.0001*
Vitamin B12 (mcg/day)	5.399 (0.088)	2.917 (0.064)	4.365 (0.089)	6.137 (0.213)	7.853 (0.162)	<0.0001*
Vitamin B6 (mg/day)	2.073 (0.016)	1.201 (0.014)	1.735 (0.023)	2.257 (0.022)	3.002 (0.034)	<0.0001*
Vitamin C (mg/day)	87.605 (1.243)	45.663 (1.266)	67.817 (1.487)	93.269 (2.107)	140.012 (2.783)	<0.0001*
Vitamin E (ATE) (mg/day)	8.541 (0.094)	4.646 (0.062)	6.841 (0.076)	9.272 (0.107)	13.008 (0.217)	<0.0001*
Calcium (mg/day)	943.926 (7.114)	561.495 (6.678)	802.827 (8.451)	1020.033 (9.917)	1349.674 (15.124)	<0.0001*
Magnesium (mg/day)	312.993 (2.121)	187.249 (1.490)	263.576 (1.754)	339.453 (2.254)	447.816 (3.871)	<0.0001*
Zinc (mg/day)	11.970 (0.093)	7.363 (0.093)	10.158 (0.129)	13.144 (0.179)	16.656 (0.199)	<0.0001*
Copper (mg/day)	1.370 (0.013)	0.813 (0.008)	1.131 (0.010)	1.516 (0.030)	1.953 (0.025)	<0.0001*
Total fat (g/day)	81.767 (0.529)	58.170 (0.658)	75.980 (0.761)	88.532 (0.908)	101.136 (1.277)	<0.0001*
Iron (mg/day)	15.497 (0.111)	9.657 (0.106)	13.070 (0.116)	16.761 (0.156)	21.854 (0.263)	<0.0001*
Selenium (mcg)	112.991 (0.640)	75.700 (0.779)	100.885 (0.966)	122.215 (1.152)	148.561 (1.737)	<0.0001*
Cotinine (ng/mL)	53.587 (2.189)	96.752 (4.360)	60.517 (3.941)	40.254 (2.780)	23.224 (2.565)	<0.0001*
Anemia						0.052
Non-Anemia	11,073 (95.011)	2,770 (93.625)	2,877 (95.461)	3,131 (94.798)	2,295 (96.072)	
Mild	649 (3.655)	201 (4.344)	166 (3.327)	169 (3.884)	113 (3.082)	
Moderate	202 (1.264)	65 (1.908)	49 (1.129)	56 (1.270)	32 (0.808)	
Severe	12 (0.070)	2 (0.123)	4 (0.083)	4 (0.047)	2 (0.037)	
CKD						<0.0001*
Yes	1,761 (12.391)	585 (16.394)	458 (13.437)	446 (11.433)	272 (8.803)	
No	10,175 (87.609)	2,453 (83.606)	2,638 (86.563)	2,914 (88.567)	2,170 (91.197)	
Hypertension						<0.0001*
Yes	5,619 (42.957)	1,613 (47.327)	1,499 (46.680)	1,521 (42.598)	986 (35.447)	
No	6,317 (57.043)	1,425 (52.673)	1,597 (53.320)	1,839 (57.402)	1,456 (64.553)	
Hyperlipidemia						<0.0001*
Yes	9,308 (78.567)	2,422 (80.606)	2,473 (81.662)	2,613 (77.810)	1,800 (74.361)	
No	9,308 (78.567)	2,422 (80.606)	2,473 (81.662)	2,613 (77.810)	1,800 (74.361)	
DM						<0.0001*
Yes	2,146 (13.133)	645 (15.302)	584 (14.848)	598 (13.336)	319 (9.070)	
No	9,790 (86.867)	2,393 (84.698)	2,512 (85.152)	2,762 (86.664)	2,123 (90.930)	
10-year ASCVD risk	0.080 (0.001)	0.094 (0.002)	0.085 (0.003)	0.078 (0.002)	0.062 (0.002)	<0.0001
High predicted 10-year ASCVD risk						<0.0001*
Yes	4,897 (33.204)	1,489 (39.133)	1,334 (37.014)	1,287 (32.205)	787 (24.970)	
No	7,039 (66.796)	1,549 (60.867)	1,762 (62.986)	2,073 (67.795)	1,655 (75.030)	

Mean ± SEs for continuous variables: *p* value was calculated by weighted Student's *t*-test. Number (%) for categorical variables: *p* value was calculated by weighted chi-square test. BMI, Body mass index; Edu, Education; LYM, Lymphocyte ratio; WBC, Leucocyte count; Neu, Neutrophile granulocyte; 10-year ASCVD risk, 10-year atherosclerotic cardiovascular disease risk; DM, Diabetes mellitus; CKD, Chronic kidney disease; TC, Cholesterin; HDL, High density lipoprotein; SBP, Systolic pressure; High predicted 10-year risk, 10-year ASCVD risk ≥ 7.5%.

Association between OBS and high ASCVD risk

After making adjustments for different models, Table 2 demonstrates the correlation between OBS and high ASCVD risk. The OBS was analyzed both as a continuous and a categorized variable. After making adjustments to different models in the ongoing model, the outcomes for Model 1 indicated aOR = 0.97 [95% CI (0.96–0.97), $p < 0.0001$]. Similarly, Model 2 showed aORs = 0.94 [95% CI (0.93–0.95), $p < 0.0001$]. In addition, Model 3 exhibited aOR = 0.94 [95% CI of (0.92–0.96), $p < 0.0001$]. After adjusting Model 3 in the OBS categorized model, the aOR and their corresponding 95% confidence intervals (CI) for different OBS categories (3–15, 16–21, 22–26, and 27–37) were as follows: 1.00 (reference), 0.79 (0.61, 1.03), ($p = 0.08$), 0.56 (0.41, 0.77), ($p < 0.0001$), and 0.39 (0.27, 0.56), ($p < 0.0001$) for high ASCVD risk.

Supplementary Table S2 showed the associations of OBS with study outcomes in age, sex, GFR, hyperlipidemia, and hypertension

TABLE 2 Multivariable logistic regression analyses demonstrating associations of OBS and 10-year ASCVD risk.

	Multivariable adjusted (OR, 95% CI)*					
	Model 1		Model 2		Model 3	
OBS	95%CI	*p*-value	95%CI	*p*-value	95%CI	*p*-value
Q1	ref		ref		ref	
Q2	0.91 (0.79,1.05)	0.21	0.83 (0.66, 1.05)	0.11	0.79 (0.61, 1.03)	0.08
Q3	0.74 (0.63,0.86)	<0.001	0.57 (0.46, 0.71)	<0.0001	0.56 (0.41, 0.77)	<0.0001
Q4	0.52 (0.45,0.60)	<0.0001	0.34 (0.27, 0.45)	<0.0001	0.39 (0.27, 0.56)	<0.0001
p trend		<0.0001		<0.0001		<0.0001
OBS	0.97 (0.96,0.97)	<0.0001	0.94 (0.93, 0.95)	<0.0001	0.94 (0.92, 0.96)	<0.0001

Model 1 comprised OBS only. Model 2 included Model 1, sex, and age, race and education. Model 3 included Model 2, creatinine, lymphocyte ratio (LYM), leucocyte count (WBC), glutamic-pyruvic transaminase (ALT), alcohol user, diabetes mellitus (DM), hypertension, hyperlipidemia, anemia, and total energy intake. *$p < 0.05$.

subjects. In model 3 adjusted for age, sex, GFR, hyperlipidemia, and hypertension, there was a significant association between OBS and high ASCVD risk. Subgroup analyses showed results with no significant interaction (All *p* interaction >0.05).

Associations of dietary OBS, lifestyle OBS with high ASCVD risk

Supplementary Table S1 displays the outcomes of a multiple logistic regression analysis that assesses the correlation between dietary OBS, lifestyle OBS, and high ASCVD risk. After adjusting for Model 3, there was a significant negative association between high ASCVD risk and dietary OBS, with a statistically significant continuous OR of 0.96 (0.93, 0.98) and a categorized OR of 0.49 (0.33, 0.72), both with $p < 0.0001$. After adjusting for Model 3, there was a significant negative association between high ASCVD risk and lifestyle OBS. The association remained statistically significant with a continuous aOR of 0.74 (0.69, 0.79), $p < 0.0001$, and categorized OR of 0.34 (0.26, 0.45), $p < 0.001$.

Supplementary Table S2 showed the associations of dietary OBS, lifestyle OBS with study outcomes in age, sex, GFR, hyperlipidemia, and hypertension subjects.

In model 3, adjusted for sex, GFR, and hypertension, there was a significant association between dietary OBS and high ASCVD risk. In the younger (<60), one-unit increasement in dietary OBS, high ASCVD risk decreased by 4.9%. In the hyperlipidemia participants, one-unit increasement in dietary OBS, high 10-year ASCVD risk decreased by 4.5%. Subgroup analyses showed results with no significant interaction (All *p* interaction >0.05).

In model 3, adjusted for sex and hypertension, there was a significant association between lifestyle OBS and high ASCVD risk. In the younger (<60), one-unit increasement in lifestyle OBS, high ASCVD risk decreased by 16.4%. In the hyperlipidemia participants, one-unit increasement in lifestyle OBS, high 10-year ASCVD risk decreased by 27.5%. In the GFR <60 mL/min/1.73 m^2 group, there was a no association between lifestyle OBS and high ASCVD risk. It was interesting to note that there was a significant association between lifestyle OBS and high 10-year ASCVD risk among those with age, GFR, and hyperlipidemia (*p* for interaction >0.05).

The high ASCVD risk is associated linearly with OBS, dietary OBS, and lifestyle OBS

Following the adjustment for Model 3, a linear correlation was detected among OBS, dietary OBS, lifestyle OBS, and the high ASCVD risk (Figure 2). According to RCS models, there is a consistent decline in the aOR for the high ASCVD risk with an increase in OBS, dietary OBS, and lifestyle OBS (Figure 2).

In both the male and female groups, there was a noticeable correlation between OBS, dietary OBS, and the high ASCVD risk, as depicted in Supplementary Figures S1, S2. In the female group, a noteworthy non-linear association was found between lifestyle OBS and the high ASCVD risk (*p* value for nonlinearity = 0.0017, as shown in Supplementary Figure S3).

Cohort study basic characteristics

Basic characteristics

In the cohort study, a total of 4,892 participants with high-risk 10-years ASCVD were included (Supplementary Table S3). The average length of follow-up was 115.0 months, with a range of 72.0–153.0 months (IQR). Out of the entire sample, 3,850 individuals (78.70%) were males, while there were 1,053 instances (19.5%) of recorded mortality. OBSs were categorized into quartiles: Q1 (3–15), Q2 (16–21), Q3 (22–26), and Q4 (27–37). It was important to note that the mortality also showed a significant trend from the lower OBS quartiles to the high OBS quartiles (*p* value <0.001) (Supplementary Table S3).

The connections between OBS and all-causes, CVD mortality in individuals with high ASCVD risk

The correlation between OBS and all-causes, CVD mortality in individuals with high ASCVD risk is depicted in Table 3. After adjusting various models in the OBS continuous model, the results showed that for Model 1, the aHRs = 0.98 (0.97–0.99), *p* value < 0.0001 (Table 3). Similarly, for Model 2, the aHRs = 0.97 (0.96–0.98), $p < 0.0001$. In addition, for Model 3, the aHRs = 0.97 (0.96–0.99), $p < 0.0001$ for all-cause mortality. For Model 1, the aHRs = 0.97 (0.95–0.99), $p < 0.0001$. Similarly, for Model 2, the aHRs = 0.95 (0.93–0.97), $p < 0.0001$. Furthermore, for Model 3, the aHRs were 0.95 (0.93–0.98) $p < 0.002$ for CVD mortality.

In the OBS categorized model, after adjustment Model 3, the aHRs and corresponding to the 95% CI for the different OBS categories (Q1, Q2, Q3, and Q4) had the following values: 1.00 (reference), 0.86 (0.67, 1.10) ($p = 0.23$), 0.89 (0.69, 1.14) ($p = 0.34$), 0.66 (0.49, 0.87) ($p = 0.004$) for all-cause mortality (*p* trend = 0.01); and 1.00 (reference), 0.66 (0.45, 0.96) ($p = 0.03$), 0.56 (0.36, 0.85)

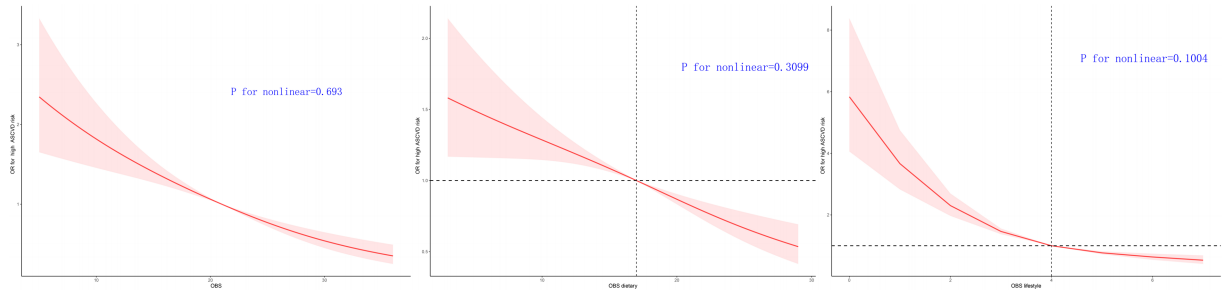

FIGURE 2
Multivariable-adjusted ORs for 10-year ASCVD risk by OBS, dietary OBS, and lifestyle OBS.

TABLE 3 Multivariable Cox regression analyses demonstrating associations of OBS and all-cause mortality, CVD mortality.

OBS	Multivariable adjusted (HR, 95% CI)*					
	Model 1		Model 2		Model 3	
	95%CI	*p*-value	95%CI	*p*-value	95%CI	*p*-value
All-cause mortality						
Q1	ref		ref		ref	
Q2	0.85 (0.68,1.07)	0.17	0.82 (0.65,1.03)	0.08	0.86 (0.67,1.10)	0.23
Q3	0.87 (0.70,1.07)	0.18	0.80 (0.65,0.99)	0.04	0.89 (0.69,1.14)	0.34
Q4	0.65 (0.50,0.84)	<0.001	0.57 (0.44,0.73)	<0.0001	0.66 (0.49,0.87)	0.004
p trend		0.002		<0.0001		0.01
OBS	0.98 (0.97,0.99)	<0.0001	0.97 (0.96,0.98)	<0.0001	0.97 (0.96,0.99)	<0.0001
CVD mortality						
Q1	ref		ref		ref	
Q2	0.71 (0.50,1.00)	0.05	0.66 (0.47,0.92)	0.01	0.66 (0.45,0.96)	0.03
Q3	0.62 (0.44,0.87)	0.01	0.54 (0.39,0.74)	<0.001	0.56 (0.36,0.85)	0.01
Q4	0.53 (0.33,0.84)	0.01	0.43 (0.27,0.69)	<0.001	0.45 (0.25,0.80)	0.01
p trend		0.004		<0.0001		0.01
OBS	0.97 (0.95,0.99)	<0.001	0.95 (0.93,0.97)	<0.0001	0.95 (0.93,0.98)	0.002

Model 1 comprised OBS only. Model 2 included Model 1, sex, and age, race and education. Model 3 included Model 2, creatinine, lymphocyte ratio (LYM), leucocyte count (WBC), glutamic-pyruvic transaminase (ALT), alcohol user, diabetes mellitus (DM), hypertension, hyperlipidemia, anemia, and total energy intake. *$p < 0.05$.

($p = 0.01$), 0.45 (0.25, 0.80) ($p = 0.01$) for CVD mortality (p trend = 0.01).

Examining the connection between dietary OBS, lifestyle OBS, and the risk of all-cause and CVD mortality in individuals with a highly predicted ASCVD risk over a 10-year period

Supplementary Table S4 displays the outcomes of cox regression analysis, which assesses the correlation between dietary OBS, lifestyle OBS, and the risk of ASCVD over a period of 10 years. After adjusting for Model 3, there was a significant negative association between dietary OBS and both all-cause mortality [HR = 0.98 (0.96, 0.99), $p = 0.002$] and CVD mortality [HR = 0.95 (0.93, 0.98), $p = 0.002$], and a significant negative association between lifestyle OBS and both all-cause mortality [HR = 0.94 (0.89, 0.99), $p = 0.01$] and CVD mortality [HR = 0.90 (0.82, 0.98), $p = 0.02$] (Supplementary Table S4).

The all-cause mortality and CVD mortality is associated linearly with OBS, dietary OBS, and lifestyle OBS

Figures 3, 4 illustrate the correlation between OBS, dietary OBS, lifestyle OBS, and both all-cause mortality and CVD mortality.

After accounting for Model 3, a linear correlation was observed between OBS, dietary OBS, and the mortality rates for all causes and cardiovascular diseases, as indicated in Table 3 and Supplementary Table S3. According to RCS models, the aHRs for mortality from any cause and cardiovascular disease exhibited a consistent decline as OBS and dietary OBS increase, as shown in Figures 3, 4.

A linear correlation was found between lifestyle OBS and CVD mortality in the OBS for lifestyle. The RCS models indicated that the aHRs for CVD mortality exhibit a consistent decline as lifestyle OBS increases (Figure 4.). However, the RCS models indicated a turning

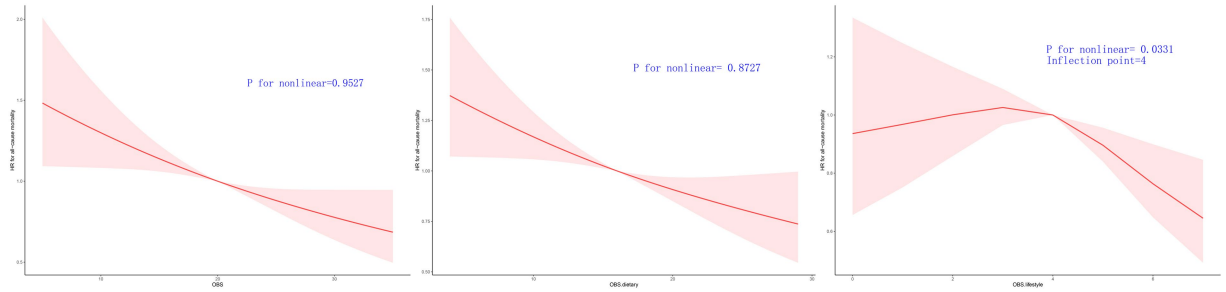

FIGURE 3
Multivariable-adjusted HRs for all cause mortality by OBS, diatery OBS, and lifestyle OBS.

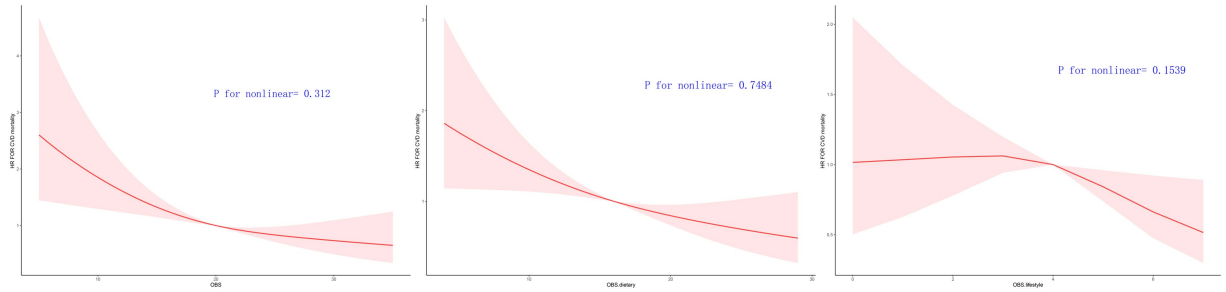

FIGURE 4
Multivariable-adjusted HRs for CVD mortality by OBS, diatery OBS, and lifestyle OBS.

point for overall mortality at 4 (Figures 3, 4). On the left side of this inflection point, the HR for all-cause mortality was 1.000 [95% CI: (0.910, 1.099), $p = 0.996$]. The HR for all-cause mortality on the right side of the inflection point was 0.836 [(95% CI 0.661, 1.057), $p = 0.134$] in Figure 3.

Supplementary Figures S4–S9 show the trend of decreasing HR for all-cause and CVD mortality with increasing OBS/dietary OBS/lifestyle OBS, which remained in male subgroups. However dietary OBS (p overall = 0.096) and lifestyle OBS (p overall = 0.6271) was not found to reduce CVD mortality in the female group (p overall = 0.096) (Supplementary Figures S7, S9).

As depicted in the Kaplan–Meier survival curves, patients with high ASCVD risk who had OBS < 15 (Q1) exhibited significantly higher rates of all-cause mortality (Figure 5) during the follow-up period.

Discussion

The current research revealed that elevated OBS was linked to a reduced 10-year ASCVD risk and exhibited a negative relationship between OBS and all-cause, CVD mortality in individuals with high ASCVD risk. The significance of a diet and lifestyle rich in antioxidants is underscored by our research, particularly in terms of reducing the 10-year ASCVD risk, all-cause and CVD mortality with high ASCVD risk. As far as we know, this research is the first to assess the correlation between OBS and all-cause mortality, as well as CVD mortality in individuals with a high ASCVD risk.

The current research shows a negative correlation between OBS and the 10-year ASCVD risk. The lifestyle and dietary factors of OBS have a significant impact on CVD (23). Previous studies have established that harmful lifestyles, such as obesity, excessive alcohol intake, and lack of physical activity can influence the development of CVD (24–26). According to the Global Burden of Disease Study (27), approximately 52% of all CVD deaths worldwide were attributed to risks associated with diet. The development of CVD and CVD mortality is widely recognized to be influenced significantly by chronic inflammation and oxidative stress (28). Studies conducted earlier have suggested that the overall eating pattern and different elements of the diet are directly linked to inflammation (29–32). Different dietary elements have also been linked to playing a significant part in the progression of different CVD (33–35). A meta-analysis of 14 studies investigated the link between the Dietary Inflammatory Index (DII) and CVD revealed compelling evidence indicating a significant association between a higher inflammatory diet and increased risk of CVD and related mortality (36). Oxidative stress refers to the in equilibrium between substances that promote oxidation and those that inhibit oxidation. The composite dietary antioxidant index (CDAI), is a score that assesses the overall antioxidant capacity of a person's diet. It takes into account different vitamins and minerals that have antioxidant properties, such as vitamins A, C, and E, as well as selenium and zinc (37, 38). A cross-sectional study discovered that multiple factors indicating a reduced occurrence of ASCVD in postmenopausal females exhibiting elevated CDAI levels, as well as a dose–response relationship in the L shape between CDAI levels and the hazards of ASCVD (16). Insufficient

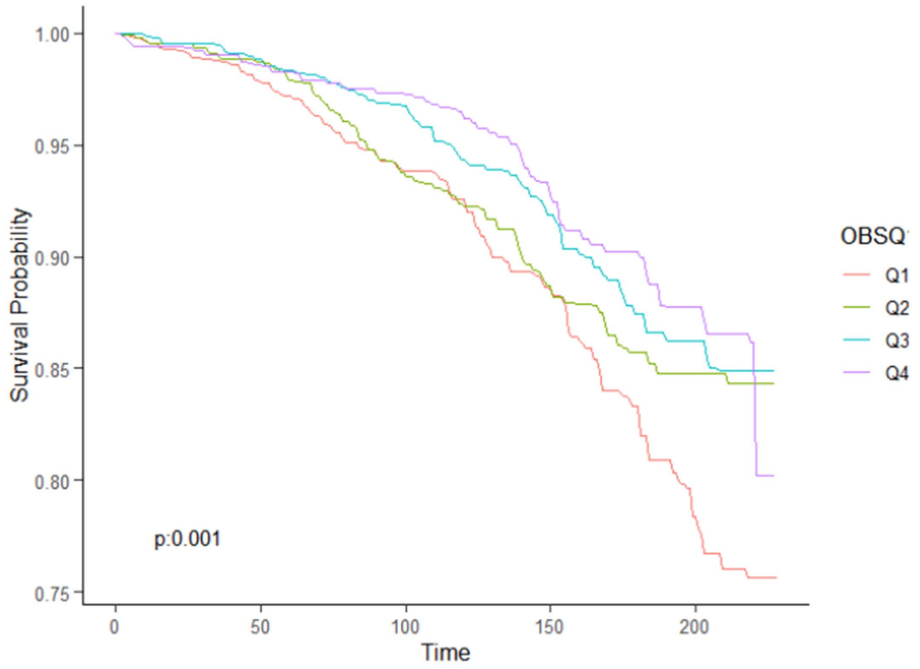

FIGURE 5
Kaplan–Meier survival curves for all cause mortality.

evidence exists to support the cardioprotective advantages of vitamin and mineral supplementation. A study in an elderly Swedish population discovered that providing selenium and coenzyme Q10 supplements to a group of elderly individuals who had low levels of selenium and coenzyme Q10 resulted in a decreased risk of CVD mortality when compared to the placebo group (39). Nevertheless, in the previous year, the US Preventive Services Task Force (USPSTF) stirred controversy when they performed a comprehensive evaluation of the effectiveness and possible drawbacks of a single nutrient, nutrient pair, or multivitamin supplementation in adults to lower the chances of developing CVD, cancer, and mortality (40). To prevent CVD (41), the USPSTF recommendation goes against specific vitamin supplements, like beta-carotene and vitamin E, which were previously believed to possess antioxidant properties, according to their findings. In a recent update of the previous systematic review and meta-analysis conducted in 2018, it was found that commonly used multivitamins, vitamin D, calcium, and vitamin C had no impact on cardiovascular disease outcomes and all-cause mortality, however, an elevated risk of all-cause mortality was observed when niacin was taken with statin medication (42). It is important to mention that nutrients are not ingested in solitude but rather as a component of a food matrix. Hence, it proves challenging to regulate the potential impacts of additional nutrients offered by a food source. In recent times, the emphasis of nutritional studies on cardiovascular disease has changed from individual nutrients and particular foods to overall eating habits (43). Examining entire dietary patterns may hold greater significance and be more easily understood when compared to analyzing individual nutrients or specific food items. This could be partly due to the fact that numerous connections between specific elements of macronutrients and the risk of CVD are not linear, resulting in perplexing and contradictory results that do not align with present dietary guidelines. Additional research on chronic illnesses has similarly discovered that the collective influence of various elements may have a greater correlation with the risk of developing diseases when compared to the impact of individual nutrients (44, 45). In this study, we opted for OBS as a measure of the overall equilibrium between pro-oxidants and antioxidants in our diet and lifestyle. In addition, we uncovered the significance of OBS in predicting the 10-year ASCVD risk. We believe that incorporating a composite measure of oxidative balance, which includes a combination of dietary patterns and lifestyle factors related to pro-oxidant and antioxidant exposures, may have a stronger association with the 10-year ASCVD risk.

Inadequate nutrition is a prominent contributing factor to all-cause mortality, CVD mortality, and cancer mortality (46). The quality of dietary intake and its correlation with the risk of mortality showed significant variations among European nations. In Spain, the Mediterranean diet showed a significant negative correlation with mortality, whereas in the Netherlands, the Healthy Nordic Dietary Pattern was found to be strongly linked to reduced mortality. These results reflect dietary cultural and pattern differences. A comprehensive analysis of multiple studies indicated that following a diet rich in inflammatory components could potentially increase the chances of developing colorectal cancer, CVD, and all-cause mortality (47). A Pan-European Cohort Study has revealed that different measures of diet quality are linked to all-cause mortality, as well as cause-specific (CVD and cancer) mortality, especially with stronger associations with CVD compared to cancer, and these scores have poor predictive performance for 10-year mortality risk when used in isolation. However, in combination with other non-invasive common risk factors such as smoking, body weight, physical activity, and educational level, these composite scores display good predictive ability (23). These studies support a view whereby it is necessary to analyze various dietary components and lifestyle as an organic whole,

due to the inseparable characteristics. In our research, we have discovered a direct correlation between OBS, dietary OBS, and lifestyle OBS and the all-causes and CVD mortality. However, we have observed a non-linear relationship between lifestyle OBS and mortality rates from all causes in individuals with a high ASCVD risk, as determined by Cox proportional hazards models. According to the RCS models, there was a consistent decline in the aHRs for all-causes and CVD mortality as the OBS, dietary OBS, and lifestyle OBS increased. In the female group, the subgroup analysis indicated that there was no reduction in CVD mortality with the use of OBS in the diet or lifestyle (Supplementary Figures S6–S9). We infer that it may be hormonally related. The antioxidant effects of diet and lifestyle on CVD mortality may be overshadowed by the protective function of estrogen, resulting in a lack of statistical significance in the correlation.

Strengths and limitations

Strengths

The present study had multiple advantages. First, the rare exploration of OBSs potential protection against ASCVD was not commonly undertaken in previous studies. Second, the extensive size of the sample and rigorous statistical analyses conducted on the overall population allowed us to extend our findings to the majority of individuals. Third, to ensure the accuracy of the results, this study controlled many confounding factors, including creatinine, WBC, ALT, alcohol user, hypertension, hyperlipidemia, and anemia. In our study, we initially performed cross-sectional analyses followed by additional cohort analyses.

Limitations

In addition, there are various drawbacks in the present investigation. Incorporating all ASCVD-associated dietary and lifestyle exposures proved challenging for the OBS; the database had restrictions on various elements, including flavonoids. Moreover, there was a possibility that certain ambiguous ASCVD-related dietary or lifestyle factors were not encompassed. Furthermore, the OBS dietary constituents were obtained from self-reported information collected through a single 24 h, which could introduce measurement inaccuracies and biases, and might not accurately reflect day-to-day variations in diet, thereby resulting in imprecise estimations. Lastly, it was not possible to utilize any biomarkers to authenticate the accuracy of the OBS in evaluating oxidative balance in the current investigation. Nevertheless, the association between 10-year ASCVD and the current OBS remained consistent and is unlikely to be significantly influenced by the excluded elements.

Conclusion

The findings of this research demonstrate that individuals with elevated OBS levels exhibited a decreased likelihood of developing ASCVD risk over a span of 10 years. Moreover, it suggests that adopting a dietary pattern and lifestyle that promotes higher OBS levels can be advantageous in mitigating the risk of mortality associated with all-cause and CVD among individuals with a high predicted 10-year ASCVD risk.

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 humans were approved by the NCHS Research Ethics Review Board at the National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

DJ: Writing – original draft. TL: Data curation, Methodology, Software, Writing – original draft. SC: Methodology, Writing – original draft. YC: Methodology, Writing – original draft. CZ: Conceptualization, Supervision, Writing – review & editing. XW: Conceptualization, Supervision, Writing – review & editing. JL: Conceptualization, Supervision, Writing – review & editing.

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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.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1422946/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Linear association between OBS and 10-years ASCVD risk by sex.

SUPPLEMENTARY FIGURE S2
Linear association between dietary OBS and 10-years ASCVD risk by sex.

SUPPLEMENTARY FIGURE S3
Linear association between lifestyle OBS and 10-years ASCVD risk by sex.

SUPPLEMENTARY FIGURE S4
Linear association between OBS and all cause mortality by sex.

SUPPLEMENTARY FIGURE S5
Linear association between OBS and CVD mortality by sex.

SUPPLEMENTARY FIGURE S6
Linear association between dietary OBS and all cause mortality by sex.

SUPPLEMENTARY FIGURE S7
Linear association between diatery OBS and CVD mortality by sex.

SUPPLEMENTARY FIGURE S8
Linear association between lifestyle OBS and all cause mortality by sex.

SUPPLEMENTARY FIGURE S9
Linear association between lifestyle OBS and CVD mortality by sex.

References

1. Tsao CW, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, et al. Heart disease and stroke Statistics-2023 update: a report from the American Heart Association. *Circulation*. (2023) 147:e93–e621. doi: 10.1161/cir.0000000000001123

2. Cauwenberghs N, Hedman K, Kobayashi Y, Vanassche T, Haddad F, Kuznetsova T. The 2013 ACC/AHA risk score and subclinical cardiac remodeling and dysfunction: complementary in cardiovascular disease prediction. *Int J Cardiol*. (2019) 297:67–74. doi: 10.1016/j.ijcard.2019.09.061

3. Mokdad AH, Ballestros K, Echko M, Glenn S, Olsen HE, Mullany E, et al. The state of US health, 1990-2016: burden of diseases, injuries, and risk factors among US states. *JAMA*. (2018) 319:1444–72. doi: 10.1001/jama.2018.0158

4. Muller DC, Murphy N, Johansson M, Ferrari P, Tsilidis KK, Boutron-Ruault MC, et al. Modifiable causes of premature death in middle-age in Western Europe: results from the EPIC cohort study. *BMC Med*. (2016) 14:87. doi: 10.1186/s12916-016-0630-6

5. Lichtenstein AH, Appel LJ, Vadiveloo M, Hu FB, Kris-Etherton PM, Rebholz CM, et al. 2021 dietary guidance to improve cardiovascular health: a scientific statement from the American Heart Association. *Circulation*. (2021) 144:e472–87. doi: 10.1161/cir.0000000000001031

6. Anand SS, Hawkes C, de Souza RJ, Mente A, Dehghan M, Nugent R, et al. Food consumption and its impact on cardiovascular disease: importance of solutions focused on the globalized food system: a report from the workshop convened by the world heart federation. *J Am Coll Cardiol*. (2015) 66:1590–614. doi: 10.1016/j.jacc.2015.07.050

7. Mozaffarian D, Liu J, Sy S, Huang Y, Rehm C, Lee Y, et al. Cost-effectiveness of financial incentives and disincentives for improving food purchases and health through the US supplemental nutrition assistance program (SNAP): a microsimulation study. *PLoS Med*. (2018) 15:e1002661. doi: 10.1371/journal.pmed.1002661

8. Nissen SEUS. Dietary guidelines: an evidence-free zone. *Ann Intern Med*. (2016) 164:558–9. doi: 10.7326/m16-0035

9. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. *N Engl J Med*. (2018) 378:e34. doi: 10.1056/NEJMoa1800389

10. Hernández-Ruiz Á, García-Villanova B, Guerra-Hernández E, Amiano P, Ruiz-Canela M, Molina-Montes E. A review of a priori defined oxidative balance scores relative to their components and impact on health outcomes. *Nutrients*. (2019) 11:774. doi: 10.3390/nu11040774

11. Golmohammadi M, Ayremlou P, Zarrin R. Higher oxidative balance score is associated with better glycemic control among Iranian adults with type-2 diabetes. *Int J Vitam Nutr Res*. (2021) 91:31–9. doi: 10.1024/0300-9831/a000596

12. Lee JH, Joo YB, Han M, Kwon SR, Park W, Park KS, et al. Relationship between oxidative balance score and quality of life in patients with osteoarthritis: data from the Korea National Health and nutrition examination survey (2014-2015). *Medicine (Baltimore)*. (2019) 98:e16355. doi: 10.1097/md.0000000000016355

13. Ilori TO, Wang X, Huang M, Gutierrez OM, Narayan KM, Goodman M, et al. Oxidative balance score and the risk of end-stage renal disease and cardiovascular disease. *Am J Nephrol*. (2017) 45:338–45. doi: 10.1159/000464257

14. Wang T, Goodman M, Sun YV, Thyagarajan B, Gross M, Bostick RM. DNA base excision repair genetic risk scores, oxidative balance, and incident, sporadic colorectal adenoma. *Mol Carcinog*. (2017) 56:1642–52. doi: 10.1002/mc.22620

15. Park YM, Shivappa N, Petimar J, Hodgson ME, Nichols HB, Steck SE, et al. Dietary inflammatory potential, oxidative balance score, and risk of breast cancer: findings from the sister study. *Int J Cancer*. (2021) 149:615–26. doi: 10.1002/ijc.33581

16. Liu C, Lai W, Zhao M, Zhang Y, Hu Y. Association between the composite dietary antioxidant index and atherosclerotic cardiovascular disease in postmenopausal women: a cross-sectional study of NHANES data, 2013-2018. *Antioxidants*. (2023) 12:1740. doi: 10.3390/antiox12091740

17. Zhang J, Jia J, Lai R, Wang X, Chen X, Tian W, et al. Association between dietary inflammatory index and atherosclerosis cardiovascular disease in U.S. adults. *Front Nutr*. (2022) 9:1044329. doi: 10.3389/fnut.2022.1044329

18. Talavera-Rodriguez I, Fernandez-Lazaro CI, Hernández-Ruiz Á, Hershey MS, Galarregui C, Sotos-Prieto M, et al. Association between an oxidative balance score and mortality: a prospective analysis in the SUN cohort. *Eur J Nutr*. (2023) 62:1667–80. doi: 10.1007/s00394-023-03099-8

19. Jeong S.Y., Wee C.C., Kovell L.C., Plante T.B., Miller E.R., 3rd; Appel L.J., Mukamal K.J., Juraschek S.P. Effects of diet on 10-year atherosclerotic cardiovascular disease risk (from the DASH trial). Am J Cardiol (2023) 187, 10–17. doi: 10.1016/j.amjcard.2022.10.019

20. Wright ME, Mayne ST, Stolzenberg-Solomon RZ, Li Z, Pietinen P, Taylor PR, et al. Development of a comprehensive dietary antioxidant index and application to lung cancer risk in a cohort of male smokers. *Am J Epidemiol*. (2004) 160:68–76. doi: 10.1093/aje/kwh173

21. Zhang W, Peng SF, Chen L, Chen HM, Cheng XE, Tang YH. Association between the oxidative balance score and telomere length from the National Health and nutrition examination survey 1999-2002. *Oxidative Med Cell Longev*. (2022) 2022:1345071. doi: 10.1155/2022/1345071

22. Goff DC, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association task force on practice guidelines. *J Am Coll Cardiol*. (2014) 63:2935–59. doi: 10.1016/j.jacc.2013.11.005

23. Lassale C, Gunter MJ, Romaguera D, Peelen LM, Van der Schouw YT, Beulens JW, et al. Diet quality scores and prediction of all-cause, cardiovascular and Cancer mortality in a Pan-European cohort study. *PLoS One*. (2016) 11:e0159025. doi: 10.1371/journal.pone.0159025

24. Dale CE, Fatemifar G, Palmer TM, White J, Prieto-Merino D, Zabaneh D, et al. Causal associations of adiposity and body fat distribution with coronary heart disease, stroke subtypes, and type 2 diabetes mellitus: a Mendelian randomization analysis. *Circulation*. (2017) 135:2373–88. doi: 10.1161/circulationaha.116.026560

25. Kamimura D, Cain-Shields LR, Clark D, Oshunbade AA, Ashley KE, Guild CS, et al. Physical activity, inflammation, coronary artery calcification, and incident coronary heart disease in African Americans: insights from the Jackson heart study. *Mayo Clin Proc*. (2021) 96:901–11. doi: 10.1016/j.mayocp.2020.09.042

26. Roerecke M, Rehm J. Alcohol consumption, drinking patterns, and ischemic heart disease: a narrative review of meta-analyses and a systematic review and meta-analysis of the impact of heavy drinking occasions on risk for moderate drinkers. *BMC Med*. (2014) 12:182. doi: 10.1186/s12916-014-0182-6

27. GBD 2017 Diet Collaborators. Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the global burden of disease study 2017. *Lancet*. (2019) 393:1958–72. doi: 10.1016/s0140-6736(19)30041-8

28. Pearson T.A., Mensah G.A., Alexander R.W., Anderson J.L., Cannon R.O., 3rd; Criqui M., Fadl Y.Y., Fortmann S.P., Hong Y., Myers G.L.; et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation (2003) 107, 499–511. doi: 10.1161/01.cir.0000052939.59093.45

29. Santos S, Oliveira A, Lopes C. Systematic review of saturated fatty acids on inflammation and circulating levels of adipokines. *Nutr Res*. (2013) 33:687–95. doi: 10.1016/j.nutres.2013.07.002

30. Bordoni A, Danesi F, Dardevet D, Dupont D, Fernandez AS, Gille D, et al. Dairy products and inflammation: a review of the clinical evidence. *Crit Rev Food Sci Nutr*. (2017) 57:2497–525. doi: 10.1080/10408398.2014.967385

31. Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. *Nutr Rev*. (2013) 71:511–27. doi: 10.1111/nure.12035

32. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother*. (2002) 56:365–79. doi: 10.1016/s0753-3322(02)00253-6

33. Chai W, Morimoto Y, Cooney RV, Franke AA, Shvetsov YB, Le Marchand L, et al. Dietary red and processed meat intake and markers of adiposity and inflammation: the

multiethnic cohort study. *J Am Coll Nutr*. (2017) 36:378–85. doi: 10.1080/07315724.2017.1318317

34. Bodén S, Wennberg M, Van Guelpen B, Johansson I, Lindahl B, Andersson J, et al. Dietary inflammatory index and risk of first myocardial infarction; a prospective population-based study. *Nutr J*. (2017) 16:21. doi: 10.1186/s12937-017-0243-8

35. Garcia-Arellano A, Ramallal R, Ruiz-Canela M, Salas-Salvadó J, Corella D, Shivappa N, et al. Dietary inflammatory index and incidence of cardiovascular disease in the PREDIMED study. *Nutrients*. (2015) 7:4124–38. doi: 10.3390/nu7064124

36. Shivappa N, Godos J, Hébert JR, Wirth MD, Piuri G, Speciani AF, et al. Dietary inflammatory index and cardiovascular risk and mortality-a meta-analysis. *Nutrients*. (2018) 10:200. doi: 10.3390/nu10020200

37. Yu YC, Paragomi P, Wang R, Jin A, Schoen RE, Sheng LT, et al. Composite dietary antioxidant index and the risk of colorectal cancer: findings from the Singapore Chinese health study. *Int J Cancer*. (2022) 150:1599–608. doi: 10.1002/ijc.33925

38. Zhao L, Sun Y, Cao R, Wu X, Huang T, Peng W. Non-linear association between composite dietary antioxidant index and depression. *Front Public Health*. (2022) 10:988727. doi: 10.3389/fpubh.2022.988727

39. Alehagen U, Aaseth J, Lindahl TL, Larsson A, Alexander J. Dietary supplementation with selenium and coenzyme Q(10) prevents increase in plasma D-dimer while lowering cardiovascular mortality in an elderly Swedish population. *Nutrients*. (2021) 13:1344. doi: 10.3390/nu13041344

40. O'Connor EA, Evans CV, Ivlev I, Rushkin MC, Thomas RG, Martin A, et al. Vitamin and mineral supplements for the primary prevention of cardiovascular disease and Cancer: updated evidence report and systematic review for the US preventive services task force. *JAMA*. (2022) 327:2334–47. doi: 10.1001/jama.2021.15650

41. Mangione CM, Barry MJ, Nicholson WK, Cabana M, Chelmow D, Coker TR, et al. Vitamin, mineral, and multivitamin supplementation to prevent cardiovascular disease and Cancer: US preventive services task force recommendation statement. *JAMA*. (2022) 327:2326–33. doi: 10.1001/jama.2022.8970

42. Jenkins DJA, Spence JD, Giovannucci EL, Kim YI, Josse RG, Vieth R, et al. Supplemental vitamins and minerals for cardiovascular disease prevention and treatment: JACC focus seminar. *J Am Coll Cardiol*. (2021) 77:423–36. doi: 10.1016/j.jacc.2020.09.619

43. Chen W, Zhang S, Hu X, Chen F, Li D. A review of healthy dietary choices for cardiovascular disease: from individual nutrients and foods to dietary patterns. *Nutrients*. (2023) 15:4898. doi: 10.3390/nu15234898

44. Slattery ML, Boucher KM, Caan BJ, Potter JD, Ma KN. Eating patterns and risk of colon cancer. *Am J Epidemiol*. (1998) 148:4–16. doi: 10.1093/aje/148.1.4-a

45. Huijbregts P, Feskens E, Räsänen L, Fidanza F, Nissinen A, Menotti A, et al. Dietary pattern and 20 year mortality in elderly men in Finland, Italy, and the Netherlands: longitudinal cohort study. *BMJ*. (1997) 315:13–7. doi: 10.1136/bmj.315.7099.13

46. Wiseman M. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc Nutr Soc*. (2008) 67:253–6. doi: 10.1017/s002966510800712x

47. Farazi M, Jayedi A, Shab-Bidar S. Dietary inflammatory index and the risk of non-communicable chronic disease and mortality: an umbrella review of meta-analyses of observational studies. *Crit Rev Food Sci Nutr*. (2023) 63:57–66. doi: 10.1080/10408398.2021.1943646

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Associations of minerals intake with colorectal cancer risk in the prostate, lung, colorectal, ovarian cancer screening trial

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Objective: Exploring the association between common mineral intake and the risk of colorectal cancer (CRC).

Methods: We utilized the multivariate Cox proportional hazards model to assess the association between intake of minerals and the risk of CRC, estimating hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: A total of 101,686 eligible participants were included in the analysis of this study, including 1,100 CRC cases. After adjusting for potential confounders, we found that total zinc intake ($HR_{Q4vs.Q1}$: 0.79, 95%CI 0.67–0.93; *P* for trend <0.05), iron intake ($HR_{Q4vs.Q1}$: 0.81, 95%CI 0.68–0.96; *P* for trend <0.05), copper intake ($HR_{Q4vs.Q1}$: 0.80, 95%CI 0.68–0.95; *P* for trend <0.05), selenium intake ($HR_{Q4vs.Q1}$: 0.83, 95%CI 0.69–0.98; *P* for trend <0.05) were significantly negatively associated with the incidence of CRC, but magnesium intake in the appropriate range is associated with a reduced risk of CRC ($HR_{Q3vs.Q1}$: 0.77, 95%CI 0.65–0.91; *P* for trend >0.05).

Conclusion: Our findings suggested that an appropriate intake of total zinc, iron, copper, selenium and magnesium were associated with lower CRC risk.

KEYWORDS

minerals, colorectal cancer, PLCO, zinc, iron, magnesium, copper, selenium

1 Introduction

Minerals intake is strongly associated with cancer, especially digestive system tumors. In recent years, colorectal cancer (CRC) has become the third most commonly diagnosed cancer, and second leading cause of cancer death worldwide (1, 2). Such a high morbidity of CRC is not only associated with the promotion of screening programs, but is also closely linked to modern dietary habits (3). The incidence of CRC is tending to occur at progressively younger ages and the proportion of young patients (age < 50 years) is increasing (4, 5). The colorectum, a vital component of the digestive system, is increasingly burdened by the disease (1). CRC is a common cancer with a significant genetic component; approximately 10–16% of patients have pathogenic variants in their cancer susceptibility genes (6). In addition, lifestyle factors such as smoking, alcohol consumption, obesity, and the intake of red and processed meats also influence CRC risk (2, 7). Several studies have demonstrated the association of minerals with carcinogenesis and the content of certain minerals are significantly differences between tumor

tissues and healthy tissues (8–10). The minerals are necessary in the metabolism of the body, but there are fewer studies on the relationship between mineral intake and CRC.

The minerals, specifically magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu) and selenium (Se), can be acquired in diet or supplements. These minerals maintain normal physiological functions and are important for maintaining human health, such as in DNA replication, immunity and energy production (11–14). The association between minerals and cancers has aroused great concern. Increasing dietary zinc intake was reported to be effective in reducing cancer risk, especially in prostate cancer (15–17). Iron deficiency and iron deficiency anemia are global health problems, whereas excessive iron intake may be associated with tumorigenesis (18–21). Copper is involved in the important process of cancer development, with lower in malignant tumors than in benign tumors, and it was reported that copper of specific structure has anti-tumor effects (22–25). The anti-cancer effects of selenium have been confirmed by several studies (9, 26, 27). Through further research, it was noted that all of these minerals have been linked to CRC. Epidemiologic studies and some meta-analyses have shown higher intake of magnesium is associated with lower CRC risk (28, 29). Iron has also been found to reduce the risk of lung cancer (30), but it may increase the risk of CRC (29). Moreover, elesclomol-mediated copper overload has been shown to inhibit CRC both *in vitro* and *in vivo* (31). A randomized controlled trial has shown that higher selenium levels are associated with a lower risk of prostate, lung and CRC (32). Some researchers have found higher levels of magnesium, zinc, copper and selenium in tumor tissue from CRC patients than in normal tissue (8). Based on these finding, we explored the association between the intake of these elements and the development of CRC.

In recent years, the effect of minerals intake on CRC has become a subject of considerable concern, but study findings have varied considerably. The Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial, a prospective cohort study, has a large number of participants, a long follow-up period and reliable mineral intake data, which can effectively show the relationship between the intake of each mineral and the development of CRC. Our study aims to examine the associations between the intake of five key minerals (magnesium, zinc, copper, iron, and selenium) and the CRC risk in the PLCO cancer screening trial.

2 Methods

2.1 Study population

The design and methodology of the PLCO cancer screening trial has been reported in several previous studies (33, 34). The PLCO Cancer Screening Trial is a randomized, controlled trial of screening tests for prostate, lung, colorectal and ovarian cancers and more than a dozen other cancers. Ten PLCO Screening Centers recruited approximately 155,000 participants aged between 55 and 75 years from November 1993 to July 2001, and all participants signed an informed consent. The Clinical Trials. gov numbers for PLCO are NCT00002540, NCT01696968, NCT01696981, and NCT01696994.

2.2 Data collection and minerals assessment

All participants were asked to complete a baseline questionnaire (BQ), is the baseline risk factor questionnaire, including participant-reported information such as sex, age, education, cancer history and medical history. The Dietary History Questionnaire (DHQ) is a food frequency questionnaire that was added in 1998 and covers daily intake of 124 foods over the past 12 months for 113,000 participants. Nutrient intake was derived from frequencies and portion sizes from the Food Frequency Questionnaire (FFQ), in which mineral values per portion multiplied by the frequency of daily intake and then summed to obtain the intake of the nutrient concerned (35). Daily intakes of nutrients were calculated based on the Nutrition Data System for Research (NDS-R). The NDS-R combines nutritional information from the USDA Standard Reference Nutrient Database, food manufacturers, scientific literature, and other published food tables (36). In this study, total intake of five minerals (magnesium, zinc, iron, copper, and selenium) was extracted from DHQ, both from food and from supplements.

2.3 Participant selection

Our study needed to identify participants eligible for the DHQ CRC analysis (Figure 1). Participants will be excluded from the study if they did not return a baseline questionnaire ($n = 48,283$); missing DHQ completed data ($n = 15,019$); their DHQ was invalid ($n = 9,798$); they have personal history of any cancer prior to the DHQ ($n = 116$). After screening, 101,686 eligible participants were identified in the analysis, including 1,100 CRC cases.

2.4 Ascertainment of CRC

The endpoint event in this study was CRC incidence. Carcinoid colorectal cancer is considered a target for CRC screening, it is therefore included in the definition of confirmed CRC. In the PLCO trial, subjects were not diagnosed with CRC at the start of the study. CRC reports were collected in a variety of ways, including self-reports, family reports and death certificates, family reports and death certificates. Cancer data collected up to December 31, 2009 and mortality data collected through 2015 for each subject in the PLCO trial, and cancers and deaths continue to accrue. The time metric chosen for the study was the number of days between completion of the DHQ and the diagnosis for participants with CRC, or to trial exit otherwise.

2.5 Statistical analysis

Hazard ratios (HRs) values in this study were calculated using data after multiple interpolation. The distribution of general characteristics of cases and controls were compared using

Abbreviations: BMI, body mass index; CI, confidence interval; CRC, colorectal cancer; DHQ, the diet history questionnaire; HR, hazard ratio; PLCO, prostate, lung, colorectal and ovarian.

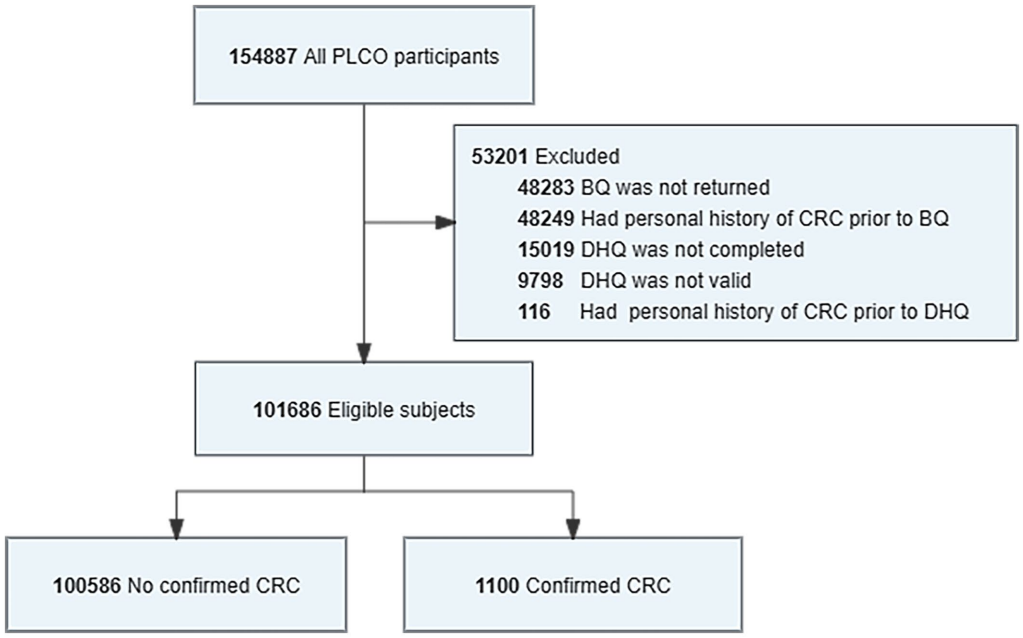

FIGURE 1
Flowchart for identifying eligible PLCO participants in study analysis. The final cohort had a total of 101,686 eligible participants included in the analysis. BQ, baseline questionnaire; DHQ, diet history questionnaire.

Chi-square (χ^2) tests for categorical variables and *t*-test for continuous variables. Intakes of total magnesium, zinc, iron, copper, and selenium were used to generate new categorical variables by quartiles.

HRs and 95% confidence intervals (CIs) were estimated for CRC risk in relation to five minerals intake using a multivariate Cox proportional hazards model. Modelling to adjust covariates for known or suspected CRC risk factors, including age (<65 vs. ≥65), sex (male vs. female), randomization arm (intervention vs. control), body mass index (BMI, <25 kg/m^2 vs. ≥25 kg/m^2), race (White, Non-Hispanic vs. Other), marital status (married vs. unmarried), education (<college vs. ≥college), smoking status (never smoked cigarettes vs. current cigarette smoker vs. former cigarette smoker), drinking status (never vs. former vs. current), family history of CRC (yes vs. no vs. possibly-relative or cancer), and family history of any cancer (yes vs. no vs. possibly-relative or cancer). Three regression models were constructed for each of the five minerals: model 1 does not make any adjustments and roughly estimates HR; model 2 just adjusted for age and sex; model 3 adjusted for all ten covariates.

Subgroup analysis was done for 5 variables such as age, sex, BMI, family history of CRC and family history of any cancer. The results were corrected for 10 confounders other than the grouping factors. Restricted spline models were fitted with three nodes to determine dose-response trends between intakes of total minerals (continuous variable) and CRC risk. Sensitivity analyses were performed by excluding events with less than 2 years of follow-up, extreme BMI values (<1% and >99%), or removing cases with missing values.

The proportional hazards assumption was graphically tested for all built models, all data are consistent with the proportional risk assumption. Statistical analyses were performed using R statistical software (http://www.R-project.org, The R Foundation), and a *p* value <0.05 (two-tailed) was considered significant.

3 Result

3.1 Participant characteristics

The characteristics of the study population are shown in Table 1. The median follow-up time for cancer diagnosis data was 11.3 years, during which 1,100 cases of CRC were diagnosed. Mean age at baseline was 64.14 years in the case group and 62.38 years in the control group. By comparing the case and control groups, it can be seen that the case group's characteristics included a majority of males, a younger age, higher BMI, a higher likelihood of having a higher education, primarily alcohol drinkers, and a family history of any cancer.

3.2 Association between intakes of total minerals and the incidence of CRC

Estimates risk of CRC associated with total intakes of magnesium, zinc, iron, copper, and selenium are shown in Table 2. Model 1 was the crude model; model 2 just adjusted for age and sex; model 3 adjusted for all ten covariates. There was a significant correlation between CRC incidence and moderate magnesium intake in the crude analysis model ($HR_{Q3vs.Q1}$: 0.76, 95%CI: 0.64–0.91, $p = 0.002$). Similar results were found in the adjusted models (model 2: $HR_{Q3vs.Q1}$: 0.75, 95%CI: 0.63–0.89, $p = 0.001$; model 3: $HR_{Q3vs.Q1}$: 0.77, 95%CI: 0.65–0.91, $p = 0.002$). Zinc intake was found to significantly associated with lower CRC risk. After adjusting for covariates, the results of models 2 and 3 were the same as model 1. After adjusting for ten covariates, the minerals iron, copper, and selenium were also shown to associate with lower risk of CRC, *P* for trend <0.05.

TABLE 1 Baseline characteristics of CRC patients and non-patient participants.

Variables	Overall (*n* = 101,686)	Non-patients (*n* = 100,586)	Patients (*n* = 1,100)	*p*
Age (years), mean (SD)	62.40 (5.28)	62.38 (5.28)	64.14 (5.19)	<0.001
Sex (*n*, %)				
Male	49,445 (48.63)	48,837 (48.55)	608 (55.27)	<0.001
Female	52,241 (51.37)	51,749 (51.45)	492 (44.73)	
Arm (*n*, %)				
Intervention	51,771 (50.91)	51,327 (51.03)	444 (40.36)	<0.001
Control	49,915 (49.09)	49,259 (48.97)	656 (59.64)	
Education (*n*, %)				
<college	42,911 (42.20)	42,387 (42.14)	524 (47.64)	0.001
>=college	58,578 (57.61)	58,005 (57.67)	573 (52.09)	
Missing	197 (0.19)	194 (0.19)	3 (0.27)	
Marital status (*n*, %)				
Married	98,286 (96.66)	78,733 (78.27)	851 (77.36)	0.306
Unmarried	3,214 (3.16)	21,671 (21.54)	245 (22.27)	
Missing	186 (0.18)	182 (0.18)	4 (0.36)	
Race (*n*, %)				
White, non-Hispanic	92,470 (90.94)	91,480 (90.95)	990 (90.00)	0.432
Other	9,179 (9.03)	9,069 (9.02)	110 (10.00)	
Missing	37 (0.04)	37 (0.04)	0 (0.00)	
BMI (*n*, %)				
<25	34,428 (33.86)	34,094 (33.90)	334 (30.36)	0.006
>=25	65,919 (64.83)	65,176 (64.80)	743 (67.55)	
Missing	1,339 (1.32)	1,316 (1.31)	23 (2.09)	
Smoking (*n*, %)				
Never	48,535 (47.73)	48,050 (47.77)	485 (44.09)	0.108
Former	43,744 (43.02)	43,237 (42.99)	507 (46.09)	
Current	9,394 (9.24)	9,286 (9.23)	108 (9.82)	
Missing	13 (0.01)	13 (0.01)	0 (0.00)	
Alcohol intake (*n*, %)				
Never	10,110 (9.94)	10,013 (9.95)	97 (8.82)	0.602
Former	14,746 (14.50)	14,581 (14.50)	165 (15.00)	
Current	73,950 (72.72)	73,141 (72.71)	809 (73.55)	
Missing	2,880 (2.83)	2,851 (2.83)	29 (2.64)	
Family history of CRC (*n*, %)				
No	88,118 (86.88)	87,190 (86.68)	928 (84.36)	0.040
Yes	10,301 (10.13)	10,178 (10.12)	123 (11.18)	
Possibly	2,493 (2.45)	2,453 (2.44)	40 (3.64)	
Missing	774 (0.76)	765 (0.76)	9 (0.82)	
Family history of any cancer (*n*, %)				
No	44,584 (43.84)	44,130 (43.87)	454 (41.27)	0.223
Yes	56,821 (55.88)	56,178 (55.85)	643 (58.45)	
Missing	281 (0.28)	278 (0.28)	3 (0.27)	

Values are means (SD) for continuous variables or number (percentage) for categorical variables. Differences in characteristics shown patients and non-patients were tested by using chi-square tests for categorical variables and ANOVA for continuous variables.

TABLE 2 Association between minerals intake and CRC risk in the PLCO cancer screening trial.

Variables	Cohort (*n*)	Cases (*n*)	Person-years	Incidence rate per 10,000 person-years	HR (95% CI), *p*-value		
					Model 1	Model 2	Model 3
Magnesium (mg/day)							
Q1 (<273.64)	25,422	304	222512.0	13.66	Ref	Ref	Ref
Q2 (≥273.64 to <354.05)	25,423	282	224442.2	12.56	0.92 (0.78–1.08), $p = 0.313$	0.91 (0.78–1.07), $p = 0.273$	0.93 (0.79–1.10), $p = 0.394$
Q3 (≥352.05 to <446.42)	25,422	235	225394.4	10.43	0.76 (0.64–0.91), $p = 0.002$	0.75 (0.63–0.89), $p = 0.001$	0.77 (0.65–0.91), $p = 0.002$
Q4 (≥446.42)	25,419	279	224155.1	12.45	0.91 (0.77–1.07), $p = 0.263$	0.87 (0.74–1.02), $p = 0.095$	0.89 (0.75–1.05), $p = 0.154$
P for trend					0.142	0.041	0.072
Zinc (mg/day)							
Q1 (<9.93)	25,428	334	223224.8	14.96	Ref	Ref	Ref
Q2 (≥9.93 to <19.82)	25,444	254	224148.8	11.33	0.76 (0.64–0.89), $p = 0.001$	0.75 (0.64–0.88), $p = 0.001$	0.76 (0.64–0.89), $p = 0.001$
Q3 (≥19.82 to <25.75)	25,399	250	224807.3	11.12	0.74 (0.63–0.88), $p < 0.001$	0.77 (0.65–0.90), $p = 0.001$	0.79 (0.67–0.93), $p = 0.004$
Q4 (≥25.75)	25,415	262	224322.8	11.68	0.78 (0.66–0.92), $p = 0.003$	0.77 (0.65–0.90), $p = 0.001$	0.79 (0.67–0.93), $p = 0.004$
P for trend					0.004	0.004	0.011
Iron (mg/day)							
Q1 (<13.65)	25,436	311	222720.2	13.96	Ref	Ref	Ref
Q2 (≥13.65 to <24.03)	25,407	275	224777.9	12.23	0.88 (0.75–1.03), $p = 0.111$	0.85 (0.72–1.00), $p = 0.053$	0.87 (0.74–1.03), $p = 0.097$
Q3 (≥24.03 to <31.72)	25,444	265	224716.4	11.79	0.84 (0.72–1.00), $p = 0.044$	0.86 (0.73–1.02), $p = 0.080$	0.89 (0.76–1.05), $p = 0.176$
Q4 (≥31.72)	25,399	249	224289.3	11.10	0.80 (0.67–0.94), $p = 0.007$	0.78 (0.66–0.92), $p = 0.003$	0.81 (0.68–0.96), $p = 0.014$
P for trend					0.007	0.008	0.028
Copper (mg/day)							
Q1 (<1.23)	25,653	316	224803.5	14.06	Ref	Ref	Ref
Q2 (≥1.23 to <2.34)	25,242	268	223027.0	12.02	0.86 (0.73–1.01), $p = 0.060$	0.83 (0.71–0.98), $p = 0.029$	0.85 (0.72–1.00), $p = 0.046$
Q3 (≥2.34 to <3.2)	25,697	273	226958.9	12.03	0.86 (0.73–1.01), $p = 0.060$	0.88 (0.75–1.04), $p = 0.138$	0.91 (0.77–1.07), $p = 0.251$
Q4 (≥3.2)	25,094	243	221714.2	10.96	0.78 (0.66–0.92), $p = 0.004$	0.77 (0.65–0.91), $p = 0.003$	0.80 (0.68–0.95), $p = 0.010$
P for trend					0.008	0.015	0.047
Selenium (mcg/day)							
Q1 (<59.52)	25,434	306	222865.6	13.73	Ref	Ref	Ref
Q2 (≥59.52 to <81.59)	25,410	272	225181.8	12.08	0.88 (0.75–1.04), $p = 0.125$	0.87 (0.73–1.02), $p = 0.086$	0.88 (0.74–1.03), $p = 0.114$
Q3 (≥81.59 to <110.78)	25,429	251	224691.4	11.17	0.81 (0.69–0.96), $p = 0.016$	0.78 (0.66–0.92), $p = 0.004$	0.79 (0.66–0.93), $p = 0.006$
Q4 (≥110.78)	25,413	271	223764.9	12.11	0.88 (0.75–1.04), $p = 0.134$	0.82 (0.69–0.98), $p = 0.028$	0.83 (0.69–0.98), $p = 0.029$
P for trend					0.151	0.032	0.031

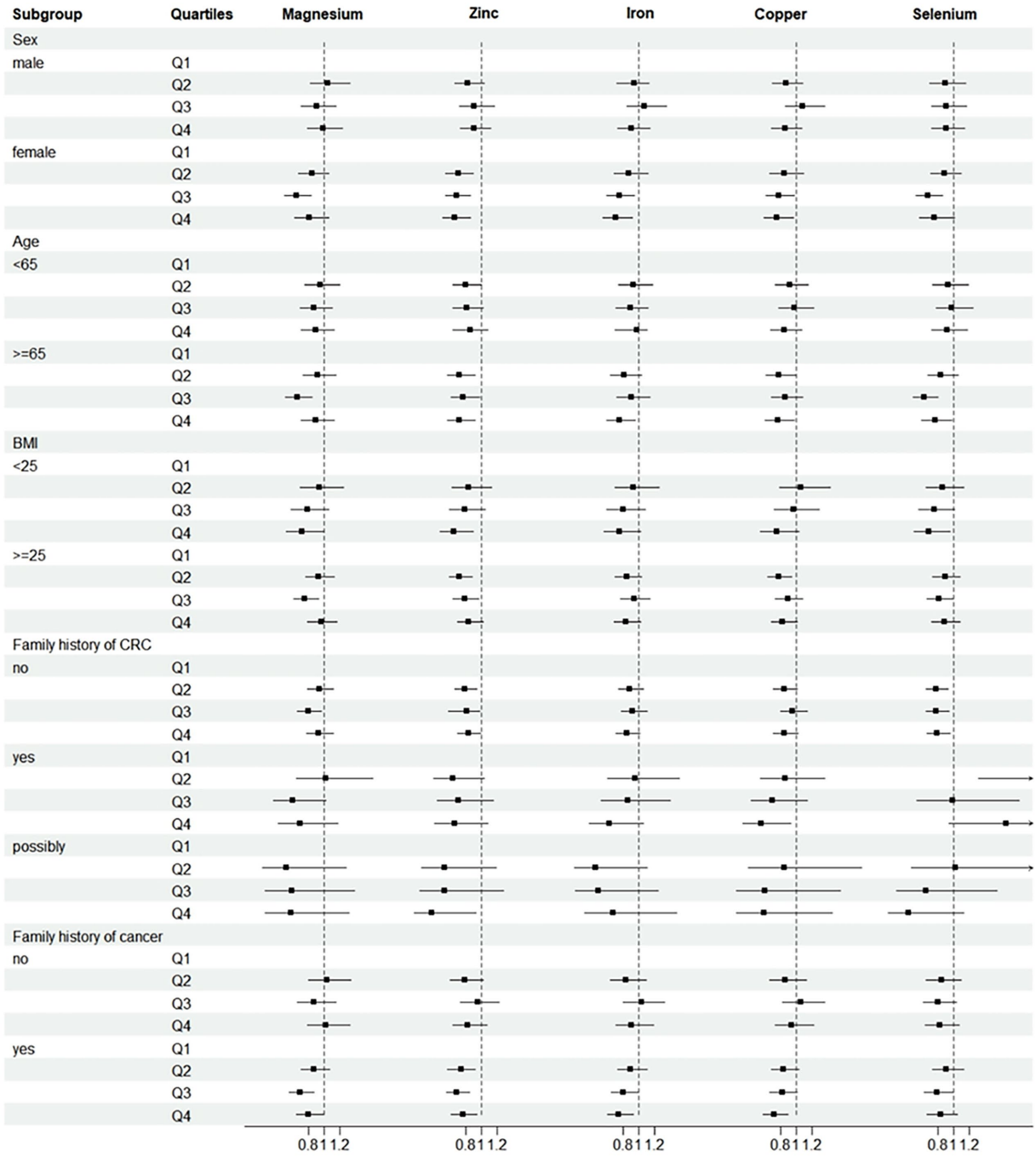

FIGURE 2
Subgroup analyses of CRC incidence. Adjusted for age (<65 vs. ≥65), sex (male vs. female), trial arm (intervention vs. control), race (White, non-Hispanic vs. other), marital status (married vs. unmarried), education (<college vs. ≥college), BMI (<25 kg/m^2 vs. ≥25 kg/m^2), smoking status (never vs. current vs. former), alcohol drinking status (never vs. current vs. former), family history of CRC (no vs. yes vs. possibly), family history of any cancer (no vs. yes). Subgroup analyses by sex, age, BMI, family history of CRC and family history of cancer. HRs were adjusted except for the stratification factor. $p > 0.05$ was considered no statistical significance.

Further analysis was performed after adjusting for confounders. Subgroup analyses suggested that the protective effect of these five minerals against CRC was more pronounced among men, age ≥ 65 years, or with family history of any cancer (Figure 2). Sensitivity analyses showed that the results of the association between minerals intake and CRC risk were generally consistent with those in Table 2 after removing missing data, removing BMI extremes, or removing cases with less than 2 years of follow-up (Table 3). Restricted cubic spline model analysis suggested that there was a nonlinear association of Mg, Fe, Zn, and Se intakes with incidence of CRC (*P*-non-linear <0.05, Figures 3A–E).

TABLE 3 Sensitivity analyses on the association between minerals intake and CRC incidence.

Processing methods	Quartiles	HR (95% CI), *p*-value				
		Magnesium	Zinc	Iron	Copper	Selenium
Primary analysis	Q1	Ref	Ref	Ref	Ref	Ref
	Q2	0.93 (0.79–1.10), $p = 0.394$	0.76 (0.64–0.89), $p = 0.001$	0.87 (0.74–1.03), $p = 0.097$	0.85 (0.72–1.00), $p = 0.046$	0.88 (0.74–1.03), $p = 0.114$
	Q3	0.77 (0.65–0.91), $p = 0.002$	0.79 (0.67–0.93), $p = 0.004$	0.89 (0.76–1.05), $p = 0.176$	0.91 (0.77–1.07), $p = 0.251$	0.79 (0.66–0.93), $p = 0.006$
	Q4	0.89 (0.75–1.05), $p = 0.154$	0.79 (0.67–0.93), $p = 0.004$	0.81 (0.68–0.96), $p = 0.014$	0.80 (0.68–0.95), $p = 0.010$	0.83 (0.69–0.98), $p = 0.029$
	P for trend	0.072	0.011	0.028	0.047	0.031
Deleting missing values	Q1	Ref	Ref	Ref	Ref	Ref
	Q2	0.91 (0.77–1.08), $p = 0.273$	0.79 (0.67–0.94), $p = 0.007$	0.86 (0.73–1.02), $p = 0.091$	0.83 (0.70–0.98), $p = 0.029$	0.92 (0.77–1.08), $p = 0.308$
	Q3	0.75 (0.62–0.89), $p = 0.001$	0.79 (0.67–0.94), $p = 0.008$	0.88 (0.75–1.05), $p = 0.159$	0.91 (0.76–1.07), $p = 0.245$	0.79 (0.66–0.95), $p = 0.011$
	Q4	0.88 (0.74–1.04), $p = 0.145$	0.79 (0.66–0.93), $p = 0.006$	0.81 (0.68–0.96), $p = 0.017$	0.80 (0.67–0.95), $p = 0.010$	0.84 (0.70–1.01), $p = 0.057$
	P for trend	0.073	0.010	0.032	0.055	0.044
Excluding participants with extreme BMI	Q1	Ref	Ref	Ref	Ref	Ref
	Q2	0.95 (0.80–1.12), $p = 0.526$	0.77 (0.65–0.91), $p = 0.002$	0.88 (0.75–1.04), $p = 0.131$	0.87 (0.73–1.02), $p = 0.088$	0.88 (0.74–1.04), $p = 0.127$
	Q3	0.78 (0.66–0.93), $p = 0.005$	0.80 (0.67–0.94), $p = 0.007$	0.91 (0.77–1.07), $p = 0.255$	0.93 (0.79–1.09), $p = 0.376$	0.80 (0.67–0.94), $p = 0.009$
	Q4	0.90 (0.76–1.07), $p = 0.236$	0.79 (0.67–0.94), $p = 0.006$	0.82 (0.69–0.97), $p = 0.022$	0.81 (0.68–0.96), $p = 0.016$	0.84 (0.70–1.00), $p = 0.045$
	P for trend	0.061	0.016	0.045	0.066	0.050
Excluding participants with a follow-up less than 2 years	Q1	Ref	Ref	Ref	Ref	Ref
	Q2	0.87 (0.72–1.04), $p = 0.134$	0.77 (0.64–0.92), $p = 0.005$	0.86 (0.72–1.04), $p = 0.113$	0.82 (0.68–0.99), $p = 0.038$	0.88 (0.73–1.06), $p = 0.174$
	Q3	0.71 (0.58–0.86), $p < 0.001$	0.74 (0.61–0.89), $p = 0.002$	0.85 (0.71–1.03), $p = 0.098$	0.88 (0.74–1.06), $p = 0.187$	0.72 (0.59–0.87), $p = 0.001$
	Q4	0.81 (0.67–0.97), $p = 0.024$	0.76 (0.63–0.92), $p = 0.004$	0.77 (0.64–0.94), $p = 0.008$	0.73 (0.60–0.88), $p = 0.001$	0.78 (0.64–0.95), $p = 0.012$
	P for trend	0.011	0.005	0.012	0.009	0.008

4 Discussion

In this study, we analyzed the relationship between total minerals intake and CRC incidence and found that total minerals intake was associated with the risk of CRC incidence. However, there is not a simple negative or positive association between intake and risk of morbidity. According to our findings, the risk of CRC is at its lowest when the total intake of minerals is as follows: magnesium between 352.05 and 446.42 mg/day (Q3), zinc between 9.93 and 19.82 mg/day (Q2), iron exceeding 31.72 mg/day (Q4), copper exceeding 3.2 mg/day (Q4), and selenium between 81.59 and 110.78 mcg/day (Q3) (Table 2). These results have been adjusted for potential confounders.

Regarding the relationship between magnesium and zinc intake and CRC incidence, our findings are in agreement with some previous studies that higher magnesium and zinc intake are associated with a lower risk of CRC incidence. The Netherlands cohort study on diet and cancer has found that higher magnesium intake is more protective for people with a BMI > 25 (37). A cohort study in Japan found that higher dietary intake of magnesium may reduce the risk of CRC in Japanese men (38). The Swedish mammography cohort, a population based prospective cohort of women, showed that high magnesium intake reduces the incidence of CRC in women (39). The findings for zinc and CRC are similar to magnesium. The Iowa Women's Health Study followed 34,708 postmenopausal women for 15 years. Based on this study, Lee et al. found that zinc intake was associated with a decreased risk of distal colon cancer (*P* for trend = 0.03) (40). Larsson et al., analyzing data from the population-based Swedish mammography cohort, proposed a relatively weak association of zinc intake with colon cancer (41).

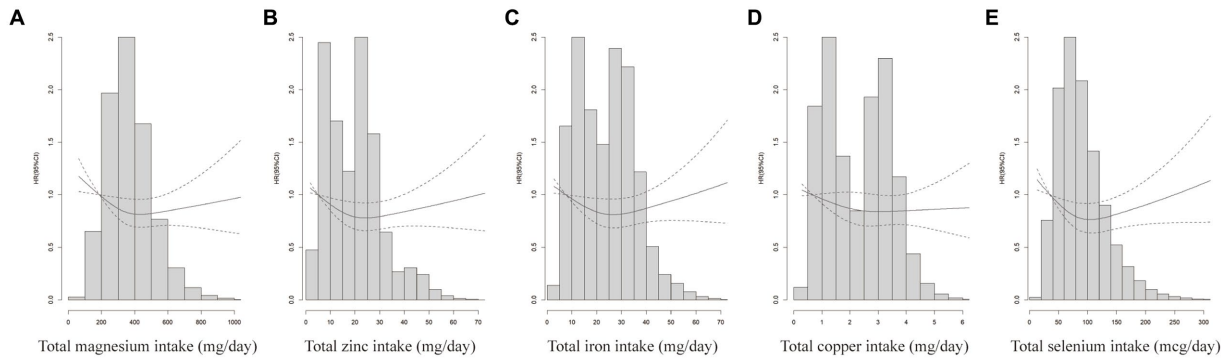

FIGURE 3
Does-response analyses for the associations between minerals intake and CRC incidence. Does-response analyses for the associations between total magnesium intake **(A)**, total zinc intake **(B)**, total iron intake **(C)** total copper intake **(D)** total selenium intake **(E)** and CRC. HRs and 95%CIs were calculated by the fully-adjusted multivariable Cox regression model, including age (<65 vs. ≥65), sex (male vs. female), trial arm (intervention vs. control), race (White, non-Hispanic vs. other), marital status (married vs. unmarried), education (<college vs. ≥college), BMI (<25 kg/m^2 vs. ≥25 kg/m^2), smoking status (never vs. current vs. former), alcohol drinking status (never vs. current vs. former), family history of CRC (no vs. yes vs. possibly), family history of any cancer (no vs. yes).

However, our study suggests that higher zinc intake is significantly associated with a reduced CRC risk. While these studies vary in population characteristics, the conclusions mostly suggest that magnesium and zinc play a protective role against CRC, and our findings contribute to the existing evidence on this topic. Distinct from the aforementioned studies, our data were derived from the PLCO database, the study population included both men and women, and with a long follow-up period and a large sample size, the results obtained were adjusted for multiple confounders.

In recent years, there have been relatively few studies on selenium and CRC. The selenium and vitamin E cancer prevention trial (SELECT) was a randomized, placebo-controlled trial of 35,533 men followed for a minimum of 7 years and a maximum of 12 years between August 22, 2001, and June 24, 2004. Lippman et al., analyzed data from this trial to assess the potential of selenium and vitamin E in preventing prostate cancer, with prespecified secondary outcomes including lung and CRC. But selenium was not statistically significantly associated with CRC (compared to the placebo group, selenium HR = 1.05 99% CI = 0.66–1.67) (42). However, our findings indicate that selenium is a protective factor against CRC when selenium intake is in the range of 81.59–110.78 mcg/day, with statistically significant results in three adjusted models. Comparing these findings with those of the above studies, we can hypothesize that the protective effect of selenium against CRC is more pronounced in women, which is confirmed in our results.

There are many differing views on the effect of iron intake on CRC. Summarizing past studies, we find that one American, one Canadian and one French case-control study all concluded that higher risks of CRC was observed for iron intake (43–45). But a case-control study from Australia suggested that iron has been observed to reduce the risk of CRC (46). All of the above are case-control studies. In addition, a European prospective cohort study finds that iron intake was not associated with CRC risk ($HR_{Q5vs.Q1}$: 0.88; 95%CI: 0.73, 1.06) (47). After our analysis of the PLCO database, we found that the risk of CRC was significantly decreased when total iron intake reached the fourth quartile (P for trend <0.05). Comparing these studies, we hypothesize that the differing findings may be due to the proportion of dietary versus supplemental iron intake, a topic that necessitates further exploration. In addition, the different study population, length of follow-up, and sample size of the cohort study may have contributed to the differences in results from previous studies.

The association between copper intake and cancer has been widely reported, but the specific effect of copper intake on the risk of CRC in human populations remains unclear. Some studies have suggested that copper may reduce the risk of lung and esophageal cancer (48), while others have reported that there is no evidence of an association between dietary copper intake and cancer development (21, 49). The results of a case-control study in Burgundy, France showed the odds ratios associated with the fourth quartile of intake were 2.4 (95%CI, 1.3–4.6) (50). In contrast, by analyzing data from 1,100 CRC patients in the PLCO database, we found that copper may reduce cancer risk. The difference between the results of previous studies and our study may be due to the small number of cases, differences in the dietary structure of the population, and unavoidable recall bias.

4.1 Strengths and limitations of this study

Strengths of this study include its prospective design, a large population sample size, a long follow-up period, a high level of confidence in the authenticity and validity of the outcome screening, and very detailed information about the diet. However, this study has some limitations. Firstly, the sample population in this study was mostly non-Hispanic whites, which may affect the generalization of the findings to other populations. Secondly, residual confounders could not be completely excluded despite adjusting for some confounders using the three models. Lastly, this study did not assess the effects of the interaction of genetic factors and various minerals in the development of CRC.

5 Conclusion

In conclusion, for the analysis of PLCO, a large US cohort, our findings not only corroborate the conclusions of previous studies on the protective role of minerals magnesium and zinc in the development of CRC, but also suggest new perspectives on the role of copper, iron, and selenium in CRC. Minerals factors play an important role in the prevention of disease, and an in-depth study of minerals can be beneficial in the prevention of cancer.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: requires PLCO approval. Requests to access these datasets should be directed to https://cdas.cancer.gov/plco/.

Ethics statement

The studies involving humans were approved by the Institutional Review Board of the National Cancer Institute (NCI) in the United States. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SL: Data curation, Formal analysis, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. QR: Methodology, Writing – review & editing, Data curation, Formal analysis, Project administration, Validation. ZS: Writing – review & editing. BL: Writing – review & editing. DW: Writing – review & editing. YS: Writing – review & editing, Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Investigation, Software. HW: Writing – review & editing, Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Funding acquisition, Investigation, Software.

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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.

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References

1. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol*. (2021) 14:101174. doi: 10.1016/j.tranon.2021.101174

2. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet*. (2019) 394:1467–80. doi: 10.1016/S0140-6736(19)32319-0

3. Ait Ouakrim D, Pizot C, Boniol M, Malvezzi M, Boniol M, Negri E, et al. Trends in colorectal cancer mortality in Europe: retrospective analysis of the WHO mortality database. *BMJ*. (2015) 351:h4970. doi: 10.1136/bmj.h4970

4. Kasi PM, Shahjehan F, Cochuyt JJ, Li Z, Colibaseanu DT, Merchea A. Rising proportion of young individuals with rectal and Colon Cancer. *Clin Colorectal Cancer*. (2019) 18:e87–95. doi: 10.1016/j.clcc.2018.10.002

5. O'Sullivan DE, Sutherland RL, Town S, Chow K, Fan J, Forbes N, et al. Risk factors for early-onset colorectal cancer: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. (2022) 20:1229–1240.e5. doi: 10.1016/j.cgh.2021.01.037

6. Hampel H, Kalady MF, Pearlman R, Stanich PP. Hereditary colorectal Cancer. *Hematol Oncol Clin North Am*. (2022) 36:429–47. doi: 10.1016/j.hoc.2022.02.002

7. Zhou X, Wang L, Xiao J, Sun J, Yu L, Zhang H, et al. Alcohol consumption, DNA methylation and colorectal cancer risk: results from pooled cohort studies and Mendelian randomization analysis. *Int J Cancer*. (2022) 151:83–94. doi: 10.1002/ijc.33945

8. Juloski JT, Rakic A, Ćuk VV, Ćuk VM, Stefanović S, Nikolić D, et al. Colorectal cancer and trace elements alteration. *J Trace Elem Med Biol*. (2020) 59:126451. doi: 10.1016/j.jtemb.2020.126451

9. Vinceti M, Filippini T, Del Giovane C, Dennert G, Zwahlen M, Brinkman M, et al. Selenium for preventing cancer. *Cochrane Database Syst Rev*. (2018) 1:Cd005195. doi: 10.1002/14651858.CD005195.pub4

10. Salnikow K. Role of iron in cancer. *Semin Cancer Biol*. (2021) 76:189–94. doi: 10.1016/j.semcancer.2021.04.001

11. Razzaque MS. Magnesium: are we consuming enough? *Nutrients*. (2018) 10:21863. doi: 10.3390/nu10121863

12. Morales M, Xue X. Targeting iron metabolism in cancer therapy. *Theranostics*. (2021) 11:8412–29. doi: 10.7150/thno.59092

13. Skrajnowska D, Bobrowska-Korczak B. Role of zinc in immune system and anti-cancer defense mechanisms. *Nutrients*. (2019) 11:2273. doi: 10.3390/nu11102273

14. Gröber U, Schmidt J, Kisters K. Magnesium in prevention and therapy. *Nutrients*. (2015) 7:8199–226. doi: 10.3390/nu7095388

15. Yang K, Han W, Jiang X, Piffko A, Bugno J, Han C, et al. Zinc cyclic di-AMP nanoparticles target and suppress tumours via endothelial STING activation and tumour-associated macrophage reinvigoration. *Nat Nanotechnol*. (2022) 17:1322–31. doi: 10.1038/s41565-022-01225-x

16. To PK, Do MH, Cho JH, Jung C. Growth modulatory role of zinc in prostate Cancer and application to Cancer therapeutics. *Int J Mol Sci*. (2020) 21:82991. doi: 10.3390/ijms21082991

17. Franklin RB, Costello LC. Zinc as an anti-tumor agent in prostate cancer and in other cancers. *Arch Biochem Biophys*. (2007) 463:211–7. doi: 10.1016/j.abb.2007.02.033

18. Petraglia F, Dolmans MM. Iron deficiency anemia: impact on women's reproductive health. *Fertil Steril*. (2022) 118:605–6. doi: 10.1016/j.fertnstert.2022.08.850

19. Kumar SB, Arnipalli SR, Mehta P, Carrau S, Ziouzenkova O. Iron deficiency Anemia: efficacy and limitations of nutritional and comprehensive mitigation strategies. *Nutrients*. (2022) 14:142976. doi: 10.3390/nu14142976

20. Kuang Y, Wang Q. Iron and lung cancer. *Cancer Lett*. (2019) 464:56–61. doi: 10.1016/j.canlet.2019.08.007

21. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer*. (2013) 13:342–55. doi: 10.1038/nrc3495

22. Bost M, Houdart S, Oberli M, Kalonji E, Huneau JF, Margaritis I. Dietary copper and human health: current evidence and unresolved issues. *J Trace Elem Med Biol*. (2016) 35:107–15. doi: 10.1016/j.jtemb.2016.02.006

23. Kucharzewski M, Braziewicz J, Majewska U, Gózdz S. Selenium, copper, and zinc concentrations in intestinal cancer tissue and in colon and rectum polyps. *Biol Trace Elem Res*. (2003) 92:1–10. doi: 10.1385/BTER:92:1:1

24. Jiang Y, Huo Z, Qi X, Zuo T, Wu Z. Copper-induced tumor cell death mechanisms and antitumor theragnostic applications of copper complexes. *Nanomedicine*. (2022) 17:303–24. doi: 10.2217/nnm-2021-0374

25. Bian C, Zheng Z, Su J, Chang S, Yu H, Bao J, et al. Copper homeostasis and cuproptosis in tumor pathogenesis and therapeutic strategies. *Front Pharmacol*. (2023) 14:1271613. doi: 10.3389/fphar.2023.1271613

26. Rayman MP. Selenium and human health. *Lancet*. (2012) 379:1256–68. doi: 10.1016/S0140-6736(11)61452-9

27. Kadkol S, Diamond AM. The interaction between dietary selenium intake and genetics in determining cancer risk and outcome. *Nutrients*. (2020) 12:82424. doi: 10.3390/nu12082424

28. Chen GC, Pang Z, Liu QF. Magnesium intake and risk of colorectal cancer: a meta-analysis of prospective studies. *Eur J Clin Nutr*. (2012) 66:1182–6. doi: 10.1038/ejcn.2012.135

29. Meng Y, Sun J, Yu J, Wang C, Su J. Dietary intakes of calcium, iron, magnesium, and potassium elements and the risk of colorectal cancer: a meta-analysis. *Biol Trace Elem Res*. (2019) 189:325–35. doi: 10.1007/s12011-018-1474-z

30. Muka T, Kraja B, Ruiter R, Lahousse L, de Keyser CE, Hofman A, et al. Dietary mineral intake and lung cancer risk: the Rotterdam study. *Eur J Nutr*. (2017) 56:1637–46. doi: 10.1007/s00394-016-1210-4

31. Gao W, Huang Z, Duan J, Nice EC, Lin J, Huang C. Elesclomol induces copper-dependent ferroptosis in colorectal cancer cells via degradation of ATP7A. *Mol Oncol*. (2021) 15:3527–44. doi: 10.1002/1878-0261.13079

32. Clark LC, Combs GF, Turnbull BW, Slate EH, Chalker DK, Chow J, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional prevention of cancer study group. *JAMA*. (1996) 276:1957–63. doi: 10.1001/jama.1996.03540240035027

33. Grubb RL, Pinsky PF, Greenlee RT, Izmirlian G, Miller AB, Hickey TP, et al. Prostate cancer screening in the prostate, lung, colorectal and ovarian cancer screening trial: update on findings from the initial four rounds of screening in a randomized trial. *BJU Int*. (2008) 102:1524–30. doi: 10.1111/j.1464-410X.2008.08214.x

34. Buys SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, et al. Effect of screening on ovarian cancer mortality: the prostate, lung, colorectal and ovarian (PLCO) cancer screening randomized controlled trial. *JAMA*. (2011) 305:2295–303. doi: 10.1001/jama.2011.766

35. Ahn J, Albanes D, Peters U, Schatzkin A, Lim U, Freedman M, et al. Dairy products, calcium intake, and risk of prostate cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev*. (2007) 16:2623–30. doi: 10.1158/1055-9965.EPI-07-0601

36. University of Minnesota Nutrition Co-ordinating Center NDSR descriptive overview. Available from: http://www.ncc.umn.edu/ [accessed 30 November 2016].

37. van den Brandt PA, Smits KM, Goldbohm RA, Weijenberg MP. Magnesium intake and colorectal cancer risk in the Netherlands cohort study. *Br J Cancer*. (2007) 96:510–3. doi: 10.1038/sj.bjc.6603577

38. Ma E, Sasazuki S, Inoue M, Iwasaki M, Sawada N, Takachi R, et al. High dietary intake of magnesium may decrease risk of colorectal cancer in Japanese men. *J Nutr*. (2010) 140:779–85. doi: 10.3945/jn.109.117747

39. Larsson SC, Bergkvist L, Wolk A. Magnesium intake in relation to risk of colorectal cancer in women. *JAMA*. (2005) 293:86–9. doi: 10.1001/jama.293.1.86

40. Lee DH, Anderson KE, Harnack LJ, Folsom AR, Jacobs DR. Heme iron, zinc, alcohol consumption, and colon cancer: Iowa Women's health study. *J Natl Cancer Inst*. (2004) 96:403–7. doi: 10.1093/jnci/djh047

41. Larsson SC, Adami HO, Giovannucci E, Wolk A. Re: Heme iron, zinc, alcohol consumption, and risk of colon cancer. *J Natl Cancer Inst*. (2005) 97:232–3. doi: 10.1093/jnci/dji032

42. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the selenium and vitamin E cancer prevention trial (SELECT). *JAMA*. (2009) 301:39–51. doi: 10.1001/jama.2008.864

43. Ruder EH, Berndt SI, Gilsing AM, Graubard BI, Burdett L, Hayes RB, et al. Dietary iron, iron homeostatic gene polymorphisms and the risk of advanced colorectal adenoma and cancer. *Carcinogenesis*. (2014) 35:1276–83. doi: 10.1093/carcin/bgu028

44. Sun Z, Zhu Y, Wang PP, Roebothan B, Zhao J, Zhao J, et al. Reported intake of selected micronutrients and risk of colorectal cancer: results from a large population-based case-control study in Newfoundland, Labrador and Ontario, Canada. *Anticancer Res*. (2012) 32:687–96. doi: 10.1186/1475-2891-11-18

45. Levi F, Pasche C, Lucchini F, La Vecchia C. Selected micronutrients and colorectal cancer. A case-control study from the canton of Vaud, Switzerland. *Eur J Cancer*. (2000) 36:2115–9. doi: 10.1016/S0959-8049(00)00195-7

46. van Lee L, Heyworth J, McNaughton S, Iacopetta B, Clayforth C, Fritschi L. Selected dietary micronutrients and the risk of right-and left-sided colorectal cancers: a case-control study in Western Australia. *Ann Epidemiol*. (2011) 21:170–7. doi: 10.1016/j.annepidem.2010.10.005

47. Aglago EK, Cross AJ, Riboli E, Fedirko V, Hughes DJ, Fournier A, et al. Dietary intake of total, heme and non-heme iron and the risk of colorectal cancer in a European prospective cohort study. *Br J Cancer*. (2023) 128:1529–40. doi: 10.1038/s41416-023-02164-7

48. Mahabir S, Spitz MR, Barrera SL, Beaver SH, Etzel C, Forman MR. Dietary zinc, copper and selenium, and risk of lung cancer. *Int J Cancer*. (2007) 120:1108–15. doi: 10.1002/ijc.22451

49. Ma X, Yang Y, Li HL, Zheng W, Gao J, Zhang W, et al. Dietary trace element intake and liver cancer risk: results from two population-based cohorts in China. *Int J Cancer*. (2017) 140:1050–9. doi: 10.1002/ijc.30522

50. Senesse P, Meance S, Cottet V, Faivre J, Boutron-Ruault MC. High dietary iron and copper and risk of colorectal cancer: a case-control study in Burgundy. *France Nutr Cancer*. (2004) 49:66–71. doi: 10.1207/s15327914nc4901_9

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The role of micronutrients and serum metabolites in intervertebral disk degeneration: insights from a Mendelian randomization study and mediation analysis

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Background: Intervertebral disk degeneration (IVDD) is a complex degenerative skeletal condition, potentially influenced by micronutrients and serum metabolites in its etiology. However, the exact causal relationship between these factors and IVDD remains ambiguous.

Methods: The research employed a Two-Sample Mendelian Randomization (2SMR) analysis to thoroughly evaluate the causal relationship between 15 micronutrients (consisting of 7 minerals and 8 vitamins) as exposure variables, 1,091 blood metabolites, and 309 metabolite ratios as intermediary factors, and IVDD as the outcome. Additionally, reverse MR analysis and mediation analysis were carried out to validate the reliability of the results and explore the underlying mechanism by which micronutrients influence the risk of IVDD by regulating metabolites.

Results: Among the micronutrients examined, vitamin B12 exhibited a noteworthy negative correlation with the incidence of IVDD (OR: 0.752, 95% [CI]: 0.573–0.987, $p = 0.040$), indicating a potential reduction in IVDD risk with increased vitamin B12 consumption. Of the 1,091 blood metabolites and 309 metabolite ratios analyzed, 52 metabolites displayed significant associations with IVDD, primarily linked to amino acid, fatty acid, nucleotide, and sphingolipid metabolic pathways. Mediation analysis identified 4-acetaminophen sulfate as a potential mediator in the protective effect of vitamin B12 against IVDD.

Conclusion: This study has shown that vitamin B12 may reduce the risk of IVDD and has identified 52 serum metabolites that are associated with IVDD. Furthermore, it proposes that 4-acetaminophen sulfate could serve as a potential mechanism by which vitamin B12 exerts its inhibitory effects on IVDD.

KEYWORDS

intervertebral disk degeneration, Mendelian randomization, mediated Mendelian randomization, micronutrients, serum metabolites, vitamin B12

1 Introduction

Intervertebral disk degeneration (IVDD) is a notable factor in the morbidity of the elderly population, significantly impacting quality of life through the presence of chronic back pain and limitations in mobility. This condition is distinguished by a progression of degenerative alterations in the structure of the disk, encompassing inflammatory mechanisms, apoptosis of disk cells, and degradation of the extracellular matrix (1). These pathological changes result in desiccation of the nucleus pulposus, fissures in the annulus fibrosus, and diminished disk height (2). Various treatment options exist, including conservative management and surgical intervention, but there is a noticeable absence of effective early prevention methods. Recent epidemiological findings indicate a possible connection between micronutrient imbalances, metabolic disturbances, and the development of skeletal disorders like IVDD (3, 4). This highlights the necessity of exploring the impact of specific micronutrients and serum metabolites on the pathogenesis of disk degeneration (5), potentially leading to innovative nutritional interventions and preventive measures.

Recent studies have shown a growing interest in exploring the correlation between micronutrient consumption and IVDD. Research has indicated a significant association between specific micronutrients, such as vitamins D, C, K, and zinc, and susceptibility to IVDD. For instance, a lack of 1,25-dihydroxyvitamin D (1,25(OH)2D) has been found to hasten the progression of IVDD by impeding the synthesis of extracellular matrix proteins and facilitating their breakdown (6). Furthermore, vitamin K2 seems to regulate inflammatory responses in IVDD by controlling the expression of Socs3 and Hmox1 genes (7). Zinc ions have been implicated in the regulation of matrix metalloproteinase activity in intervertebral disk cells, indicating that alterations in intracellular zinc levels may have a significant impact on the development of IVDD (8). Additionally, the FokI polymorphism within the vitamin D receptor (VDR) gene has been shown to modulate the response of intervertebral disk cells to vitamin D; specifically, cells with the FF genotype exhibit increased synthesis of matrix proteins and reduced expression of degrading enzymes in response to vitamin D, in contrast to those with the Ff genotype (9). This discovery offers novel insights into the potential advantages of vitamin D supplementation for individuals with IVDD. Mechanical overload is acknowledged as a risk factor for IVDD. Recent research indicates that selenium supplementation may mitigate ferroptosis in disk cells caused by mechanical stress by activating the Se-GPX4 and Se-SelK pathways, thus preserving extracellular matrix integrity (10). However, research on the association between other micronutrients and IVDD remains limited. Recent studies have also focused on the role of serum metabolites in IVDD. Notable fluctuations in metabolite concentrations have been observed in individuals with IVDD, suggesting a metabolic component in the disease's pathogenesis. For instance, a study conducted by Wang et al. (4) demonstrated that a high-fat diet not only improved symptoms of IVDD in rats but also positively altered their serum metabolite profiles, underscoring the potential impact of metabolic alterations on IVDD. Moreover, Ji et al. (11) performed a Mendelian randomization (MR) analysis on a cohort of 157 Nordic individuals, revealing robust correlations between 13 serum metabolites, specifically within lipid and amino acid metabolic pathways, and IVDD. Despite these findings, further investigation into the relationship between serum metabolites and IVDD is in its nascent stages, highlighting a notable deficiency in thorough, prospective metabolomic research.

TABLE 1 The detailed information on the GWAS of micronutrients.

Micronutrients	Sample size	Population	GWAS ID
Copper	2,603	European	ieu-a-1073
Calcium	64,979	European	ukb-b-8951
Carotene	64,979	European	ukb-b-16202
Folate	64,979	European	ukb-b-11349
Iron	64,979	European	ukb-b-20447
Magnesium	64,979	European	ukb-b-7372
Potassium	64,979	European	ukb-b-17881
Selenium	2,603	European	ieu-a-1077
Zinc	2,603	European	ieu-a-1079
Vitamin A	460,351	European	ukb-b-9596
Vitamin B12	64,979	European	ukb-b-19524
Vitamin B6	64,979	European	ukb-b-7864
Vitamin C	64,979	European	ukb-b-19390
Vitamin D	64,979	European	ukb-b-18593
Vitamin E	64,979	European	ukb-b-6888

MR analysis utilizes genetic variations as instrumental variables to mimic the structure of conventional randomized controlled trials, thereby mitigating the impact of confounding variables (12). The increased prevalence of genome-wide association studies (GWAS) and the larger sample sizes in GWAS meta-analyses have bolstered the credibility of MR analysis in pinpointing risk factors associated with intricate diseases. Furthermore, the emergence of high-quality genetic instrumental variables derived from GWAS summary data on micronutrients and metabolites has broadened the applicability of MR analysis (13). Furthermore, mediation analysis offers a rigorous quantitative technique for assessing the impact of mediators on the causal pathways linking exposure variables and outcomes, grounded in principles of causal inference. This methodology is essential for elucidating the underlying mechanisms of causal associations (14). Consequently, the present study will employ a two-sample Mendelian randomization (2SMR) approach, leveraging recent GWAS data on micronutrients, serum metabolites, and IVDD. The aim is to examine the causal relationships between micronutrients and IVDD, as well as to investigate the potential mediating effects of metabolites through MR mediation analysis.

2 Methods

2.1 Data sources

This research examined summary data for 15 micronutrients, consisting of seven minerals and eight vitamins, obtained from the GWAS database[1] as outlined in Table 1. Furthermore, we integrated genome-wide association findings for 1,091 blood metabolites and 309 metabolite ratios as reported by Zhao et al. (13). In order to validate the

1 https://gwas.mrcieu.ac.uk

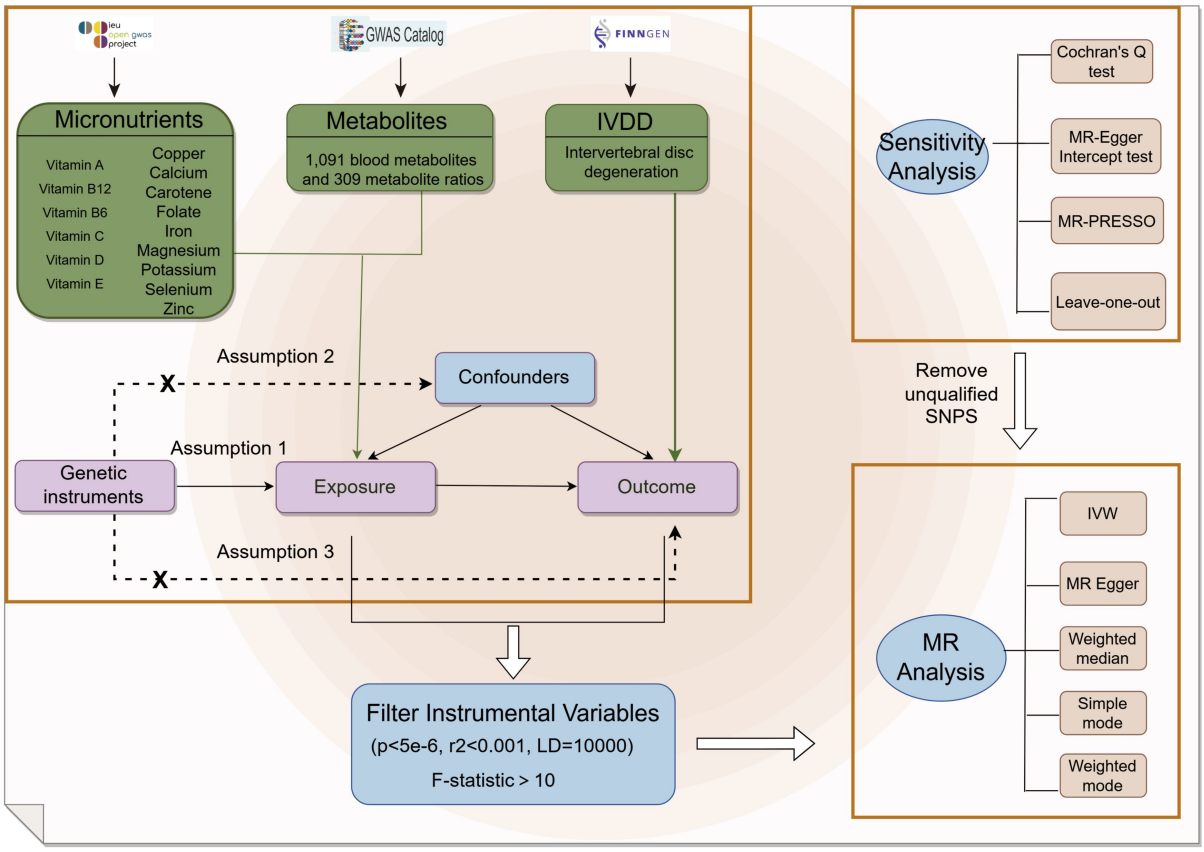


FIGURE 1
Flow chart of the study. Mendelian randomization study rationale: assumption 1, genetic instruments are associated with exposure; assumption 2, genetic instruments are not associated with confounders; assumption 3, genetic instruments are not associated with outcome, and genetic instruments act on outcome only through exposure.

genetic instrumental variables, stringent selection criteria were employed. Only loci meeting the following criteria were included: (1) demonstrating genome-wide significance ($p < 5 \times 10^{-6}$) in GWAS; (2) exhibiting low linkage disequilibrium (LD) with an $r^2 < 0.001$, and spatially separated by more than 10,000 kb; and (3) possessing an F-statistic exceeding 10, indicating strong instrument strength, calculated as $F = R^2(n-2)/(1-R^2)$, where n represents the effective sample size for single nucleotide polymorphisms (SNPs) association analysis in GWAS and R^2 represents the explained phenotypic variance by the instrumental variable (15). The GWAS summary data for IVDD, identified as "finn-b-M13_INTERVERTEB," were acquired from the FinnGen consortium,[2] which included 20,001 cases and 164,682 controls (Figure 1).

2.2 MR analysis

In this research, we utilized the 2SMR method with summary-level data from GWAS to investigate potential causal associations between 15 micronutrients and IVDD (16). Our selection of SNPs as genetic instrumental variables for each micronutrient was based on rigorous criteria, with these SNPs acting as instrumental variables for the exposure and IVDD serving as the outcome variable. Univariate 2SMR analyses were performed for each micronutrient using the "TwoSampleMR" package (17) in R (version 4.1.3), with a primary focus on employing the Inverse Variance Weighted (IVW) method to estimate causal effects. The robustness of our results was additionally confirmed through various sensitivity analyses, such as MR-Egger regression, the weighted median method, simple mode, and weighted mode approaches, as well as Cochrane's Q test to evaluate heterogeneity among the instrumental variables for each micronutrient (18–20). Further sensitivity analyses were performed to assess the potential impact of horizontal pleiotropy, utilizing the MR-Egger intercept test, the Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) global test (21), and Leave-One-Out Analysis. These assessments were designed to identify and correct for any pleiotropic influences that may introduce bias into the causal inferences. All statistical tests were carried out using a significance threshold of $p < 0.05$ to ensure the reliability of our results.

2.3 Reverse MR analysis

In order to explore the potential mediating effect of serum metabolites on the relationship between trace elements and IVDD,

2 https://www.finngen.fi/en

we performed a reverse MR analysis on trace elements that did not show a reverse causal relationship with IVDD in an initial MR evaluation of 15 trace elements. This analysis was conducted to eliminate the possibility of reverse causality between trace elements and IVDD. We employed the IVW method as our principal statistical technique, supplemented by various sensitivity analyses such as MR-Egger regression, the weighted median method, simple mode, and weighted mode methods to thoroughly evaluate the robustness of the reverse causal relationships. Within the framework of reverse MR, IVDD was considered as the exposure variable, while the concentrations of trace elements were regarded as the outcome variable. Through the integration of results from various methodologies, we pinpointed trace elements that did not exhibit substantial reverse causal relationships with IVDD. This specific selection allowed for a more in-depth examination of the mediating influences of serum metabolites in subsequent mediation MR analyses. A significance threshold of $p < 0.05$ was consistently upheld for all statistical analyses.

2.4 Mediation analysis

In order to investigate potential serum metabolite mediators in the confirmed positive correlations between trace elements and IVDD, a two-step mediation analysis approach was employed. Initially, univariable 2SMR analysis was carried out utilizing the "TwoSampleMR" package. This analysis employed screened serum metabolite SNPs as exposures and IVDD as the outcome. The IVW method was selected as the primary method to estimate causal effects, and was supported by sensitivity analyses, including MR-Egger regression, the weighted median method, simple mode, and weighted mode methods. Additionally, heterogeneity among genetic instrumental variables was assessed using Cochrane's Q test. The influence of horizontal pleiotropy and outliers was evaluated using the MR-Egger intercept test, MR-PRESSO global test, and leave-one-out analysis to ensure the robustness and validity of our findings.

In the second phase of our analysis, serum metabolites demonstrating significant causal effects from the initial analysis were designated as outcome variables, while trace elements exhibiting a unidirectional causal relationship with IVDD risk were used as exposure variables. We conducted a univariable 2SMR analysis using the IVW method to identify potential mediators. For estimating the effect size of each mediator, we employed the product of coefficients method (22). This approach first calculates the effect value beta1 of trace elements on mediators, then determines the adjusted effect value beta 2 of mediators on IVDD. The product of beta1 and beta2 yields the indirect effect, representing the influence of trace elements on IVDD through mediators (23). To uphold a fundamental assumption of mediation MR analysis—that the genetic instrumental variables for estimating beta1 and beta2 should be independent—we excluded SNPs significantly influenced by trace elements in the beta2 estimation to prevent redundancy. The proportion of the total effect of trace elements on IVDD accounted for by each mediator is quantified by dividing the indirect effect by the total effect. Standard errors were calculated using the delta method, drawing on the effect estimates from the 2SMR analysis.

3 Results

3.1 MR analysis of micronutrients and IVDD

A 2SMR analysis was utilized to examine the association between micronutrients and IVDD, with 15 micronutrients being categorized into two groups consisting of 7 minerals and 8 vitamins (Figure 2A). The findings indicated that, of all the micronutrients examined, only vitamin B12 exhibited a statistically significant negative correlation with the likelihood of developing IVDD (OR: 0.752, 95%CI: 0.573–0.987, $p = 0.040$). These results suggest that augmenting one's intake of vitamin B12 may potentially decrease the risk of IVDD (Figures 2B,C). The Cochrane's Q test results ($Q = 5.710$, $p = 0.573$) revealed no significant heterogeneity among the instrumental variables used in the analysis. Additionally, the MR-Egger intercept test (egger_intercept = 0.0043, $p = 0.773$) and MR-PRESSO ($p = 0.598$) detected no horizontal pleiotropy, affirming the reliability of the MR findings in this study.

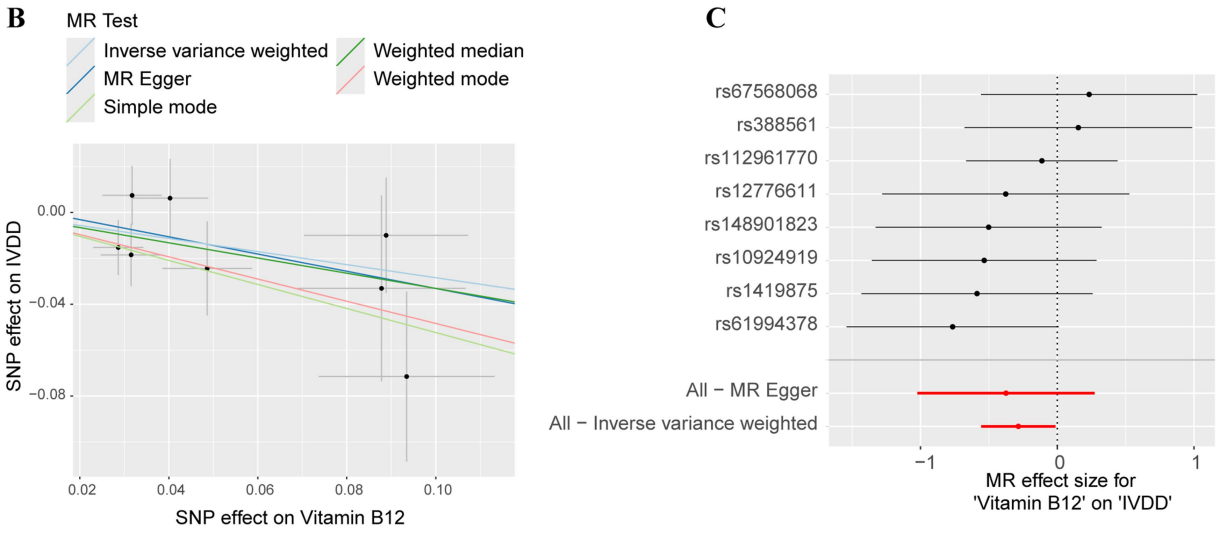
In order to address the potential for reverse causation between vitamin B12 and IVDD, we conducted a reverse MR analysis. Our analysis demonstrated that when considering IVDD as the exposure and vitamin B12 as the outcome, there was no significant causal relationship (Odds Ratio: 0.997, 95% Confidence Interval: 0.971–1.024, $p = 0.877$) (Figure 2D). These findings substantiate a unidirectional causal relationship from vitamin B12 to IVDD, unaffected by reverse causality, thus providing a robust basis for further mediation analysis.

3.2 MR analysis of serum metabolites and IVDD

To elucidate the causal relationship between blood metabolites and IVDD, we systematically analyzed 1,091 blood metabolites and 309 metabolite ratios employing a 2SMR approach. Prior to the analysis, genetic loci with linkage disequilibrium and weak instrumental variables were excluded to preserve the integrity of the study. Each metabolite then underwent univariable two-sample MR analysis, followed by tests for pleiotropy to identify metabolites with robust associations. Finally, we identified 52 serum metabolites significantly associated with IVDD (Figure 3). These include seven amino acid derivatives (S-α-amino-ω-caprolactam, 2-hydroxy-3-methylvalerate, alpha-hydroxyisocaproate, butyrylglycine, N-acetyl-L-glutamine, N-delta-acetylornithine, vanillic acid glycine); 14 fatty acid derivatives (1-(1-enyl-palmitoyl)-2-linoleoyl-GPE [p-16:0/18:2], 1-(1-enyl-stearoyl)-GPE [p-18:0], 1-palmitoleoyl-GPC [16:1], 1-palmitoyl-2-arachidonoyl-GPC [16:0/20:4n6], 1-stearoyl-2-arachidonoyl-GPC [18:0/20:4], 1-stearoyl-2-linoleoyl-GPI [18:0/18:2], 2-hydroxydecanoate, 3-hydroxyhexanoate, 3β-hydroxy-5-cholenoic acid, 5-dodecenoate [12,1n7], ceramide [d18:1/17:0, d17:1/18:0], glycosyl-N-behenoyl-sphingadienine [d18:2/22:0], octadecenedioate [C18:1-DC], taurodeoxycholate); five nucleotide derivatives (5-methylthioadenosine [MTA], 5,6-dihydrothymine, N6-carbamoylthreonyladenosine, N2-acetyl, N6,N6-dimethyllysine, N6,N6-dimethyllysine); four sphingolipids (sphingomyelin [d18:1/20:2, d18:2/20:1, d16:1/22:2], sphingomyelin [d18:1/21:0, d17:1/22:0, d16:1/23:0], sphingomyelin [d18:2/23:1],

A

exposure	nsnp	method	pval	OR(95% CI)	outcome
Calcium	19	Inverse variance weighted	0.539	1.066 (0.869 to 1.308)	IVDD
Carotene	15	Inverse variance weighted	0.146	1.198 (0.939 to 1.527)	IVDD
Copper	6	Inverse variance weighted	0.326	1.016 (0.984 to 1.049)	IVDD
Folate	12	Inverse variance weighted	0.753	1.042 (0.805 to 1.350)	IVDD
Iron	11	Inverse variance weighted	0.812	0.958 (0.671 to 1.366)	IVDD
Magnesium	17	Inverse variance weighted	0.644	1.071 (0.801 to 1.431)	IVDD
Potassium	13	Inverse variance weighted	0.621	1.101 (0.753 to 1.609)	IVDD
Selenium	6	Inverse variance weighted	0.580	0.984 (0.931 to 1.041)	IVDD
Vitamin A	12	Inverse variance weighted	0.429	0.145 (0.001 to 17.228)	IVDD
Vitamin B12	**8**	**Inverse variance weighted**	**0.040**	**0.752 (0.573 to 0.987)**	**IVDD**
Vitamin B6	16	Inverse variance weighted	0.306	0.884 (0.697 to 1.120)	IVDD
Vitamin C	10	Inverse variance weighted	0.616	0.916 (0.650 to 1.290)	IVDD
Vitamin D	13	Inverse variance weighted	0.714	1.047 (0.819 to 1.338)	IVDD
Vitamin E	11	Inverse variance weighted	0.348	1.109 (0.894 to 1.375)	IVDD
Zinc	8	Inverse variance weighted	0.090	1.059 (0.991 to 1.132)	IVDD



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exposure	nsnp	method	pval	OR(95% CI)	outcome
IVDD	44	MR Egger	0.664	1.009 (0.968 to 1.052)	Vitamin B12
	44	Weighted median	0.816	1.005 (0.967 to 1.043)	Vitamin B12
	44	Inverse variance weighted	0.877	0.998 (0.972 to 1.025)	Vitamin B12
	44	Simple mode	0.492	1.025 (0.956 to 1.098)	Vitamin B12
	44	Weighted mode	0.579	1.010 (0.975 to 1.047)	Vitamin B12

FIGURE 2
Mendelian randomization (MR) and reverse MR analyses of 15 micronutrients and IVDD. **(A)** Forest plot of the MR analyses between 15 micronutrients and IVDD. **(B)** Scatter plot of the MR analysis between vitamin B12 and IVDD. **(C)** Forest plot of the MR analysis between vitamin B12 and IVDD. **(D)** Forest plot of the reverse MR analyses between 15 micronutrients and IVDD.

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exposure	nsnp	method	pval	OR(95% CI)	outcome
(S)-a-amino-omega-caprolactam levels	13	IVW	**0.003**	1.084 (1.029 to 1.142)	IVDD
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (p-16:0/18:2) levels	22	IVW	**0.042**	1.063 (1.002 to 1.127)	IVDD
1-(1-enyl-stearoyl)-GPE (p-18:0) levels	24	IVW	**0.018**	0.926 (0.869 to 0.987)	IVDD
1-palmitoleoyl-GPC (16:1) levels	32	IVW	**0.020**	0.929 (0.872 to 0.988)	IVDD
1-palmitoyl-2-arachidonoyl-gpc (16:0/20:4n6) levels	31	IVW	**0.048**	0.970 (0.942 to 1.000)	IVDD
1-stearoyl-2-arachidonoyl-gpc (18:0/20:4) levels	36	IVW	**0.045**	0.976 (0.953 to 0.999)	IVDD
1-stearoyl-2-linoleoyl-GPI (18:0/18:2) levels	28	IVW	**0.046**	0.943 (0.890 to 0.999)	IVDD
2-hydroxy-3-methylvalerate levels	30	IVW	**0.021**	1.068 (1.010 to 1.129)	IVDD
2-hydroxydecanoate levels	19	IVW	**0.021**	1.092 (1.013 to 1.178)	IVDD
2,3-dihydroxy-2-methylbutyrate levels	15	IVW	**0.004**	0.871 (0.792 to 0.957)	IVDD
3-hydroxyhexanoate levels	19	IVW	**0.009**	1.105 (1.025 to 1.191)	IVDD
3,5-dichloro-2,6-dihydroxybenzoic acid levels	21	IVW	**0.041**	1.066 (1.002 to 1.135)	IVDD
3b-hydroxy-5-cholenoic acid levels	30	IVW	**0.004**	0.925 (0.878 to 0.975)	IVDD
4-acetaminophen sulfate levels	25	IVW	**0.038**	0.953 (0.910 to 0.997)	IVDD
5-dodecenoate (12:1n7) levels	15	IVW	**0.015**	0.893 (0.815 to 0.978)	IVDD
5-methylthioadenosine (mta) levels	27	IVW	**0.030**	1.069 (1.006 to 1.136)	IVDD
5,6-dihydrothymine levels	19	IVW	**0.010**	0.909 (0.846 to 0.977)	IVDD
5alpha-androstan-3beta,17alpha-diol disulfate levels	23	IVW	**0.006**	1.072 (1.020 to 1.126)	IVDD
Alpha-hydroxyisocaproate levels	22	IVW	0.050	1.072 (1.000 to 1.149)	IVDD
Butyrylglycine levels	22	IVW	**<0.001**	1.063 (1.029 to 1.099)	IVDD
Ceramide (d18:1/17:0, d17:1/18:0) levels	23	IVW	**0.025**	1.060 (1.007 to 1.115)	IVDD
Cortisol levels (plasma)	18	IVW	**0.023**	1.086 (1.012 to 1.166)	IVDD
Glycosyl-N-behenoyl-sphingadienine (d18:2/22:0) levels	27	IVW	**0.035**	0.944 (0.894 to 0.996)	IVDD
Hypotaurine levels	30	IVW	**0.018**	0.936 (0.887 to 0.989)	IVDD
Isovalerylcarnitine (C5) levels	24	IVW	**0.030**	1.067 (1.006 to 1.131)	IVDD
Mannonate levels	22	IVW	**0.021**	0.966 (0.937 to 0.995)	IVDD
N-acetyl-L-glutamine levels	23	IVW	**0.001**	0.950 (0.920 to 0.981)	IVDD
N-delta-acetylornithine levels	25	IVW	**<0.001**	1.059 (1.026 to 1.093)	IVDD
N2-acetyl,N6,N6-dimethyllysine levels	24	IVW	**0.032**	0.975 (0.953 to 0.998)	IVDD
N6-acetyllysine levels	19	IVW	**0.011**	0.933 (0.885 to 0.984)	IVDD
N6-carbamoylthreonyladenosine levels	32	IVW	**0.036**	1.065 (1.004 to 1.129)	IVDD
N6-methyllysine levels	32	IVW	**0.012**	0.959 (0.928 to 0.991)	IVDD
N6,N6-dimethyllysine levels	26	IVW	**0.018**	0.954 (0.917 to 0.992)	IVDD
Octadecenedioate (C18:1-DC) levels	22	IVW	**0.002**	0.925 (0.881 to 0.971)	IVDD
Sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2) levels	25	IVW	**0.004**	1.078 (1.024 to 1.135)	IVDD
Sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0) levels	19	IVW	**0.048**	1.059 (1.001 to 1.122)	IVDD
Sphingomyelin (d18:2/23:1) levels	25	IVW	**<0.001**	1.110 (1.047 to 1.176)	IVDD
Taurodeoxycholate levels	22	IVW	**0.004**	1.102 (1.032 to 1.177)	IVDD
Vanillic acid glycine levels	23	IVW	**0.018**	1.057 (1.010 to 1.107)	IVDD
X-12112 levels	37	IVW	**0.007**	0.955 (0.924 to 0.988)	IVDD
X-12731 levels	28	IVW	**0.046**	0.942 (0.889 to 0.999)	IVDD
X-12812 levels	18	IVW	0.050	1.060 (1.000 to 1.124)	IVDD
X-13431 levels	28	IVW	**0.002**	1.050 (1.018 to 1.084)	IVDD
X-13553 levels	24	IVW	**0.015**	1.075 (1.014 to 1.140)	IVDD
X-17653 levels	24	IVW	**0.031**	0.949 (0.905 to 0.995)	IVDD
X-17676 levels	30	IVW	**0.024**	0.948 (0.905 to 0.993)	IVDD
X-18888 levels	26	IVW	**0.027**	0.939 (0.888 to 0.993)	IVDD
X-18901 levels	29	IVW	**0.007**	1.078 (1.021 to 1.138)	IVDD
X-21283 levels	17	IVW	**0.046**	0.969 (0.940 to 0.999)	IVDD
X-21742 levels	20	IVW	**0.038**	1.066 (1.004 to 1.133)	IVDD
X-23641 levels	28	IVW	**0.045**	1.042 (1.001 to 1.085)	IVDD
X-25217 levels	14	IVW	**0.035**	1.065 (1.004 to 1.128)	IVDD

FIGURE 3
Forest plot illustrating the positive results from the MR analyses between 1,091 blood metabolites, 309 metabolite ratios, and IVDD.

isovalerylcarnitine [C5]); seven other known metabolites (5α-androstan-3β,17α-diol disulfate, cortisol, 2,3-dihydroxy-2-methylbutyrate, 3,5-dichloro-2,6-dihydroxybenzoic acid, 4-acetaminophen sulfate, hypotaurine, mannonate); and 15 unknown metabolites. These findings provide valuable insights into the metabolic pathways potentially contributing to IVDD.

3.3 Mediation analysis of 52 serum metabolites in the causal relationship between vitamin B12 and IVDD

In order to explore the potential mediating effect of serum metabolites on the causal association between vitamin B12 and IVDD, we conducted a systematic mediation analysis using a two-step methodology. Initially, 2SMR analyses were performed with vitamin B12 as the exposure and 52 IVDD-associated serum metabolites as outcomes to estimate the effect size (beta1). Among these metabolites, only 4-acetaminophen sulfate demonstrated a significant causal relationship with vitamin B12 (OR: 2.121, 95% CI: 1.237–3.636, $p = 0.006$, beta1 = 0.752) (Figure 4A). The results of the Cochrane's Q test ($Q = 3.609$, $p = 0.890$) indicated an absence of significant heterogeneity across the instrumental variables utilized in the analysis. Further, both the MR-Egger intercept test (egger_intercept = −0.00027, $p = 0.992$) and MR-PRESSO ($p = 0.915$) showed no evidence of horizontal pleiotropy. In the second step, excluding significant genetic instrumental variables involved in calculating beta1, we assessed the effect of 4-acetaminophen sulfate on IVDD (beta2). This analysis revealed that increased levels of 4-acetaminophen sulfate significantly reduced the risk of IVDD (OR: 0.952, 95% CI: 0.909–0.997, $p = 0.038$, beta2 = −0.048) (Figure 4B). The Cochrane's Q test ($Q = 22.659$, $p = 0.539$) showed no significant heterogeneity among the instrumental variables. Additionally, the MR-Egger intercept test (egger_intercept = 0.00343, $p = 0.719$) and MR-PRESSO ($p = 0.547$) found no horizontal pleiotropy, confirming the reliability of the MR results in this study. To determine the total effect of vitamin B12 on IVDD, a direct MR analysis was performed, showing a significant reduction in IVDD risk with increased vitamin B12 levels (OR: 0.752, 95% CI: 0.573–0.987, $p = 0.040$, beta total = −0.284) (Figure 4C). Finally, we incorporated beta1, beta2, and beta total into the mediation model to calculate the mediated effect of 4-acetaminophen sulfate. Our findings suggest that vitamin B12 may reduce the risk of IVDD by promoting increased levels of 4-acetaminophen sulfate [Mediated effect (beta1*beta2): −0.0365 (−0.0573, −0.0158)] [Mediated proportion: 12.8% (20.1, 5.56%)] (Figure 5).

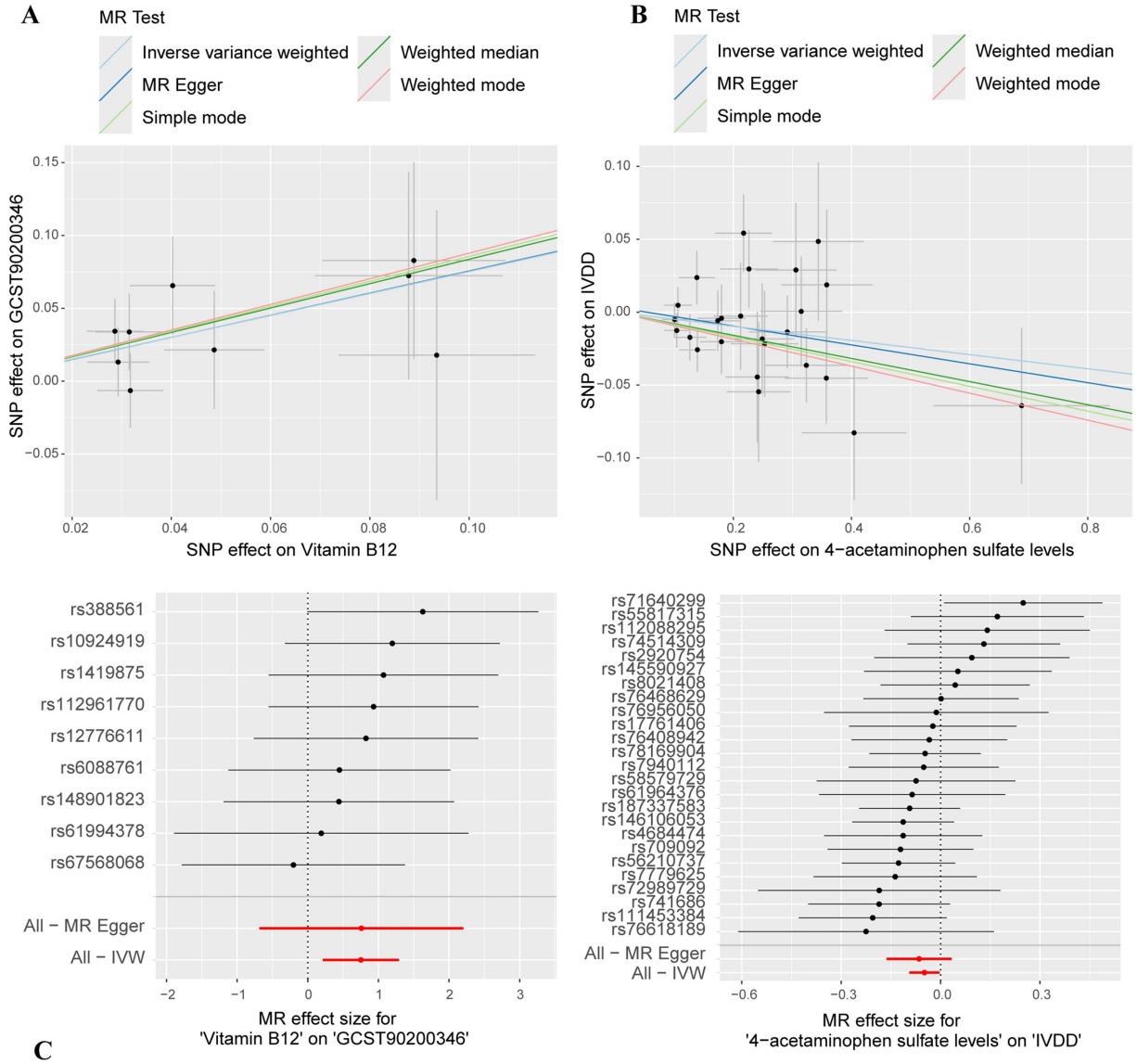
4 Discussion

The purpose of this study was to identify micronutrients that may reduce the risk of IVDD and to elucidate the serum metabolites mediating this protective effect. We employed several analytic methods, including 2SMR, reverse MR, and mediation MR. Our results suggest that vitamin B12 could decrease the risk of IVDD by enhancing the expression of the serum metabolite 4-acetaminophen sulfate.

The correlation between micronutrients and IVDD is becoming more acknowledged in the fields of orthopedics and nutrition. Micronutrients play a critical role in numerous metabolic processes and physiological functions, aiding in the maintenance of the structural and functional integrity of connective tissues like bone and cartilage (24). Therefore, the examination of how micronutrients influence IVDD is essential for the advancement of nutritional interventions and preventative measures. The present study examined 15 micronutrients, comprising 7 minerals and 8 vitamins, with the objective of elucidating their potential causal relationships with susceptibility to IVDD. Previous research has underscored the correlation between zinc and the advancement of IVDD, wherein elevated zinc concentrations in disk cells trigger the activation of matrix metalloproteinases (MMPs), thereby hastening the degradation of the extracellular matrix. Conversely, diminished zinc levels under hypoxic conditions may impede MMP function, suggesting a plausible protective mechanism against cellular injury (8). Selenium, a cofactor essential for the activity of antioxidant enzymes like glutathione peroxidase (GPX4), is pivotal in attenuating ferroptosis and oxidative stress in intervertebral disk cells. Research indicates that mechanical stress can trigger Piezo1 ion channels, resulting in calcium overload and endoplasmic reticulum stress in these cells, thereby exacerbating ferroptosis. Supplementation with selenium has been demonstrated to decrease intracellular free calcium levels and ameliorate oxidative stress and ferroptosis, consequently reducing the likelihood of IVDD. These results emphasize the considerable protective effects of micronutrients in preventing disk degeneration (10, 25). However, the MR analysis did not find a causal relationship between the seven minerals examined and the risk of IVDD. This lack of association may be due to constraints related to the genetic instrumental variables utilized and the limited sample size.

The results of our study demonstrate a notable causal association between vitamin B12 and IVDD, suggesting a potentially more pronounced connection with vitamins compared to minerals. Epidemiological research has established a robust correlation between vitamin D insufficiency and degeneration of the lumbar disks, with evidence indicating that vitamin D supplementation effectively reduces this risk (26). *In vitro* investigations indicate that vitamin D promotes the integrity of nucleus pulposus cells by increasing Sirt1 expression, suppressing the NF-κB inflammatory pathway, and decreasing MMPs and inflammatory mediators (6). The depletion of VDR has been shown to markedly diminish cell proliferation and matrix synthesis, hastening apoptosis and senescence. Nevertheless, administration of vitamin D has been found to partially counteract these deteriorative effects by activating Sirt1 and inhibiting the NF-κB pathway (9, 27). Furthermore, the deficiency of vitamin C, frequently seen alongside inadequate levels of vitamin D, poses a potential risk factor for IVDD in the elderly population. Vitamin C plays a crucial role in collagen synthesis, and its insufficiency can impair the functionality of connective tissues by diminishing collagen production and compromising structural integrity. Additionally, the progression of IVDD may lead to localized tissue inflammation, which can exacerbate the depletion of vitamin C levels, perpetuating a detrimental cycle that accelerates disk degeneration (28).

Furthermore, alongside vitamins D and C, vitamin B12 is being recognized as a potentially significant factor in the development of IVDD. While direct evidence linking vitamin B12 to IVDD is currently limited, several studies suggest that vitamin B12 may offer protection against IVDD through mechanisms involving anti-inflammatory, antioxidative, and autophagy-regulating pathways. Inflammation and oxidative stress play crucial roles in the pathogenesis of IVDD. Experimental models have shown that supplementation with vitamin B12 can reduce levels of inflammatory mediators such as IL-1β and IL-6, as well as decrease malondialdehyde (MDA) levels and enhance glutathione content. Furthermore, vitamin B12 has been shown to suppress the TLR-4/NF-κB signaling pathway, leading to the inhibition of subsequent inflammatory reactions (29). A study also demonstrated that supplementation with a B-vitamin



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exposure	outcome	nsnp	method	pval	OR(95% CI)
Vitamin B12	4−acetaminophen sulfate levels	9	MR Egger	0.337	2.135 (0.504 to 9.051)
		9	Weighted median	**0.019**	2.309 (1.147 to 4.647)
		9	Inverse variance weighted	**0.006**	2.121 (1.238 to 3.636)
		9	Simple mode	0.136	2.354 (0.856 to 6.475)
		9	Weighted mode	0.136	2.407 (0.852 to 6.797)
4−acetaminophen sulfate levels	IVDD	25	MR Egger	0.211	0.937 (0.849 to 1.034)
		25	Weighted median	**0.022**	0.924 (0.863 to 0.989)
		25	Inverse variance weighted	**0.038**	0.953 (0.910 to 0.997)
		25	Simple mode	0.183	0.919 (0.814 to 1.037)
		25	Weighted mode	0.064	0.912 (0.830 to 1.001)
Vitamin B12	IVDD	8	MR Egger	0.300	0.687 (0.360 to 1.314)
		8	Weighted median	0.079	0.718 (0.496 to 1.040)
		8	Inverse variance weighted	**0.040**	0.752 (0.573 to 0.987)
		8	Simple mode	0.156	0.593 (0.311 to 1.129)
		8	Weighted mode	0.121	0.616 (0.360 to 1.055)

FIGURE 4
Mediation MR analyses. **(A)** Scatter plot and forest plot of the MR analysis between vitamin B12 and 4-acetaminophen sulfate levels. **(B)** Scatter plot and forest plot of the MR analysis between 4-acetaminophen sulfate levels and IVDD. **(C)** Summary forest plot of the mediation analyses.

complex, which includes folic acid, vitamin B6, and B12, improved neurobehavioral deficits in offspring mice exposed to maternal PM2.5 by regulating neuroinflammatory markers and decreasing oxidative stress (30). Studies in diabetic rat models have also demonstrated that vitamin B12, combined with folic acid, alleviates nicotine-induced insulin resistance and pancreatic β-cell apoptosis through

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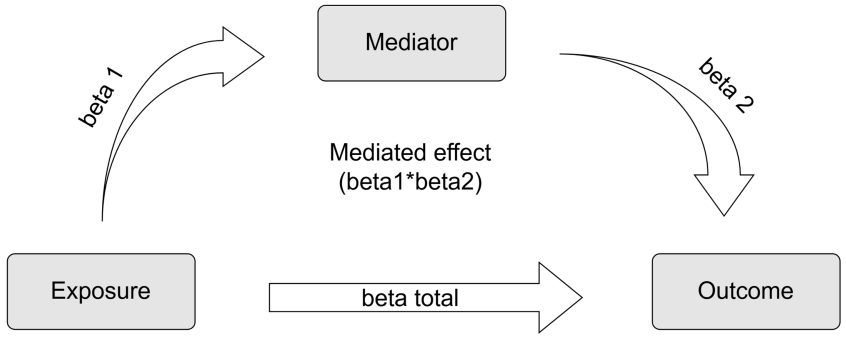

Exposure	Mediator	outcome	Mediated effect (beta1*beta2)	Mediated proportion
Vitamin B12	4-acetaminophen sulfate levels	IVDD	-0.0365 (-0.0573, -0.0158)	12.8% (20.1%, 5.56%)

FIGURE 5
Mediation effects and proportion mediated by serum metabolites.

antioxidative and anti-inflammatory actions (31). Moreover, research has indicated that vitamin B12 can provide protective benefits against tissue damage by regulating autophagy. High doses of vitamin B12 have been found to effectively decrease oxidative stress and apoptosis in cases of myocardial ischemia–reperfusion injuries by increasing SIRT3 and AMPK activity, inhibiting Nox2 expression, and reducing levels of cleaved caspase-3 (32). *In vitro*, vitamin B12 has also shown to safeguard RIN-m5F pancreatic β-cells from high glucose-induced apoptosis by activating autophagy (33). Moreover, vitamin B12 was found to reduce infarct volume in a rat model of focal cerebral ischemia by upregulating the ERK1/2 pathway and Beclin1 expression, while downregulating Bax and cleaved caspase-3 (34). Collectively, these results collectively indicate that vitamin B12 may provide protective effects in conditions like IVDD by reducing inflammation, alleviating oxidative stress, and regulating autophagy.

In addition to its known anti-inflammatory, antioxidative, and autophagy-regulating effects, vitamin B12 may play a role in the development and progression of IVDD by influencing metabolite levels in the body. Using 2SMR, we investigated the potential causal relationships between 1,091 blood metabolites and 309 metabolite ratios with IVDD. Our analysis revealed 52 serum metabolites that were significantly linked to IVDD, including amino acid derivatives, fatty acids, nucleotides, sphingosine, and sphingolipids. Prior research supports the association between the progression of IVDD and notable metabolic changes that can impact the development of the condition by influencing multiple pathological mechanisms, including inflammation, apoptosis, autophagy, and matrix degradation in disk cells. Specifically, the accumulation of lactate, a byproduct of glycolysis, in degenerated disks has been identified as a potential inducer of senescence and oxidative stress in nucleus pulposus cells, potentially through the modulation of the PI3K/Akt pathway (35). Furthermore, metabolomic investigations have revealed disturbances in lipid metabolism, particularly involving phospholipids, cholesterol esters, and triglycerides, as well as in bile acid metabolism among individuals with IVDD. These findings indicate the potential of these metabolites as biomarkers and targets for therapeutic intervention (4, 11). The metabolites identified in this study, including various fatty acids (e.g., 2-hydroxydecanoate, octadecenedioate) and sphingolipids (e.g., sphingomyelin), align with prior metabolomic findings (36). Furthermore, certain metabolites, including 5′-methylthioadenosine (MTA), a sulfur-containing nucleoside, have been observed in other musculoskeletal disorders and may impact IVDD through comparable mechanisms. Recent studies have shown that MTA has the potential to alleviate inflammation-induced bone loss by inhibiting the RANKL-induced NFκB-NFATc1 signaling pathway and suppressing osteoclast differentiation and activity, thus regulating inflammatory reactions in IVDD (37).

The results of further mediation analysis suggest that heightened levels of vitamin B12 may have a significant impact on reducing the risk of IVDD, potentially through the mediation of increased levels of 4-acetaminophen sulfate. Vitamin B12, an essential coenzyme involved in the methionine cycle, plays a crucial role in the regulation of homocysteine metabolism and the maintenance of sulfate homeostasis within the body (38). This investigation highlights the ability of vitamin B12 to substantially raise levels of 4-acetaminophen sulfate, potentially by enhancing the methionine cycle, increasing sulfate availability, and preserving sulfotransferase function. Moreover, previous studies have indicated that vitamin B12 can improve liver detoxification functions by enhancing acetaminophen sulfation metabolism (39). This process may lead to the inhibition of IVDD progression through the modulation of inflammatory responses, regulation of matrix metabolism, and protection of intervertebral disk cell function. Additionally, elevated levels of the sulfated metabolite, 4-acetaminophen sulfate, suggest an increased detoxification capacity, which may aid in the efficient clearance of toxic substances related to IVDD and reduce their detrimental impact on intervertebral disks (40).

Although this study employed MR analysis to investigate the causal relationships between micronutrients, metabolites, and IVDD, several limitations exist. First, selection bias and the limited strength of genetic instrumental variables may affect the analytical power.

Second, due to gaps in current metabolomic and nutriomic studies, many micronutrients and metabolites lack suitable genetic instruments and were excluded, potentially underestimating their impact on IVDD. Additionally, we did not apply multiple testing corrections such as Bonferroni adjustment, as our primary objective was to identify potential biomarkers or therapeutic targets for IVDD. The stringent Bonferroni criteria might exclude meaningful indicators. Furthermore, while mediation analysis suggests a mediating effect of 4-acetaminophen sulfate in the association between vitamin B12 and IVDD, the precise molecular mechanisms remain unclear. The effects of vitamin B12 may involve various pathological pathways not comprehensively explored in this study. Future research should expand sample sizes, include diverse populations, conduct prospective cohort studies and randomized controlled trials, and incorporate histopathological assessments to further validate the causal relationships and underlying mechanisms.

5 Conclusion

This study utilized MR analysis to establish a significant association between vitamin B12 and a decreased risk of IVDD. Out of the 1,091 blood metabolites and 309 metabolite ratios analyzed, 52 were found to be correlated with IVDD risk. Particularly noteworthy is the potential role of 4-acetaminophen sulfate in mediating the protective effects of vitamin B12. This discovery unveils a new pathway by which vitamin B12 may reduce IVDD risk by influencing the metabolism of 4-acetaminophen sulfate, thereby enhancing our comprehension of the mechanisms involved in IVDD progression.

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

NJ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. QW: Data curation, Formal analysis, Funding acquisition, Methodology, Writing – review & editing. JJ: Conceptualization, Funding acquisition, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. LL: Funding acquisition, Writing – review & editing.

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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.

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References

1. Kang L, Zhang H, Jia C, Zhang R, Shen C. Epigenetic modifications of inflammation in intervertebral disc degeneration. *Ageing Res Rev*. (2023) 87:101902. doi: 10.1016/j.arr.2023.101902

2. Fine N, Lively S, Séguin CA, Perruccio AV, Kapoor M, Rampersaud R. Intervertebral disc degeneration and osteoarthritis: a common molecular disease spectrum. *Nat Rev Rheumatol*. (2023) 19:136–52. doi: 10.1038/s41584-022-00888-z

3. Fenech MF, Bull CF, Van Klinken BJ. Protective effects of micronutrient supplements, phytochemicals and phytochemical-rich beverages and foods against DNA damage in humans: a systematic review of randomized controlled trials and prospective studies. *Adv Nutr (Bethesda, MD)*. (2023) 14:1337–58. doi: 10.1016/j.advnut.2023.08.004

4. Wang N, Chen S, Xie Y, Liu X, Xi Z, Li J, et al. The Sanbi decoction alleviates intervertebral disc degeneration in rats through intestinal flora and serum metabolic homeostasis modulation. *Phytomedicine*. (2024) 127:155480. doi: 10.1016/j.phymed.2024.155480

5. Lanham-New SA. Importance of calcium, vitamin D and vitamin K for osteoporosis prevention and treatment. *Proc Nutr Soc*. (2008) 67:163–76. doi: 10.1017/s0029665108007003

6. Wang P, Yang C, Lu J, Ren Y, Goltzman D, Miao D. Sirt1 protects against intervertebral disc degeneration induced by 1,25-dihydroxyvitamin D insufficiency in mice by inhibiting the NF-κB inflammatory pathway. *J Orthop Translat*. (2023) 40:13–26. doi: 10.1016/j.jot.2023.04.003

7. Mahmoud M, Kokozidou M, Gögele C, Werner C, Auffarth A, Kohl B, et al. Does vitamin K2 influence the interplay between diabetes mellitus and intervertebral disc degeneration in a rat model? *Nutrients*. (2023) 15:2872. doi: 10.3390/nu15132872

8. Yin XF, Jiang LB, Ma YQ, Xu J, Gu HJ, Wu XH, et al. Decreased Zn(2+) influx underlies the protective role of hypoxia in rat nucleus pulposus cells. *Biol Trace Elem Res*. (2015) 168:196–205. doi: 10.1007/s12011-015-0335-2

9. Colombini A, De Luca P, Cangelosi D, Perucca Orfei C, Ragni E, Viganò M, et al. High-throughput gene and Protein analysis revealed the response of disc cells to vitamin D, depending on the VDR FokI variants. *Int J Mol Sci*. (2021) 22:9603. doi: 10.3390/ijms22179603

10. Jia C, Xiang Z, Zhang P, Liu L, Zhu X, Yu R, et al. Selenium-SelK-GPX4 axis protects nucleus pulposus cells against mechanical overloading-induced ferroptosis and attenuates senescence of intervertebral disc. *Cell Mol Life Sci*. (2024) 81:49. doi: 10.1007/s00018-023-05067-1

11. Ji L, Wang Y, Lu T, Yang J, Luo C, Qiu B. Identification of blood metabolites linked to the risk of intervertebral disc diseases: a comprehensive Mendelian randomization study. *Postgrad Med J*. (2023) 99:1148–53. doi: 10.1093/postmj/qgad052

12. Emdin CA, Khera AV, Kathiresan S. Mendelian Randomization. *JAMA*. (2017) 318:1925–6. doi: 10.1001/jama.2017.17219

13. Zhao JH, Stacey D, Eriksson N, Macdonald-Dunlop E, Hedman ÅK, Kalnapenkis A, et al. Genetics of circulating inflammatory proteins identifies drivers of immune-mediated disease risk and therapeutic targets. *Nat Immunol*. (2023) 24:1540–51. doi: 10.1038/s41590-023-01588-w

14. Xu M, Zheng J, Hou T, Lin H, Wang T, Wang S, et al. SGLT2 inhibition, choline metabolites, and Cardiometabolic diseases: a mediation Mendelian randomization study. *Diabetes Care*. (2022) 45:2718–28. doi: 10.2337/dc22-0323

15. Chen Y, Lu T, Pettersson-Kymmer U, Stewart ID, Butler-Laporte G, Nakanishi T, et al. Genomic atlas of the plasma metabolome prioritizes metabolites implicated in human diseases. *Nat Genet*. (2023) 55:44–53. doi: 10.1038/s41588-022-01270-1

16. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. (2003) 32:1–22. doi: 10.1093/ije/dyg070

17. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-base platform supports systematic causal inference across the human phenome. *eLife*. (2018) 7:e34408. doi: 10.7554/eLife.34408

18. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-egger method. *Eur J Epidemiol*. (2017) 32:377–89. doi: 10.1007/s10654-017-0255-x

19. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol*. (2017) 46:1985–98. doi: 10.1093/ije/dyx102

20. Sanderson E, Spiller W, Bowden J. Testing and correcting for weak and pleiotropic instruments in two-sample multivariable Mendelian randomization. *Stat Med*. (2021) 40:5434–52. doi: 10.1002/sim.9133

21. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7

22. Vander Weele TJ. Mediation analysis: a Practitioner's guide. *Annu Rev Public Health*. (2016) 37:17–32. doi: 10.1146/annurev-publhealth-032315-021402

23. Burgess S, Daniel RM, Butterworth AS, Thompson SG. Network Mendelian randomization: using genetic variants as instrumental variables to investigate mediation in causal pathways. *Int J Epidemiol*. (2015) 44:484–95. doi: 10.1093/ije/dyu176

24. Casaer MP, Bellomo R. Micronutrient deficiency in critical illness: an invisible foe? *Intensive Care Med*. (2019) 45:1136–9. doi: 10.1007/s00134-019-05678-y

25. Hu T, Shi Z, Sun Y, Hu F, Rong Y, Wang J, et al. SEPHS1 attenuates intervertebral disc degeneration by delaying nucleus pulposus cell senescence through the hippo-yap/Taz pathway. *Am J Physiol Cell Physiol*. (2024) 326:C386–c399. doi: 10.1152/ajpcell.00571.2023

26. Tegeder I, Lötsch J. Current evidence for a modulation of low back pain by human genetic variants. *J Cell Mol Med*. (2009) 13:1605–19. doi: 10.1111/j.1582-4934.2009.00703.x

27. Tong T, Liu Z, Zhang H, Sun J, Zhang D, Wang F, et al. Age-dependent expression of the vitamin D receptor and the protective effect of vitamin D receptor activation on H(2)O(2)-induced apoptosis in rat intervertebral disc cells. *J Steroid Biochem Mol Biol*. (2019) 190:126–38. doi: 10.1016/j.jsbmb.2019.03.013

28. Smith VH. Vitamin C deficiency is an under-diagnosed contributor to degenerative disc disease in the elderly. *Med Hypotheses*. (2010) 74:695–7. doi: 10.1016/j.mehy.2009.10.041

29. Deniz E, Topcu A, Ozturk A, Ozturk SD, Arpa M, Atak M. The effects of vitamin B12 on the TLR-4/NF-κB signaling pathway in ovarian ischemia-reperfusion injury-related inflammation. *Int Immunopharmacol*. (2022) 107:108676. doi: 10.1016/j.intimp.2022.108676

30. Wang T, Zhang T, Sun L, Li W, Zhang C, Yu L, et al. Gestational B-vitamin supplementation alleviates PM(2.5)-induced autism-like behavior and hippocampal neurodevelopmental impairment in mice offspring. *Ecotoxicol Environ Saf*. (2019) 185:109686. doi: 10.1016/j.ecoenv.2019.109686

31. Bhattacharjee A, Prasad SK, Pal S, Maji B, Syamal AK, Mukherjee S. Synergistic protective effect of folic acid and vitamin B12 against nicotine-induced oxidative stress and apoptosis in pancreatic islets of the rat. *Pharm Biol*. (2016) 54:433–44. doi: 10.3109/13880209.2015.1043561

32. Qin Y, Shi Y, Yu Q, Yang S, Wang Y, Dai X, et al. Vitamin B12 alleviates myocardial ischemia/reperfusion injury via the SIRT3/AMPK signaling pathway. *Biomed Pharmacother*. (2023) 163:114761. doi: 10.1016/j.biopha.2023.114761

33. Zhang Y, Chu L, Zhou X, Xu T, Shen Q, Li T, et al. Vitamin B12-induced autophagy alleviates high glucose-mediated apoptosis of islet β cells. *Int J Mol Sci*. (2023) 24:15217. doi: 10.3390/ijms242015217

34. Li Y, Zheng J, Zhu Y, Qu Y, Suo R, Zhu Y. Neuroprotective effects of methylcobalamin in cerebral ischemia/reperfusion injury through activation of the ERK1/2 signaling pathway. *Int Immunopharmacol*. (2021) 99:108040. doi: 10.1016/j.intimp.2021.108040

35. Zhang Y, Liu L, Qi Y, Lou J, Chen Y, Liu C, et al. Lactic acid promotes nucleus pulposus cell senescence and corresponding intervertebral disc degeneration via interacting with Akt. *Cell Mol Life Sci*. (2024) 81:24. doi: 10.1007/s00018-023-05094-y

36. Francisco V, Ait Eldjoudi D, González-Rodríguez M, Ruiz-Fernández C, Cordero-Barreal A, Marques P, et al. Metabolomic signature and molecular profile of normal and degenerated human intervertebral disc cells. *Spine J*. (2023) 23:1549–62. doi: 10.1016/j.spinee.2023.06.005

37. Rattajak P, Aroonkesorn A, Smythe C, Wititsuwannakul R, Pitakpornpreecha T. 5′-Methylthioadenosine strongly suppresses RANKL-induced osteoclast differentiation and function via inhibition of RANK-NFATc1 signalling pathways. *Heliyon*. (2023) 9:e22365. doi: 10.1016/j.heliyon.2023.e22365

38. Froese DS, Fowler B, Baumgartner MR. Vitamin B (12), folate, and the methionine remethylation cycle-biochemistry, pathways, and regulation. *J Inherit Metab Dis*. (2019) 42:673–85. doi: 10.1002/jimd.12009

39. Hannibal L, DiBello PM, Jacobsen DW. Proteomics of vitamin B12 processing. *Clin Chem Lab Med*. (2013) 51:477–88. doi: 10.1515/cclm-2012-0568

40. Goresky CA, Pang KS, Schwab AJ, Barker F 3rd, Cherry WF, Bach GG. Uptake of a protein-bound polar compound, acetaminophen sulfate, by perfused rat liver. *Hepatology (Baltimore, MD)*. (1992) 16:173–90. doi: 10.1002/hep.1840160129

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Associations of dietary sources of antioxidant intake and NAFLD: NHANES 2017–2020 and Mendelian randomization

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Purpose: To determine the association between dietary antioxidant sources and non-alcoholic fatty liver disease (NAFLD).

Methods: In this observational study, we utilized NHANES 2017–2020 data to identify the factors associated with NAFLD in dietary antioxidant sources via weighted multivariate logistic regression models. Then, Mendelian randomization (MR) was applied to investigate the effect of dietary antioxidant sources on NAFLD at the genetic level.

Results: Of the six dietary sources of antioxidants, only vitamin E (Vit E) was significantly associated with NAFLD (OR = 0.98; 95% CI: 0.97–0.99; $p = 0.001$). Upon adjusting for all covariates, it was determined that the highest quartile of dietary Vit E intake was associated with a decreased NAFLD occurrence compared with the lowest quartile of dietary Vit E intake ($p < 0.001$). The results of IVW-MR analysis revealed an association between Vit E and NAFLD (OR = 0.028; $p = 0.039$).

Conclusion: Our research indicates a negative and linear relationship between daily vitamin E intake and NAFLD.

KEYWORDS

dietary sources of antioxidant, non-alcoholic fatty liver disease, NHANES, vitamin E, Mendelian randomization

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver conditions worldwide. Studies suggest that the prevalence of NAFLD is more than 30% among adults in the United States and China (1, 2). Based on its histological type, NAFLD can be classified into two types: non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), and NASH primarily arises from the advancement of NAFL (3, 4). At present, end-stage liver disease triggered by NAFLD is the primary reason for liver transplantation, accounting for 8.4% of liver transplant cases in Europe and 17.38% in the United States (5, 6). This has been showing an increasing trend each year. NAFLD imposes a significant strain on global public health. Therefore, determining ways to effectively prevent or ameliorate NAFLD is urgently warranted.

Options for the pharmacological treatment of NAFLD are limited; however, evidence suggests the effectiveness of resmetirom in treating NASH (7, 8). Nevertheless, it has not yet

received clinical approval. Therefore, the current treatment for NAFLD mainly involves lifestyle intervention and management of comorbidities. Oxidative stress is defined as an imbalance between oxidant production in the body and cellular antioxidant defenses; this ultimately leads to cellular dysfunction and tissue damage, and is an important mechanism in the development of NAFLD (9–11). Oxidative stress can cause increased expression of IRE1a and ATF4, which leads to increased autophagy and activation of hepatic stellate cells (12). The exogenous antioxidants can modulate the damage caused by oxidative stress by neutralising reactive oxygen species (13). Many studies on mice have revealed that NAFLD incidence can be decreased by controlling oxidative stress (14–16). So, we conjecture that there is a possible correlation between the intake of exogenous antioxidants in the daily diet and the onset of NAFLD. Vitamin A (Vit A), vitamin C (Vit C), vitamin E (Vit E), carotenoids, selenium and zinc are common exogenous antioxidants found in our daily diet. Dietary antioxidant sources intervene in hepatic metabolism through multiple metabolic pathways. Vit A can inhibit fatty acid oxidation in the liver (17). Xie ZQ's study showed that increased serum levels of Vit C reduced the risk of NAFLD (18). Furthermore, Panera et al. have reported that exogenous Vit E supplementation can ameliorate NAFLD-induced liver fibrosis (19). Reduced serum zinc levels can lead to increased hepatic fibrosis in NAFLD patients (20). Dietary selenium consumption can reduce oxidative stress in hepatocytes by enhancing the synthesis of selenoprotein P1 (21). These evidence suggest that there may be some association between antioxidants and NAFLD. After consulting relevant literature, we discovered a lack of studies investigating the relationship between dietary intake of exogenous antioxidants and NAFLD.

In this study, we plan to identify exogenous antioxidants in the daily diet related to NAFLD. Then further analyze the relationship between these selected antioxidants and NAFLD.

Methods

Study population

All data were derived from The National Health and Nutrition Examination Survey (NHANES). The ethics review board of the National Center for Health Statistics approved the NHANES survey protocol, with all participants providing written informed consent before the survey. We selected participants between 2017 and 2020. The exclusion criteria were as follows: (1) age < 20 years, (2) absence of complete dietary data, and (3) incomplete follow-up data.

Exposure and outcomes

Two nonconsecutive 24 h dietary recall interviews were conducted to collect information on the intake of NHANES dietary sources of exogenous antioxidants. Subsequently, the data were converted into estimates of nutrient intake via the Food and Nutrient Database for Dietary Studies of the United States Department of Agriculture. Vit A, Vit C, Vit E, carotenoids, selenium, and zinc are the primary exogenous antioxidants ingested in the daily diet. Based on the NHANES questionnaire results, we determined the intake of dietary sources for each participant.

To further investigate the overall effect of antioxidants from multiple dietary sources, the composite dietary antioxidant index (CDAI) for each participant in NHANES was calculated based on their dietary records. The CDAI represents a composite score of the above six dietary antioxidant intakes. CDAI calculations follow a previously established and validated methodology (22), as follows:

$$CADI = \sum_{i=1}^{6} \frac{x_i - u_i}{S_i}$$

x_i is the daily intake of each antioxidant, u_i is the mean value of x_i, and S_i is the standard deviation of u_i (23). NAFLD was defined in this study as a Controlled Attenuation Parameter (CAP) score of ≥248 dB/m in the absence of alcoholism and liver disease (24). Participants with NAFLD were defined as NAFLD group and patients without NAFLD were defined as No NAFLD group.

Covariates

Based on the participant-related data contained in the NHANES database, we selected the following variables as covariates: sex, age, education, BMI, smoking history, diabetes history, fasting blood glucose, red blood cell count (RBC), hemoglobin A1C (HbA1C), lymphocyte count (LN), neutrophil count (NEUT), platelet count (PLT), hemoglobin (Hb), white blood cell count (WBC), C-reactive protein (CRP), Alaninetransaminase (ALT), Alaninetransaminase (AST), and blood lipids.

Mendelian randomization

Based on the results of the cross-sectional study, antioxidants associated with NAFLD were selected as the exposure factor. Single nucleotide polymorphisms (SNPs) closely associated with specific exogenous antioxidants and with correlations satisfying $p < 5 \times 10^{-5}$ were selected as instrumental variables (IVs). We used $r^2 < 0.001$ and kb >10,000 as linkage disequilibrium thresholds to ensure the independence of IV (25). To quantify the strength of the genetic tool, SNP with F-statistic less than 10 are excluded. Inverse variance weighted (IVW) was used as the primary method for MR analysis. Furthermore, MR-Egger regression, weighted median, and other IVW-complementing methods were used. Cochran's Q test was used to assess heterogeneity among SNPs, whereas MR-Egger regression intercepts were used to assess horizontal pleiotropy. A leave-one-out analysis was performed to estimate the potential effect of individual SNPs on the causal relationship between NAFLD and associated antioxidant intake. Figure 1 illustrates the flow chart of MR analysis.

Statistical analysis

R (4.2.2) was used to perform statistical analysis. A p-value of <0.05 was considered a statistically significant difference. Continuous variables were expressed using mean (standard deviation), whereas categorical variables were expressed using n (%). For continuous variables, group comparisons were conducted using the student t-test. In contrast, the chi-square test was used for categorical variables.

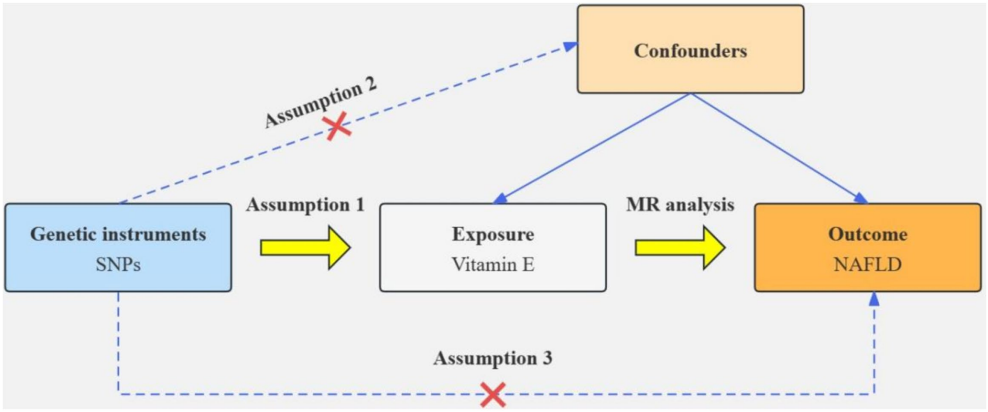

FIGURE 1
Mendelian randomization flow chart.

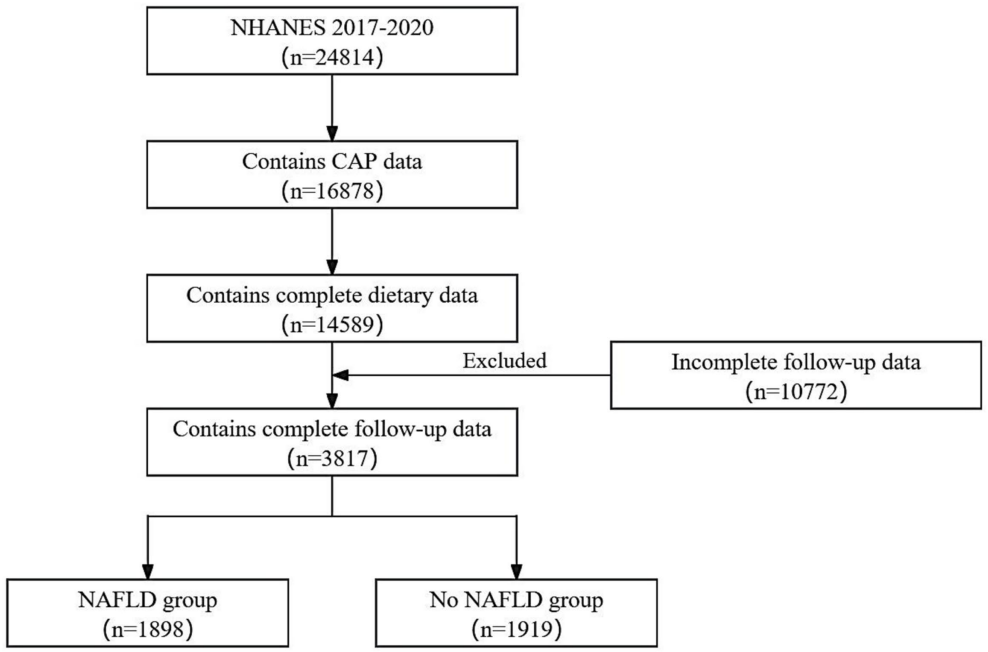

FIGURE 2
The participant selection flow chart.

Logistic regression models were utilized to analyze the relationship between exogenous antioxidants and NAFLD. The covariates in the crude model were not adjusted, and model 1 comprised all covariates. Restricted cubic splines analysis was used to determine if a non-linear relationship exists between antioxidant intake and NAFLD.

Results

Baseline characteristics

This study included 3,817 respondents, of which 1926 (50.5%) were males and 1891 (49.5%) were females. Figure 2 illustrates the detailed flowchart. Table 1 presents the baseline demographic characteristics. The NAFLD and the No NAFLD groups exhibited significant differences in age, race, education level, body mass index, hypertension prevalence, and diabetes prevalence ($p < 0.05$ for all). Patients with NAFLD exhibited higher age, education level, and prevalence of diabetes and hypertension.

Association of exogenous antioxidants and NAFLD

Table 2 summarizes the results of logistic regression analysis. Among the six exogenous antioxidants included in this study, only Vit E was noted to be associated with NAFLD occurrence (OR = 0.98; 95% CI: 0.97–0.99; $p = 0.001$).

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TABLE 1 Baseline demographic characteristics.

Variables	Total (*n* = 3,817)	NAFLD (*n* = 1898)	No NAFLD (*n* = 1919)	*p*
Age, years	51.0 ± 16.8	52.1 ± 16.2	49.9 ± 17.3	<0.001
Gender, *n* (%)				0.202
Female	1891 (49.5)	960 (50.6)	931 (48.5)	
Male	1926 (50.5)	938 (49.4)	988 (51.5)	
Race/Ethnicity, *n* (%)				<0.001
Mexican American	641 (16.8)	318 (16.8)	323 (16.8)	
Other Hispanic	469 (12.3)	192 (10.1)	277 (14.4)	
Non-Hispanic White	1,370 (35.9)	695 (36.6)	675 (35.2)	
Non-Hispanic Black	759 (19.9)	384 (20.2)	375 (19.5)	
Other race	989 (25.9)	483 (25.4)	506 (26.4)	
Education level, *n* (%)				<0.001
<9 years	352 (9.2)	140 (7.4)	212 (11)	
9–11 years	406 (10.6)	192 (10.1)	214 (11.2)	
Senior high schools or GED	871 (22.8)	437 (23)	434 (22.6)	
Some college or associate degree	1,199 (31.4)	646 (34)	553 (28.8)	
Colleges or above	989 (25.9)	483 (25.4)	506 (26.4)	
BMI, *n* (%)				<0.001
<25 Kg/m^2	733 (19.2)	223 (11.7)	510 (26.6)	
≥25 Kg/m^2	3,084 (80.8)	1,675 (88.3)	1,409 (73.4)	
Smoke, *n* (%)				0.15
Yes	1,631 (42.7)	789 (41.6)	842 (43.9)	
No	2,186 (57.3)	1,109 (58.4)	1,077 (56.1)	
Hypertension, *n* (%)				<0.001
Yes	1,528 (40.0)	829 (43.7)	699 (36.4)	
No	2,289 (60.0)	1,069 (56.3)	1,220 (63.6)	
Diabetes, *n* (%)				<0.001
Yes	685 (17.9)	389 (20.5)	296 (15.4)	
No	3,132 (82.1)	1,509 (79.5)	1,623 (84.6)	
WBC, 1000 cells/uL	6.9 ± 2.7	6.9 ± 2.0	6.9 ± 3.2	0.738
LN, 1000 cells/uL	2.1 ± 1.9	2.1 ± 0.7	2.1 ± 2.6	0.504
NEUT, 1000 cells/uL	4.0 ± 1.6	4.0 ± 1.6	4.0 ± 1.5	0.238
RBC, 1000 cells/uL	4.8 ± 0.5	4.8 ± 0.5	4.8 ± 0.5	0.039
Hb, g/dL	14.2 ± 1.5	14.2 ± 1.5	14.1 ± 1.5	0.002
PLT, 1000 cells/uL	238.7 ± 61.8	243.5 ± 62.0	234.0 ± 61.2	<0.001
CRP, mg/L	4.4 ± 8.0	4.8 ± 8.1	4.1 ± 7.9	0.005
ALT, U/L	24.9 ± 15.8	24.7 ± 15.8	25.1 ± 15.8	0.372
AST, U/L	23.9 ± 18.0	22.2 ± 12.8	25.6 ± 21.9	<0.001
Glu, mmol/L	6.4 ± 2.3	6.6 ± 2.3	6.3 ± 2.3	<0.001
HbA1c, %	5.9 ± 1.3	6.0 ± 1.3	5.9 ± 1.3	<0.001
TC, mg/dL	4.9 ± 1.1	4.9 ± 1.1	4.9 ± 1.1	0.087
HDL, mg/dL	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.5	<0.001
TG, mg/dL	1.3 ± 0.8	1.4 ± 0.8	1.2 ± 0.7	<0.001
LDL, mg/dL	2.9 ± 0.9	2.9 ± 0.9	2.9 ± 0.9	0.692
Vit A, μg	584.9 ± 562.9	589.2 ± 527.7	580.7 ± 595.8	0.639

(Continued)

TABLE 1 (Continued)

Variables	Total (n = 3,817)	NAFLD (n = 1898)	No NAFLD (n = 1919)	p
Vit C, mg	78.7 ± 91.6	80.4 ± 96.8	77.0 ± 86.1	0.249
Vit E, mg	8.8 ± 6.1	9.1 ± 6.3	8.5 ± 5.8	<0.001
Se, μg	114.7 ± 68.1	113.7 ± 70.6	115.6 ± 65.5	0.377
Zn, mg	10.8 ± 12.2	10.9 ± 16.2	10.6 ± 6.0	0.471
Carotenoid, μg	2267.5 ± 4282.5	2334.3 ± 4343.5	2201.6 ± 4221.4	0.339

WBC, white blood cell count; LN, lymphocyte count; NEUT, neutrophil count; RBC, red blood cell count; Hb, hemoglobin; PLT, platelet count; CRP, C-reactive protein; ALT, alaninetransaminase; AST, alaninetransaminase; Glu, fasting blood glucose; HbA1c, hemoglobin A1C; TC, total cholesterol; HDL, high density lipoprotein; TG, triglyceride; LDL, low density lipoprotein; Vit A, vitamin A; Vit C, vitamin C; Vit E, vitamin E.

Association between Vit E and NAFLD

To investigate the correlation between Vit E and NAFLD, two weighted models were constructed. The results are summarized in Table 3. The crude model's findings showed that a daily intake of Vit E in the Q4 range reduced the likelihood of developing NAFLD by 26% (OR = 0.74; 95% CI: 0.62–0.89; p = 0.001). After we adjusting for all covariates in model 1, we noted that Vit E intake in Q3 (OR = 0.78; 95% CI: 0.63–0.96; p = 0.019) and Q4 (OR = 0.63; 95% CI: 0.5–0.79; p < 0.001) was significantly associated with a reduced risk of NAFLD. Using a restricted cubic spline plot, we further analyzed the relationship between Vit E intake and NAFLD and noted a linear relationship (p = 0.124; Figure 3).

Subgroup analysis of the association between Vit E and NAFLD

In males, Q3 doses of Vit E reduced NAFLD incidence by 32% (OR = 0.68; 95% CI: 0.52–0.89; p = 0.006), and Q4 doses by 41% (OR = 0.59; 95% CI: 0.46–0.77; p < 0.001). After adjusting for covariates, these effects were amplified. However, no significant reduction was observed in females.

For participants over 50 years, Q3 (OR = 0.73; 95% CI: 0.57–0.94; p = 0.013) and Q4 (OR = 0.59; 95% CI: 0.46–0.76; p < 0.001) doses significantly reduced NAFLD risk, even after covariate adjustment. No reduction was found in those under 50 years. Table 4 and Figure 4 summarize the results.

MR analysis

Genome-wide association study (GWAS) statistics for NAFLD were obtained from a genome-wide meta-analysis predominantly comprising European populations, which was conducted by Ghodsian et al. (26). GWAS data for Vit E supplementation were collected from UK Biobank: ukb-a-463. Based on the selection criteria for SNPs, 24 SNPs were ultimately included to investigate the association between Vit E intake and NAFLD (Supplementary Table S1). IVW analysis revealed a causal connection between Vit E supplementation and NAFLD risk (OR = 0.028; p = 0.039). Analysis of scatter and funnel plots suggested a relatively balanced sample selection, with no evident bias (Figures 5, 6). IVW and MR-Egger regression analyses indicated that Cochran's Q statistic was 17.313 (p = 0.794) and 17.312 (p = 0.746), respectively. This suggests the absence of heterogeneity among SNPs.

TABLE 2 Association of dietary antioxidants and NAFLD.

Dietary antioxidants	OR (95% CI)	p
Vitamin A	1.00 (1.00 ~ 1.00)	0.639
Vitamin C	1.00 (1.00 ~ 1.00)	0.250
Vitamin E	0.98 (0.97 ~ 0.99)	0.001
Selenium	1.00 (1.00 ~ 1.00)	0.378
Zinc	1.00 (0.99 ~ 1.00)	0.482
Carotenoid	1.00 (1.00 ~ 1.00)	0.339

OR, odds ratio; CI, confidence interval.

Leave-one-out analysis revealed that upon removing any one SNP, the results of the remaining SNPs consistently fell on the same side of the invalid line. This verifies the robustness of the results of the MR analysis (Supplementary Figure S1).

Discussion

Studies have confirmed a close association between exogenous antioxidants and the occurrence and development of various diseases (27, 28). However, studies on the association between consuming exogenous antioxidants from dietary sources and NAFLD are limited. NAFLD progression is intricately associated with dietary patterns (29). In the absence of effective pharmacological interventions for NAFLD, we aimed to explore potential treatment modalities for NAFLD by improving lifestyle habits and dietary changes, among other factors.

Our study is the first attempt to utilize NHANES data to investigate the correlation between exogenous antioxidant intake and NAFLD and to establish the association between them at the genetic level via MR analysis. By analyzing exogenous antioxidants from the six common dietary sources included in this study, we discovered that only Vit E intake is significantly associated with the risk of developing NAFLD (p < 0.05). Vit E, an essential nutrient for the human body, comprises the benzodihydropyran structure and exhibits α-tocopherol bioactivity. It belongs to a class of substances primarily found in various plants (30). In addition, Vit E acts as a chain-breaking antioxidant, combating free radicals and scavenging them (31).

Oxidative stress may fundamentally affect NAFLD progression, with a strong correlation between excessive reactive oxygen species generation and NAFLD-associated hepatocyte death (32). Moreover, oxidative stress can increase the levels of IL-2, TNF, and IL-2, which may contribute to hepatic fibrosis (33, 34). Exogenous Vit E

TABLE 3 Association between Vit E and NAFLD.

Characteristics	Crude model		Model 1	
	OR (95% CI)	*p*	Adj. OR (95% CI)	*p*
Vit E	0.98 (0.97 ~ 0.99)	0.001	0.97 (0.96 ~ 0.98)	<0.001
Categorical Vit E				
Q 1	Ref		Ref	
Q 2	1.04 (0.87 ~ 1.24)	0.7	1.01 (0.83 ~ 1.23)	0.89
Q 3	0.84 (0.7 ~ 1.01)	0.063	0.78 (0.63 ~ 0.96)	0.019
Q 4	0.74 (0.62 ~ 0.89)	0.001	0.63 (0.5 ~ 0.79)	<0.001

Ref, reference. Crude model: none variables were adjusted. Model 1: adjusted for all variables.

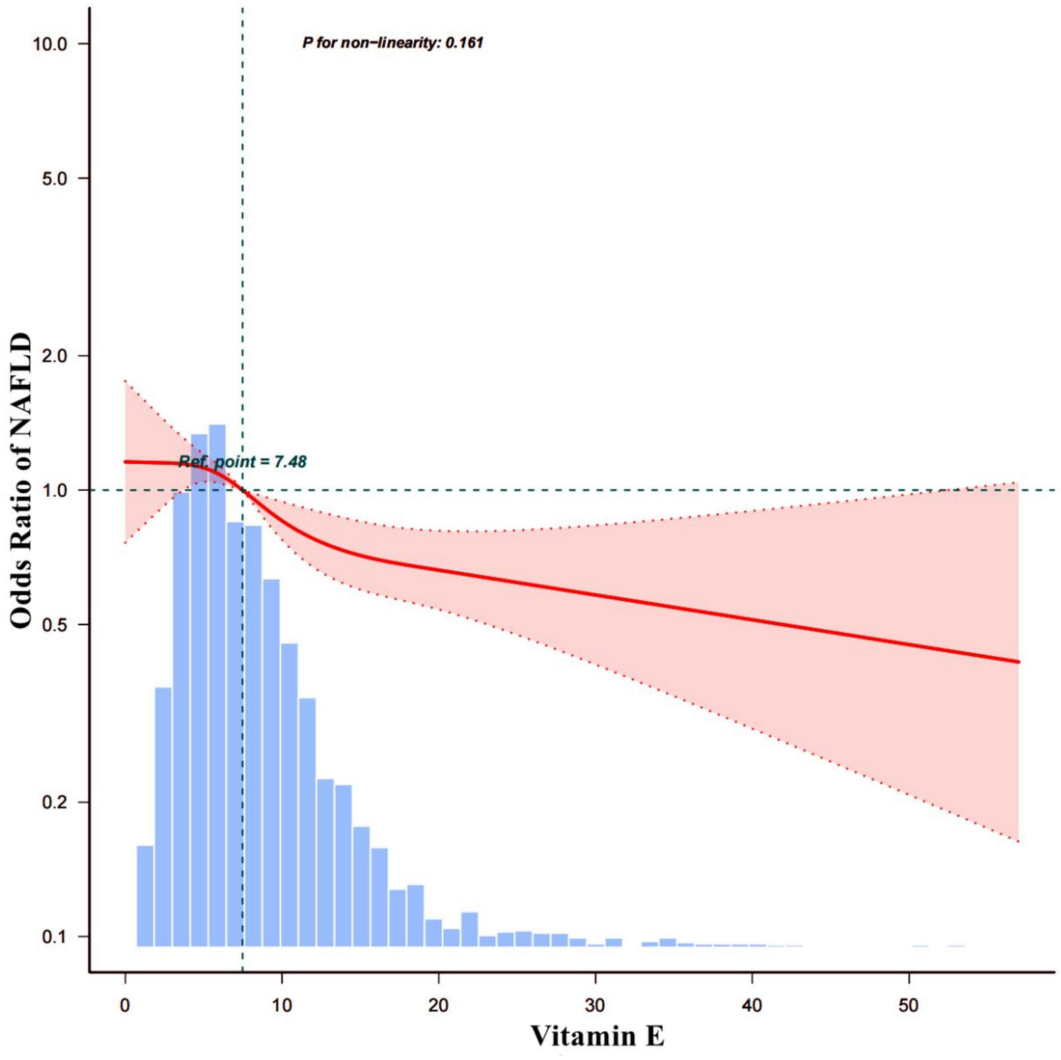

FIGURE 3
The dose–response relationship between vitamin E and the prevalence of NAFLD.

supplementation can inhibit JNK-mediated inflammatory signaling pathways to attenuate inflammation levels in patients with NAFLD (35). Vit E supplementation, as demonstrated in a study by Bai Y et al., was found to activate the AMPK pathway and reduce oxidative stress, resulting in improved NAFLD in rats (36).

Several studies have indicated a close relationship between Vit E and NAFLD. To determine into the correlation between the intake of various amounts of Vit E and NAFLD, we developed two distinct models. Both models revealed that the risk of NAFLD was significantly decreased ($p < 0.05$) with Vit E intake up to Q3 and Q4. Sanyal AJ et al.

TABLE 4 Subgroup analysis for the association between Vit E and NAFLD.

Characteristics	Crude model		Model 1	
	OR (95% CI)	*p*	Adj. OR (95% CI)	*p*
Male				
Q 1	Ref		Ref	
Q 2	0.83 (0.63 ~ 1.09)	0.184	0.76 (0.56 ~ 1.03)	0.078
Q 3	0.68 (0.52 ~ 0.89)	0.006	0.59 (0.44 ~ 0.81)	0.001
Q 4	0.59 (0.46 ~ 0.77)	<0.001	0.49 (0.71 ~ 0.88)	<0.001
P for trend	<0.001		<0.001	
Female				
Q 1	Ref		Ref	
Q 2	1.22 (0.96 ~ 1.55)	0.103	1.18 (0.91 ~ 1.54)	0.215
Q 3	0.99 (0.78 ~ 1.27)	0.963	0.90 (0.68 ~ 1.21)	0.495
Q 4	0.92 (0.71 ~ 1.20)	0.555	0.72 (0.51 ~ 1.00)	0.050
P for trend	0.369		0.031	
Age≤50years				
Q 1	Ref		Ref	
Q 2	1.16 (0.89 ~ 1.50)	0.270	1.07 (0.80 ~ 1.43)	0.650
Q 3	0.98 (0.75 ~ 1.27)	0.881	0.89 (0.66 ~ 1.21)	0.462
Q 4	0.90 (0.70 ~ 1.17)	0.433	0.81 (0.58 ~ 1.13)	0.207
P for trend	0.230		0.126	
Age>50years				
Q 1	Ref		Ref	
Q 2	0.92 (0.72 ~ 1.18)	0.519	0.97 (0.74 ~ 1.28)	0.839
Q 3	0.73 (0.57 ~ 0.94)	0.013	0.68 (0.51 ~ 0.92)	0.011
Q 4	0.59 (0.46 ~ 0.76)	<0.001	0.49 (0.35 ~ 0.68)	<0.001
P for trend	<0.001		<0.001	

Ref, reference. Crude model adjusted for: none. Model 1 adjusted for: all variables.

have suggested that the daily supplementation of 800 IU of Vit E can be used to treat NAFLD (37). Therefore, increasing exogenous Vit E intake may be an effective measure to prevent NAFLD development.

Subgroup analyses revealed a significant association between Vit E and NAFLD in men and those older than 50 years ($p < 0.05$), but not in women and those younger than 50 years ($p > 0.05$). Studies have revealed that the dietary patterns of men are dominated by "convenience, red meat, and alcohol," which often results in oxidative stress and chronic inflammation in the body (38–41). At the same time women produce higher levels of oestrogen which can act as an antioxidant (42). Therefore, in male patients, supplementing Vit E is more essential to decrease oxidative stress in the body and subsequently decrease NAFLD risk. Vit E can efficiently enhance age-related disruption of immune and inflammatory reactions (43). In the present study, we suggested that Vit E can effectively decrease NAFLD risk in the elderly population. Hemilä H's findings showed that a daily intake of 50 mg of vitamin E reduced the risk of pneumonia by 67% in older people, but did not have the same effect in younger people (44). Study has shown that aging causes elevated levels of reactive oxygen species in the body (45), which promotes oxidative stress. Therefore, age can serve as a determining factor in assessing the need for Vit E, and additional studies are warranted to substantiate this. Although studies on using Vit E for treating NAFLD are available, critical evidence to support the use of Vit E as a key measure for treating and preventing NAFLD is lacking.

Observational studies are often affected by confounding factors; however, MR can analyze the relationship between exposure factors and outcomes while decreasing the effect of confounding factors (46). Based on the conclusions drawn from the cross-sectional study, we further validated the relationship between Vit E intake and NAFLD using MR analysis. IVW-MR analysis revealed an association between Vit E intake and NAFLD. Furthermore, it revealed that Vit E intake decreases NAFLD risk. The results of observational studies and MR analyses support the idea that Vit E supplementation decreases NAFLD risk. Combined with the findings of other related studies (47–49), we hypothesize that Vit E intervenes in NAFLD development primarily by regulating oxidative stress, inflammatory responses, gene expression, and cell signaling. However, the specific molecular mechanism underlying the role of Vit E in NAFLD remains unclear. Therefore, additional experiments are warranted to verify this.

In our study, we analyzed the common exogenous antioxidants in the daily diet and finally observed that Vit E can effectively decrease NAFLD risk. This provides some reference for treating and preventing NAFLD in clinical settings. Our study is the first to

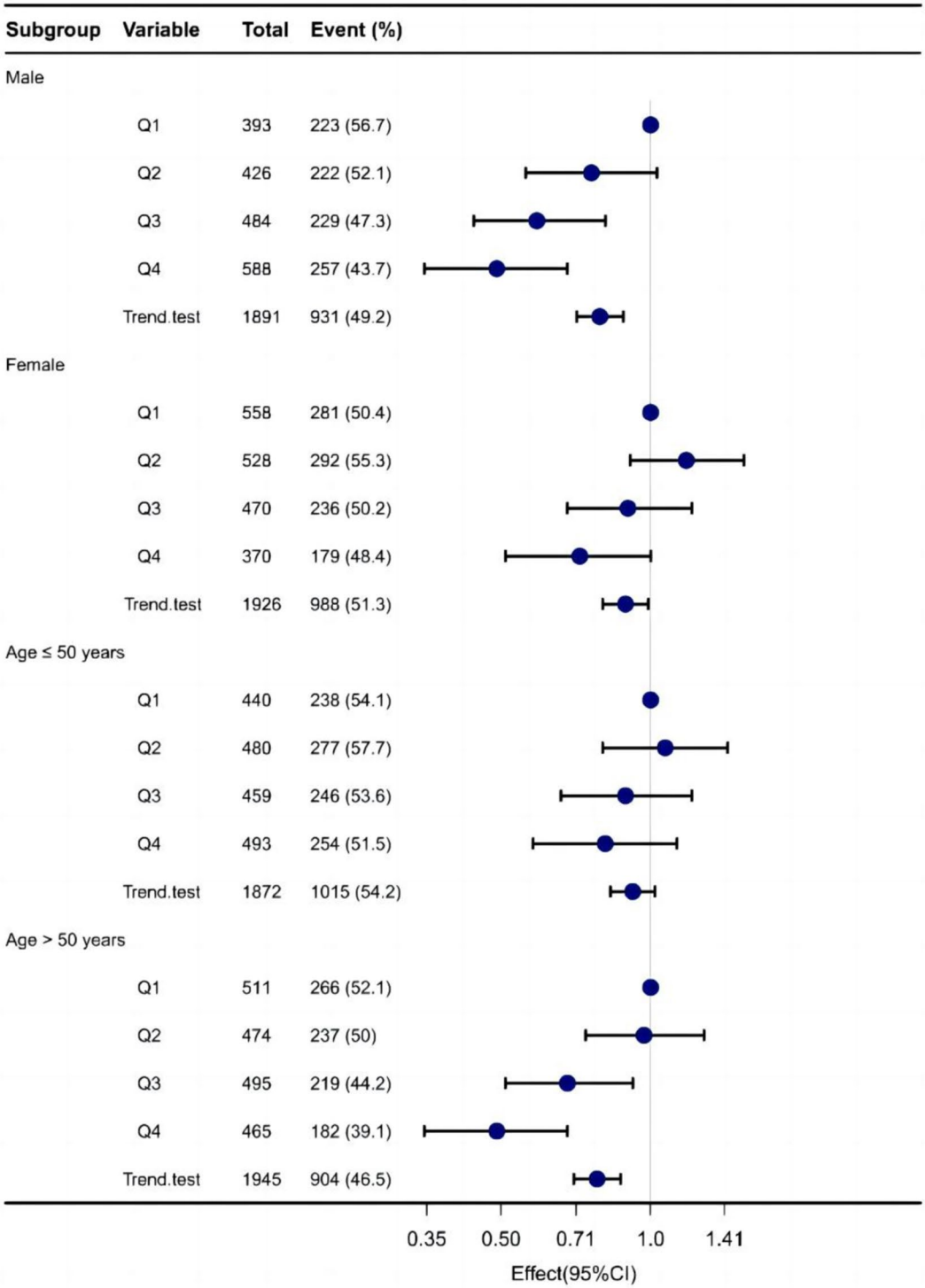

FIGURE 4
Forest plot demonstrates the risk association between different doses of dietary sources of vitamin E and NAFLD across various subgroups.

use a cross-sectional design combined with MR analysis to explore the relationship between dietary antioxidant sources and NAFLD. The combination of both research methods provided more convincing results. However, our study still has some limitations that should be acknowledged. First information on the intake of exogenous antioxidants was obtained from the 24 h diet recall in the questionnaire, resulting in possible bias from the real situation. Second, owing to the absence of age- and sex-stratified GWAS data, we could not use MR analyses to validate the association between Vit E intake and NAFLD across sex and age

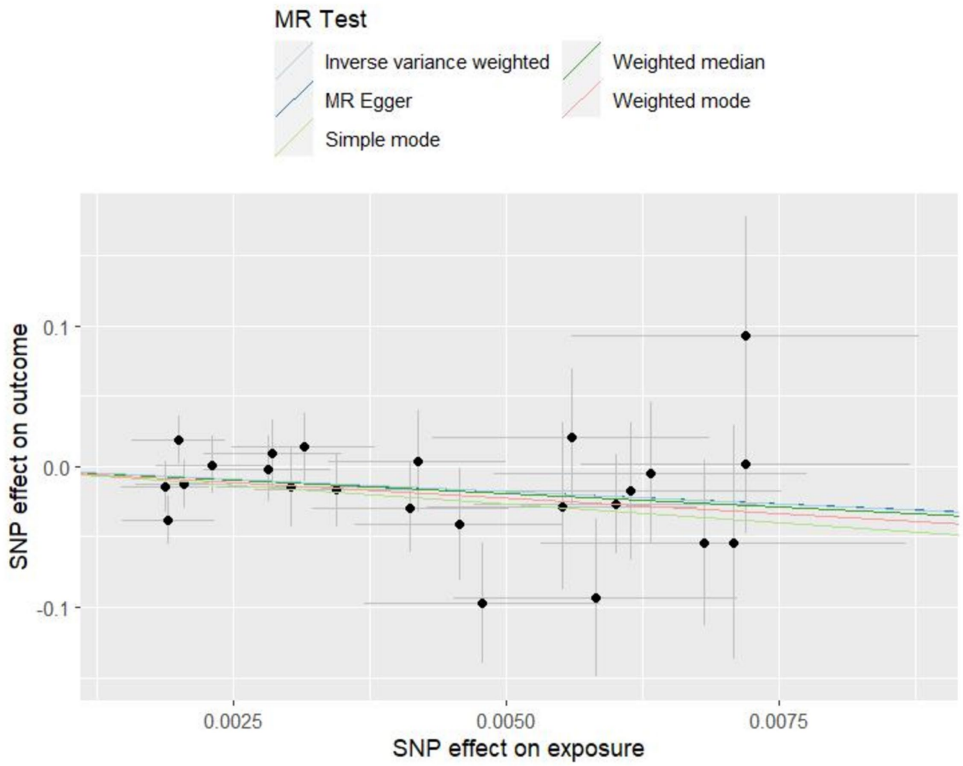

FIGURE 5
Scatter plots of Mendelian randomization tests assessing the effect of dietary sources of vitamin E on NAFLD.

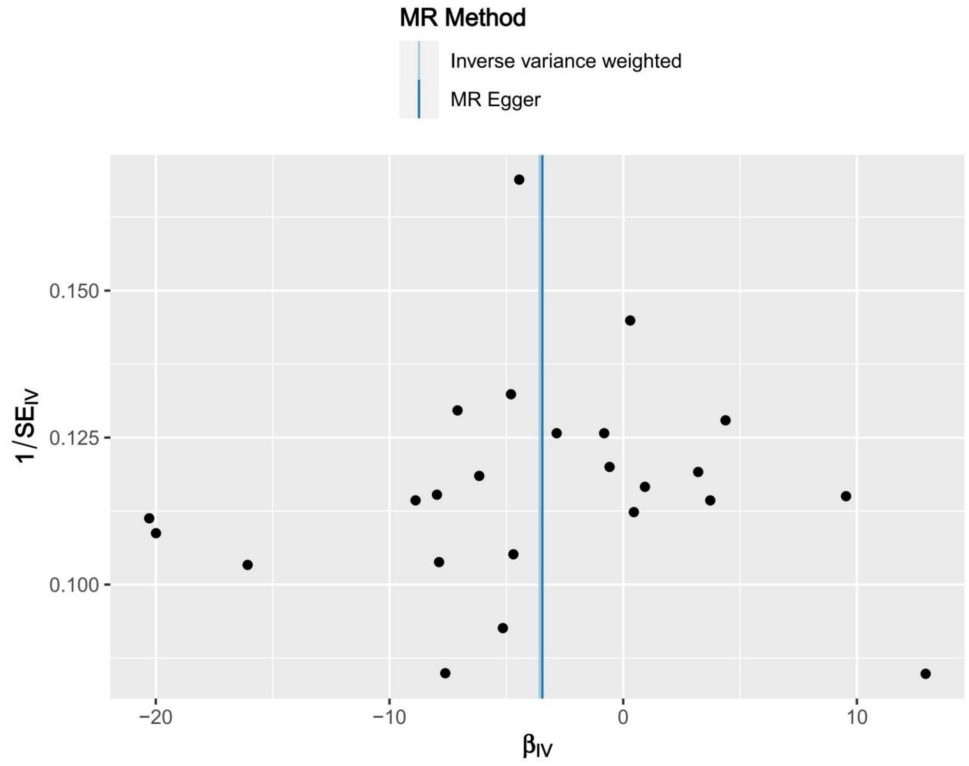

FIGURE 6
Funnel plots of Mendelian randomization tests assessing the effect of dietary sources of vitamin E on NAFLD.

found in cross-sectional studies. The subjects of the MR study were all Europeans, so we cannot be sure whether similar results can be obtained in other populations. In addition, MR analysis is a statistical method study and cannot elucidate the intrinsic pathogenesis, thus further relevant clinical and experimental validation is needed.

Conclusion

Our research indicates a negative and linear relationship between daily vitamin E intake and NAFLD. Furthermore, Mendelian randomization results imply a connection between daily consumption of vitamin E and the occurrence of NAFLD. The recommended supplemental dose of vitamin E remains to be determined.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: NHANES.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

ZY: Conceptualization, Data curation, Investigation, Software, Validation, Visualization, Writing – original draft. ZJ: Conceptualization, Data curation, Formal analysis, Project administration, Supervision, Writing – review & editing. LQ: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing. LL: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – review & editing. XQ: Data curation, Formal analysis, Methodology, Writing – review & editing. KH: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Software, Visualization, Writing – review & editing.

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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.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1447524/full#supplementary-material

References

1. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*. (2018) 67:328–57. doi: 10.1002/hep.29367

2. Zhou J, Zhou F, Wang W, Zhang XJ, Ji YX, Zhang P, et al. Epidemiological features of NAFLD from 1999 to 2018 in China. *Hepatology*. (2020) 71:1851–64. doi: 10.1002/hep.31150

3. Bence KK, Birnbaum MJ. Metabolic drivers of non-alcoholic fatty liver disease. *Mol Metab*. (2021) 50:101143. doi: 10.1016/j.molmet.2020.101143

4. Huang F, Lyu B, Xie F, Li F, Xing Y, Han Z, et al. From gut to liver: unveiling the differences of intestinal microbiota in NAFL and NASH patients. *Front Microbiol*. (2024) 15:1366744. doi: 10.3389/fmicb.2024.1366744

5. Haldar D, Kern B, Hodson J, Armstrong MJ, Adam R, Berlakovich G, et al. Outcomes of liver transplantation for non-alcoholic steatohepatitis: a European liver transplant registry study. *J Hepatol*. (2019) 71:313–22. doi: 10.1016/j.jhep.2019.04.011

6. Cholankeril G, Wong RJ, Hu M, Perumpail RB, Yoo ER, Puri P, et al. Liver transplantation for nonalcoholic steatohepatitis in the US: temporal trends and outcomes. *Dig Dis Sci*. (2017) 62:2915–22. doi: 10.1007/s10620-017-4684-x

7. Harrison SA, Bashir M, Moussa SE, McCarty K, Pablo Frias J, Taub R, et al. Effects of Resmetirom on noninvasive endpoints in a 36-week phase 2 active treatment extension study in patients with NASH. *Hepatol Commun*. (2021) 5:573–88. doi: 10.1002/hep4.1657

8. Harrison SA, Bashir MR, Guy CD, Zhou R, Moylan CA, Frias JP, et al. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre,

randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet*. (2019) 394:2012–24. doi: 10.1016/S0140-6736(19)32517-6

9. Fois AG, Paliogiannis P, Sotgia S, Mangoni AA, Zinellu E, Pirina P, et al. Evaluation of oxidative stress biomarkers in idiopathic pulmonary fibrosis and therapeutic applications: a systematic review. *Respir Res*. (2018) 19:51. doi: 10.1186/s12931-018-0754-7

10. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med*. (2018) 24:908–22. doi: 10.1038/s41591-018-0104-9

11. Takaki A, Kawai D, Yamamoto K. Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). *Int J Mol Sci*. (2013) 14:20704–28. doi: 10.3390/ijms141020704

12. Hernández-Gea V, Hilscher M, Rozenfeld R, Lim MP, Nieto N, Werner S, et al. Endoplasmic reticulum stress induces fibrogenic activity in hepatic stellate cells through autophagy. *J Hepatol*. (2013) 59:98–104. doi: 10.1016/j.jhep.2013.02.016

13. Meulmeester FL, Luo J, Martens LG, Mills K, van Heemst D, Noordam R. Antioxidant supplementation in oxidative stress-related diseases: what have we learned from studies on alpha-tocopherol. *Antioxidants (Basel)*. (2022) 11:2322. doi: 10.3390/antiox11122322

14. Ji Y, Gao Y, Chen H, Yin Y, Zhang W. Indole-3-acetic acid alleviates nonalcoholic fatty liver disease in mice via attenuation of hepatic lipogenesis, and oxidative and inflammatory stress. *Nutrients*. (2019) 11:2062. doi: 10.3390/nu11092062

15. Pereira E, Araujo BP, Rodrigues KL, Silvares RR, Martins CSM, Flores EEI, et al. Simvastatin improves microcirculatory function in nonalcoholic fatty liver disease and downregulates oxidative and ALE-RAGE stress. *Nutrients*. (2022) 14:716. doi: 10.3390/nu14030716

16. Zhang JK, Zhou XL, Wang XQ, Zhang JX, Yang ML, Liu YP, et al. Que Zui tea ameliorates hepatic lipid accumulation and oxidative stress in high fat diet induced nonalcoholic fatty liver disease. *Food Res Int*. (2022) 156:111196. doi: 10.1016/j.foodres.2022.111196

17. Zhu S, Zhang J, Zhu D, Jiang X, Wei L, Wang W, et al. Adipose tissue plays a major role in retinoic acid-mediated metabolic homoeostasis. *Adipocytes*. (2022) 11:47–55. doi: 10.1080/21623945.2021.2015864

18. Xie ZQ, Li HX, Tan WL, Yang L, Ma XW, Li WX, et al. Association of Serum Vitamin C with NAFLD and MAFLD among adults in the United States. *Front Nutr*. (2021) 8:795391. doi: 10.3389/fnut.2021.795391

19. Panera N, Braghini MR, Crudele A, Smeriglio A, Bianchi M, Condorelli AG, et al. Combination treatment with Hydroxytyrosol and vitamin E improves NAFLD-related fibrosis. *Nutrients*. (2022) 14:3791. doi: 10.3390/nu14183791

20. Hafez A, Anwar Rashed S, Amin YA, Hemdan SB, et al. Decreased serum zinc, selenium, and vitamin E as possible risk factors of hepatic fibrosis in non-alcoholic fatty liver disease. *Nutr Health*. (2022) 22:2601060221103032. doi: 10.1177/02601060221103032

21. Wang Y, Liu B, Wu P, Chu Y, Gui S, Zheng Y, et al. Dietary selenium alleviated mouse liver oxidative stress and NAFLD induced by obesity by regulating the KEAP1/NRF2 pathway. *Antioxidants (Basel)*. (2022) 11:349. doi: 10.3390/antiox11020349

22. Wright ME, Mayne ST, Stolzenberg-Solomon RZ, Li Z, Pietinen P, Taylor PR, et al. Development of a comprehensive dietary antioxidant index and application to lung cancer risk in a cohort of male smokers. *Am J Epidemiol*. (2004) 160:68–76. doi: 10.1093/aje/kwh173

23. Yu YC, Paragomi P, Wang R, Jin A, Schoen RE, Sheng LT, et al. Composite dietary antioxidant index and the risk of colorectal cancer: findings from the Singapore Chinese health study. *Int J Cancer*. (2022) 150:1599–608. doi: 10.1002/ijc.33925

24. Zhang X, Heredia NI, Balakrishnan M, Thrift AP. Prevalence and factors associated with NAFLD detected by vibration controlled transient elastography among US adults: results from NHANES 2017–2018. *PLoS One*. (2021) 16:e0252164. doi: 10.1371/journal.pone.0252164

25. Chen R, Liu H, Zhang G, Zhang Q, Hua W, Zhang L, et al. Antioxidants and the risk of stroke: results from NHANES and two-sample Mendelian randomization study. *Eur J Med Res*. (2024) 29:50. doi: 10.1186/s40001-024-01646-5

26. Ghodsian N, Abner E, Emdin CA, Gobeil É, Taba N, Haas ME, et al. Electronic health record-based genome-wide meta-analysis provides insights on the genetic architecture of non-alcoholic fatty liver disease. *Cell Rep Med*. (2021) 2:100437. doi: 10.1016/j.xcrm.2021.100437

27. Qi X, Jha SK, Jha NK, Dewanjee S, Dey A, Deka R, et al. Antioxidants in brain tumors: current therapeutic significance and future prospects. *Mol Cancer*. (2022) 21:204. doi: 10.1186/s12943-022-01668-9

28. Kolarzyk E, Skop-Lewandowska A, Jaworska J, Ostachowska-Gąsior A, Krzeszowska-Rosiek T. Dietary intake of antioxidants and fats in the context of coronary heart disease prevention among elderly people. *Ann Agric Environ Med*. (2018) 25:131–6. doi: 10.5604/12321966.1233574

29. Petermann-Rocha F, Wirth MD, Boonpor J, Parra-Soto S, Zhou Z, Mathers JC, et al. Associations between an inflammatory diet index and severe non-alcoholic fatty liver disease: a prospective study of 171,544 UK biobank participants. *BMC Med*. (2023) 21:123. doi: 10.1186/s12916-023-02793-y

30. Violi F, Nocella C, Loffredo L, Carnevale R, Pignatelli P. Interventional study with vitamin E in cardiovascular disease and meta-analysis. *Free Radic Biol Med*. (2022) 178:26–41. doi: 10.1016/j.freeradbiomed.2021.11.027

31. Miyazawa T, Burdeos GC, Itaya M, Nakagawa K, Miyazawa T. Vitamin E: regulatory redox interactions. *IUBMB Life*. (2019) 71:430–41. doi: 10.1002/iub.2008

32. Mantena SK, King AL, Andringa KK, Eccleston HB, Bailey SM. Mitochondrial dysfunction and oxidative stress in the pathogenesis of alcohol- and obesity-induced fatty liver diseases. *Free Radic Biol Med*. (2008) 44:1259–72. doi: 10.1016/j.freeradbiomed.2007.12.029

33. Chen Z, Tian R, She Z, Cai J, Li H. Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. *Free Radic Biol Med*. (2020) 152:116–41. doi: 10.1016/j.freeradbiomed.2020.02.025

34. Paradies G, Paradies V, Ruggiero FM, Petrosillo G. Oxidative stress, cardiolipin and mitochondrial dysfunction in nonalcoholic fatty liver disease. *World J Gastroenterol*. (2014) 20:14205–18. doi: 10.3748/wjg.v20.i39.14205

35. Demirel-Yalciner T, Sozen E, Ozaltin E, Sahin A, Ozer NK. Alpha-tocopherol supplementation reduces inflammation and apoptosis in high cholesterol mediated nonalcoholic steatohepatitis. *Biofactors*. (2021) 47:403–13. doi: 10.1002/biof.1700

36. Bai Y, Li T, Liu J, Wang Y, Wang C, Ju S, et al. Aerobic exercise and vitamin E improve high-fat diet-induced NAFLD in rats by regulating the AMPK pathway and oxidative stress. *Eur J Nutr*. (2023) 62:2621–32. doi: 10.1007/s00394-023-03179-9

37. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med*. (2010) 362:1675–85. doi: 10.1056/NEJMoa0907929

38. Sprake EF, Russell JM, Cecil JE, Cooper RJ, Grabowski P, Pourshahidi LK, et al. Dietary patterns of university students in the UK: a cross-sectional study. *Nutr J*. (2018) 17:90. doi: 10.1186/s12937-018-0398-y

39. Zelber-Sagi S, Ivancovsky-Wajcman D, Fliss Isakov N, Webb M, Orenstein D, Shibolet O, et al. High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance. *J Hepatol*. (2018) 68:1239–46. doi: 10.1016/j.jhep.2018.01.015

40. Kim HG, Huang M, Xin Y, Zhang Y, Zhang X, Wang G, et al. The epigenetic regulator SIRT6 protects the liver from alcohol-induced tissue injury by reducing oxidative stress in mice. *J Hepatol*. (2019) 71:960–9. doi: 10.1016/j.jhep.2019.06.019

41. Guo X, Yin X, Liu Z, Wang J. Non-alcoholic fatty liver disease (NAFLD) pathogenesis and natural products for prevention and treatment. *Int J Mol Sci*. (2022) 23:15489. doi: 10.3390/ijms232415489

42. Kim SY. Oxidative stress and gender disparity in cancer. *Free Radic Res*. (2022) 56:90–105. doi: 10.1080/10715762.2022.2038789

43. Meydani SN, Lewis ED, Wu D. Perspective: should vitamin E recommendations for older adults be increased. *Adv Nutr*. (2018) 9:533–43. doi: 10.1093/advances/nmy035

44. Hemilä H. Vitamin E administration may decrease the incidence of pneumonia in elderly males. *Clin Interv Aging*. (2016) 11:1379–85. doi: 10.2147/CIA.S114515

45. Korovesis D, Rubio-Tomás T, Tavernarakis N. Oxidative stress in age-related neurodegenerative diseases: an overview of recent tools and findings. *Antioxidants (Basel)*. (2023) 12:131. doi: 10.3390/antiox12010131

46. Skrivankova VW, Richmond RC, Woolf B, Davies NM, Swanson SA, VanderWeele TJ, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomisation (STROBE-MR): explanation and elaboration. *BMJ*. (2021) 375:n2233. doi: 10.1136/bmj.n2233

47. El Hadi H, Vettor R, Rossato M. Vitamin E as a treatment for nonalcoholic fatty liver disease: reality or myth. *Antioxidants (Basel)*. (2018) 7:12. doi: 10.3390/antiox7010012

48. Phung N, Pera N, Farrell G, Leclercq I, Hou JY, George J. Pro-oxidant-mediated hepatic fibrosis and effects of antioxidant intervention in murine dietary steatohepatitis. *Int J Mol Med*. (2009) 24:171–80. doi: 10.3892/ijmm_00000220

49. Lavine JE, Schwimmer JB, van Natta M, Molleston JP, Murray KF, Rosenthal P, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA*. (2011) 305:1659–68. doi: 10.1001/jama.2011.520

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The relationship between dietary vitamin B1 intake and severe abdominal aortic calcification among the general population in the United States

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Background: Vitamin B1 deficiency is closely associated with vascular system damage, but the relationship between dietary vitamin B1 intake and abdominal aortic calcification (AAC) remains unclear and warrants further investigation.
Methods: 2,640 participants from the National Health and Nutrition Examination Survey (NHANES) 2013–2014 were included in the study. Severe AAC was defined as Kauppila score >5. Multivariable logistic regression analysis and restricted cubic splines (RCS) were used to examine the relationship between dietary vitamin B1 and severe AAC.
Results: The increase in dietary intake of vitamin B1 is significantly correlated with a decrease in the risk of severe AAC (OR: 0.601, 95% CI: 0.406, 0.892). Compared to the first quartile of dietary vitamin B1 intake, the fourth quartile had a significantly reduced risk of severe AAC (OR: 0.358, 95% CI: 0.172, 0.744). RCS indicated a decreasing trend in the risk of severe AAC with increasing dietary vitamin B1 intake.
Conclusion: Our research findings indicate that the increase in dietary intake of vitamin B1 is significantly associated with a decrease in the risk of severe AAC. Thus, increasing dietary vitamin B1 intake appropriately may reduce the risk of severe AAC.

KEYWORDS

dietary vitamin B1 intake, severe abdominal aortic calcification, NHANES, multivariable logistic regression, RCS

Introduction

Abdominal aortic calcification (AAC) is a complex pathological process influenced by multiple factors, characterized by the pathological deposition of calcium phosphate crystals within the arterial intima (1). Research findings suggest that with advancing age, both the prevalence and severity of AAC tend to increase gradually (2). Currently known factors closely associated with the onset of AAC include age, chronic kidney disease, gender, diabetes, and hypertension, among others (3–6). Increasing evidence indicates that severe AAC serves as a risk factor for cardiovascular diseases (CVD) and is highly correlated with the rupture of atherosclerotic plaques, adverse cardiovascular events, and increased all-cause mortality risk (7, 8). Despite advancements, our

understanding of the mechanisms underlying AAC remains incomplete, and effective preventive and treatment strategies are still lacking. Considering the significant correlation between severe vascular calcification and CVD as well as mortality rates (9), along with the challenges in treatment, further exploration of prevention and improvement methods for AAC is warranted.

Diet plays a crucial role in vascular health. Vitamin B1, as a water-soluble vitamin, is believed to be closely related to vascular health (10). Previous studies on the relationship between dietary vitamins and abdominal aortic calcification have mainly focused on dietary vitamins C, D, and K2 (11–14). However, there is still a lack of research on the relationship between dietary vitamin B1 intake and severe abdominal aortic calcification. Vitamin B1 plays an important role in regulating energy metabolism, maintaining endothelial cell function, and promoting nerve conduction (15). Previous studies have suggested an association between dietary vitamin B1 intake and increased risk of stroke and cardiovascular mortality (16). Despite the biological plausibility, the relationship between dietary vitamin B1 intake and severe AAC remains underexplored. Understanding this relationship is crucial, as it could inform dietary recommendations and public health strategies aimed at preventing or slowing the progression of AAC and its associated cardiovascular risks. Additionally, changing dietary habits as a non-invasive and cost-effective strategy for preventing severe abdominal aortic calcification is more acceptable and easier to implement for the general population.

National Health and Nutrition Examination Survey (NHANES) is a nationally representative survey that evaluates the health and nutritional status of the general United States population by gathering comprehensive data on diet, nutrition, and overall health. This study aims to evaluate the relationship between dietary vitamin B1 intake and AAC in the general population of the United States, utilizing data from NHANES 2013–2014. To fill the gap in research on the relationship between dietary vitamin B1 intake and severe abdominal aortic calcification.

Methods

Study population

NHANES is a cross-sectional study conducted on the general population of the United States aimed at investigating the nutritional and health-related information of the general population. We utilized relevant data on dietary vitamin B1 intake and AAC from NHANES 2013–2014. The 2013–2014 NHANES research protocol received approval from the National Center for Health Statistics. All subjects provided written informed consent forms. The deidentified data from the NHANES initiative is accessible to the general public at no cost. Under local regulations, this subsequent analysis does not necessitate additional authorization from the institutional review committee. A total of 10,175 participants were identified in NHANES 2011–2016. After excluding participants with missing AAC assessments ($n = 7{,}035$) and those lacking dietary vitamin B1 data ($n = 500$), a total of 2,640 participants were included in the final analysis (Figure 1).

Table 1 provides a detailed overview of the baseline characteristics of the 2,640 participants eligible for the study on dietary vitamin B1 and AAC, grouped by the presence or absence of severe AAC. Among them, there were 288 participants with severe AAC and 2,352 participants without severe AAC. The average age of the non-severe AAC group was 59.29 ± 0.27, while the average age of the severe AAC group was 71.03 ± 0.93. There were significant differences between the two groups in terms of age, total cholesterol levels, smoking history, alcohol consumption history, history of CVD, diabetes, and hypertension ($P < 0.05$).

Abdominal aortic calcification

AAC was defined using dual-energy x-ray absorptiometry. AAC was visually identified as diffuse white spots in the abdominal aorta. The severity of AAC was quantified using the Kauppila score. Severe AAC was defined as a Kauppila score >5.

Dietary vitamin B1 intake

Dietary vitamin B1 intake was determined by recalling and calculating the food intake of participants over the past 24 h. The NHANES dietary interview procedures manual provided detailed instructions for this process.

Covariates

The study included the following covariates: age, gender, race, BMI, smoking history (never, former, current), alcohol

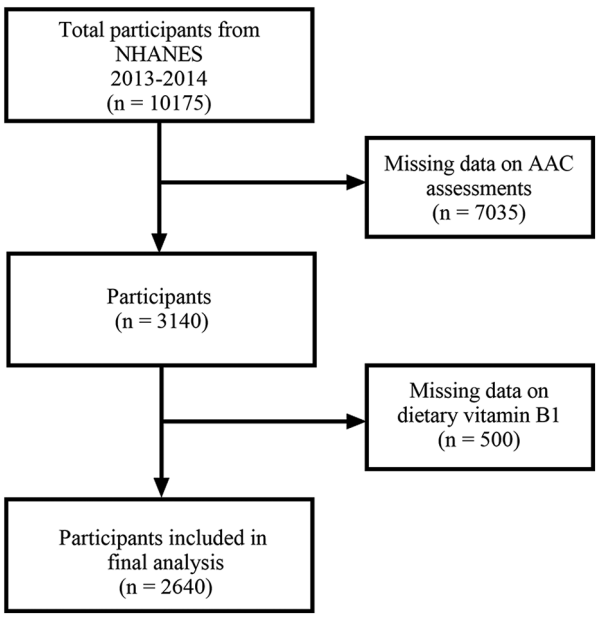

FIGURE 1
Flowchart depicting participant selection in the study.

TABLE 1 Population characteristics stratified by severe AAC.

Variable	Non-Severe AAC	Severe AAC	*P*-value
Age (years)	56.29 (0.27)	71.03 (0.93)	<0.01
Sex			0.56
Male	1,110 (47.96)	133 (44.91)	
Female	1,242 (52.04)	155 (55.09)	
Race			0.35
White	1,036 (69.67)	185 (75.10)	
Black	483 (10.75)	40 (7.36)	
Mexican American	312 (7.24)	25 (4.36)	
Other Race	521 (12.34)	38 (13.19)	
BMI ($kg/m^{2)}$	28.71 (0.16)	27.30 (0.68)	0.07
Total cholesterol (mg/dL)	198.05 (1.35)	186.20 (3.35)	0.01
Smoke			0.01
Former	630 (26.39)	117 (41.09)	
Never	1,316 (57.46)	118 (42.98)	
Now	405 (16.15)	52 (15.93)	
Alcohol consumption			0.06
Former	447 (16.91)	91 (26.13)	
Never	336 (11.51)	32 (9.04)	
Mild	859 (40.19)	107 (43.78)	
Moderate	332 (18.16)	19 (8.43)	
Heavy	299 (13.24)	34 (12.63)	
CVD			<0.01
Yes	246 (9.64)	99 (31.92)	
No	2,105 (90.36)	189 (68.08)	
Diabetes			<0.01
Yes	485 (14.41)	122 (37.68)	
No	1,867 (85.59)	166 (62.32)	
Hypertension			<0.01
Yes	1,200 (48.01)	232 (77.70)	
No	1,152 (51.99)	56 (22.30)	

consumption history (never, former, mild, moderate, heavy), total cholesterol, CVD, hypertension, and diabetes status. In terms of smoking, never smoking is defined as smoking less than 100 cigarettes in one's lifetime. Former smoking was defined as having smoked more than 100 cigarettes in one's lifetime but now having completely quit smoking. Now smoking is defined as having smoked more than 100 cigarettes in one's lifetime and currently smoking on some days or every day. In terms of alcohol consumption, never alcohol consumption is defined as drinking less than 12 glasses in one's lifetime. Former alcohol consumption is defined as drinking at least 12 times within a year and not drinking in the previous year, or not drinking in the previous year but drinking at least 12 glasses throughout one's life. Mild alcohol consumption was defined as 0–2 drinks/day for males and 0–1 drinks/day for females. Moderate alcohol consumption was defined as 3 drinks/day for males and 2 drinks/day for females, or binge drinking at least 2 times a month and less than 5 times. Heavy alcohol consumption is defined as men drinking at least 4 drinks per day, women drinking at least 3 drinks per day, or binge drinking at least 5 times a month. CVD was determined through a questionnaire. Hypertension was defined as an average systolic blood pressure ≥140 mmHg and/or an average diastolic blood pressure ≥90 mmHg, diagnosed by a doctor, or the use of antihypertensive drugs. Diabetes was defined as a random blood glucose level ≥11.1 mmol/L, fasting blood glucose ≥7 mmol/L, glycosylated hemoglobin ≥6.5%, two-hour OGTT blood glucose ≥11.1 mmol/L, diagnosed by a doctor, or the use of hypoglycemic drugs.

Statistical analysis

Statistical analysis was performed using R Studio (version 4.2.1) with weighted analysis based on dietary factors. Weighted Student's *t*-test, Mann Whitney *U*-test, and Chi-squared test were used to compare the differences between the two groups. Continuous variables were presented as mean (standard error), while categorical variables were presented as numbers (weighted percentages). We divide the intake of vitamin B1 into quartiles, Q1 < 1.09 mg, 1.09 mg ≤ Q2 < 1.43 mg, 1.43 mg ≤ Q2 < 1.89 mg, Q2 ≥ 1.89 mg. The OR and 95% CI are calculated in the "survey" R package. The relationship between dietary vitamin B1 and AAC was explored using multivariable logistic regression. We used an restricted cubic splines (RCS) model with four knots to further explore the association between dietary vitamin B1 and AAC after adjusting for all covariates. We selected the median of dietary vitamin B1 intake as the reference value (1.42 mg). Subgroup analyses were conducted based on age, gender, smoking, alcohol consumption, hypertension, CVD, and diabetes status to investigate the relationship between dietary vitamin B1 intake and severe AAC. The *P* for interaction is obtained using a likelihood ratio test.

Results

Dietary vitamin B1 intake and severe AAC

To validate the relationship between dietary vitamin B1 intake and AAC, we conducted a multivariable logistic regression analysis. In the unadjusted model (OR: 0.662, 95% CI: 0.511, 0.857), after adjusting for age, gender, race (OR: 0.583, 95% CI: 0.370, 0.917), and adjusting for all covariates (OR: 0.601, 95% CI: 0.406, 0.892), the increase in dietary intake of vitamin B1 is significantly correlated with a decrease in the risk of severe AAC. After full adjustment for covariates, compared to the first quartile of dietary vitamin B1 intake, the fourth quartile showed a significantly reduced risk of severe AAC (OR: 0.358, 95% CI: 0.172, 0.744) (Table 2).

Restricted cubic splines

RCS depicted the dose-response relationship between dietary vitamin B1 intake and severe AAC. The results showed a linear negative correlation between dietary vitamin B1 intake and the risk of severe AAC (*P* for overall = 0.003 < 0.05, *P* for nonlinearity = 0.852 > 0.05) (Figure 2).

TABLE 2 Relationship between dietary vitamin B1 and severe AAC.

Result	Model 1		Model 2		Model 3	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Dietary vitamin B1 and severe AAC	0.662 (0.511, 0.857)	0.004	0.583 (0.370, 0.917)	0.025	0.601 (0.406, 0.892)	0.015
Q1	ref	ref	ref	ref	ref	ref
Q2	0.655 (0.275, 1.562)	0.310	0.619 (0.231, 1.660)	0.288	0.716 (0.285, 1.795)	0.450
Q3	0.840 (0.556, 1.268)	0.375	0.658 (0.402, 1.077)	0.084	0.741 (0.466, 1.179)	0.189
Q4	0.426 (0.259, 0.701)	0.003	0.354 (0.153, 0.820)	0.022	0.358 (0.172, 0.744)	0.009

Adjusted variables: Model 1: unadjusted. Model 2: age, sex, race. Model 3: age, sex, race, BMI, smoking history, alcohol consumption history, total cholesterol, cardiovascular diseases, hypertension, and diabetes.
OR, odds ratio; CI, confidence interval.

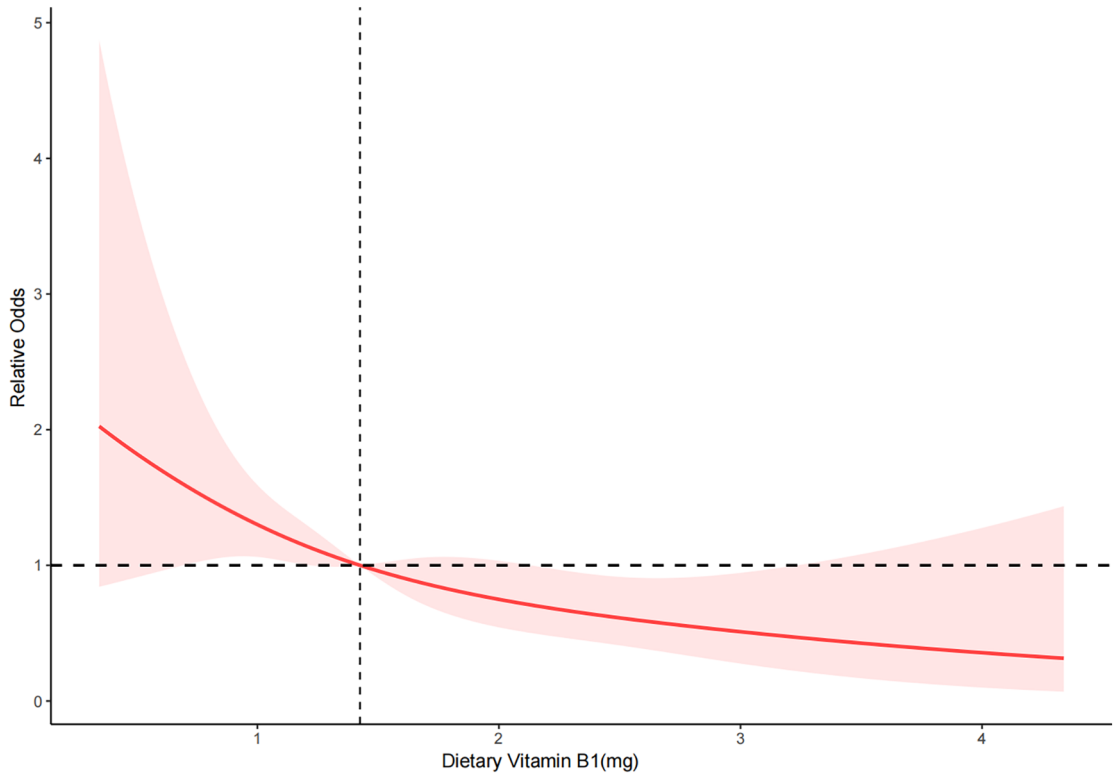

FIGURE 2
RCS of dietary vitamin B1 and severe AAC. The red solid line represents the smooth curve fitting between variables. The light red area represents the fitted 95% confidence interval.

Subgroup analysis

A subgroup analysis was conducted to examine the relationship between dietary vitamin B1 intake and severe AAC based on age, gender, smoking status, alcohol consumption, presence of diabetes, hypertension, and CVD. All analyses were adjusted for all covariates, except for the stratifying variables (Table 3). The results indicate that the relationship between dietary vitamin B1 intake and severe AAC remains significant in participants aged 60 years and younger, females, current smokers, and those without CVD and diabetes. Interaction analysis revealed that the negative correlation between dietary vitamin B1 intake and severe AAC was more pronounced in participants aged 60 years and younger, females, and current smokers (P for interaction <0.05). Additionally, all subgroup analysis results consistently showed the increase in dietary intake of vitamin B1 is significantly correlated with a decrease in the risk of severe AAC, indicating robust findings across subgroups.

Discussion

This study aimed to investigate the relationship between dietary vitamin B1 intake and severe AAC in the general population of the United States. Our study results demonstrate that the increase in dietary intake of vitamin B1 is significantly

TABLE 3 Subgroup analysis and interaction test of dietary vitamin B1 and severe AAC.

Variable	OR (95% CI)	*P*-value	*P* for interaction
Age			0.025
>60 years old	0.730 (0.461, 1.154)	0.164	
≤60 years old	0.195 (0.049, 0.782)	0.024	
Sex			0.026
Male	0.774 (0.484, 1.239)	0.264	
Female	0.375 (0.234, 0.602)	<0.001	
Smoke			0.023
Former	0.761 (0.353, 1.642)	0.461	
Never	0.699 (0.408, 1.197)	0.177	
Now	0.188 (0.072, 0.489)	0.002	
Alcohol consumption			0.433
Former	0.905 (0.342, 2.394)	0.701	
Never	0.972 (0.187, 5.059)	0.948	
Mild	0.627 (0.137, 2.881)	0.319	
Moderate	0.173 (0.001, 29.355)	0.279	
Heavy	0.566 (0.066, 4.884)	0.373	
CVD			0.343
Yes	0.853 (0.479, 1.521)	0.567	
No	0.568 (0.381, 0.846)	0.008	
Hypertension			0.74
Yes	0.617 (0.359, 1.061)	0.077	
No	0.551 (0.239, 1.275)	0.151	
Diabetes			0.255
Yes	0.805 (0.346, 1.874)	0.592	
No	0.480 (0.319, 0.722)	0.002	

OR, odds ratio; CI, confidence interval.

associated with a decrease in the risk of severe AAC. Insufficient dietary vitamin B1 intake may increase the risk of severe AAC. For populations with low dietary intake of vitamin B1, it is recommended to increase the intake of vitamin B1 to reduce the risk of severe AAC. To our knowledge, this is the first study exploring the relationship between dietary vitamin B1 intake and AAC in the general population.

In fact, epidemiological research on AAC is limited. Existing studies have shown that the incidence of AAC is very high. The Framingham Heart Study results indicate that the age of onset of AAC is mainly concentrated between 45 and 65 years old. More than 90% of people aged 65 and above suffer from varying degrees of AAC (2). The Multi-Ethnic Study of Atherosclerosis found that 80% of non-Hispanic Whites, 68% of Hispanic Americans and 63% of African Americans had AAC through the study of 1,957 participants with an average age of 65 years (17). However, more and more evidence suggests that AAC can be treated or even reversed in terms of calcification degree. This prompts people to search for possible treatment methods (18).

Vascular calcification is a process where vascular smooth muscle cells undergo transdifferentiation into osteoblast-like cells under various pathological factors, mediating the abnormal deposition of calcium salts in the vascular wall (19). The pathophysiological mechanisms of this disease are extraordinarily complex and remain incompletely understood, involving cellular osteogenic differentiation, inflammation, oxidative stress, apoptosis, and autophagy (20). Among these, oxidative stress and inflammation play crucial roles in the development of vascular calcification (21). Studies have shown that inflammation is associated with osteogenic activity in the cardiovascular system and vascular calcification. Oxidative stress can activate signaling molecules in the inflammation pathway, such as nuclear transcription factor κB, and a series of inflammatory mediators, such as tumor necrosis factor-α, interleukin-1 (22–24). The release of these inflammatory mediators and activation of inflammatory cells trigger an inflammatory response in the vascular wall, accelerating endothelial cell damage and proliferation, ultimately promoting calcium salt deposition in the vascular wall and the formation of vascular calcification.

Vitamin B1, also known as thiamine, is the first B-vitamin discovered (25). It is a complex organic molecule that acts as a coenzyme in various reactions of glycolysis and the tricarboxylic acid cycle. The primary physiological function of vitamin B1 is to participate in energy metabolism, while also playing important roles in maintaining the normal function of nerves, muscles, especially cardiac muscles, as well as regulating normal appetite, gastrointestinal motility, and digestive secretion (26). In the past, vitamin B1 has been shown to improve endothelial and smooth muscle cell function by reducing the formation of glycolytic metabolism products and inhibiting the proliferation of vascular smooth muscle cells, and it has protective effects against glucose and insulin-mediated proliferation of human vascular smooth muscle cells (27–29). Additionally, vitamin B1 can reduce the degree of oxidative stress in the body, inhibit the production of free radicals, thus protecting endothelial cells and other cells in the vascular wall from oxidative damage (30). Despite some preliminary research findings, there is currently no literature reporting the effect of vitamin B1 on AAC. Our study results suggest a significant correlation between dietary vitamin B1 intake and AAC, indicating that increasing dietary vitamin B1 intake may have a positive effect on reducing the risk of AAC. However, this association and its specific mechanisms require further investigation.

We must acknowledge the limitations of this study. One of the major limiting factors is that this is a cross-sectional study, and therefore, it cannot determine the specific mechanisms underlying the correlation between dietary vitamin B1 intake and AAC. Additionally, the study population included individuals aged 40 and above, so further exploration is needed for the relationship between dietary vitamin B1 intake and AAC in adults under 40 years of age. The study population was limited to the general population in the United States, so caution should be exercised when generalizing these results to other populations. In addition, there is a lack of more recent data on abdominal aortic calcification. The data on abdominal aortic calcification is only available in NHANES 2013–2014, which may result in potential selection bias.

Conclusion

Our research findings indicate that the increase in dietary intake of vitamin B1 is significantly associated with a decrease in

the risk of severe AAC. Insufficient dietary vitamin B1 intake may increase the risk of severe AAC.

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 humans were approved by The Institutional Review Board of National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HL: Data curation, Writing – original draft. RL: Data curation, Investigation, Writing – original draft. CG: Methodology, Supervision, Writing – original draft. ZW: Software, Validation, Writing – review & editing. QJ: Visualization, Writing – review & editing.

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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.

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References

1. Bardeesi ASA, Gao J, Zhang K, Yu S, Wei M, Liu P, et al. A novel role of cellular interactions in vascular calcification. *J Transl Med*. (2017) 15:95. doi: 10.1186/s12967-017-1190-z

2. Chuang ML, Massaro JM, Levitzky YS, Fox CS, Manders ES, Hoffmann U, et al. Prevalence and distribution of abdominal aortic calcium by gender and age group in a community-based cohort (from the Framingham heart study). *Am J Cardiol*. (2012) 110:891–6. doi: 10.1016/j.amjcard.2012.05.020

3. Jayalath RW, Mangan SH, Golledge J. Aortic calcification. *Eur J Vasc Endovasc Surg*. (2005) 30:476–88. doi: 10.1016/j.ejvs.2005.04.030

4. Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. *JAMA*. (2000) 283:2810–5. doi: 10.1001/jama.283.21.2810

5. Rodríguez AJ, Leow K, Szulc P, Scott D, Ebeling P, Sim M, et al. Abdominal aortic calcification, bone mineral density and fractures: a systematic review and meta-analysis protocol. *BMJ Open*. (2019) 9:e026232. doi: 10.1136/bmjopen-2018-026232

6. Golledge J. Abdominal aortic calcification: clinical significance, mechanisms and therapies. *Curr Pharm Des*. (2014) 20:5834–8. doi: 10.2174/1381612820666140212195309

7. Sethi A, Taylor DL, Ruby JG, Venkataraman J, Sorokin E, Cule M, et al. Calcification of the abdominal aorta is an under-appreciated cardiovascular disease risk factor in the general population. *Front Cardiovasc Med*. (2022) 9:1003246. doi: 10.3389/fcvm.2022.1003246

8. Bastos Gonçalves F, Voûte MT, Hoeks SE, Chonchol MB, Boersma EE, Stolker RJ, et al. Calcification of the abdominal aorta as an independent predictor of cardiovascular events: a meta-analysis. *Heart*. (2012) 98:988–94. doi: 10.1136/heartjnl-2011-301464

9. Tian WB, Zhang WS, Jiang CQ, Liu XY, Jin YL, Lam TH, et al. Aortic arch calcification and risk of all-cause mortality and cardiovascular disease: the Guangzhou biobank cohort study. *Lancet Reg Health West Pac*. (2022) 23:100460. doi: 10.1016/j.lanwpc.2022.100460

10. Yue S, Wang J, Zhao Y, Ye E, Niu D, Huang J, et al. Thiamine administration may increase survival benefit in critically ill patients with myocardial infarction. *Front Nutr*. (2023) 10:1227974. doi: 10.3389/fnut.2023.1227974

11. Jia J, Zhang J, He Q, Wang M, Liu Q, Wang T, et al. Association between dietary vitamin C and abdominal aortic calcification among the US adults. *Nutr J*. (2023) 22:58. doi: 10.1186/s12937-023-00889-y

12. Maghraoui AE, Hamza T, Sadni S, El Maataoui A, Majjad A, Rezqi A, et al. Vitamin D status and abdominal aortic calcification in postmenopausal women. *J Bone Miner Metab*. (2018) 36:229–37. doi: 10.1007/s00774-017-0832-9

13. Kristensen JSS, Melholt L, Kristensen KL, Dahl M, Lindholt JS. Vitamin K2 dependent matrix gla protein relating to abdominal aortic aneurysm and overall mortality: a combined case control and cohort study. *Eur J Vasc Endovasc Surg*. (2021) 62:267–74. doi: 10.1016/j.ejvs.2021.03.016

14. Oyama S, Okamoto N, Koide S, Hayashi H, Nakai S, Takahashi K, et al. Vitamin K2 supplementation and the progression of abdominal aortic calcification in dialysis patients. *Fujita Med J*. (2021) 7:136–8. doi: 10.20407/fmj.2020-020

15. Smith TJ, Johnson CR, Koshy R, Hess SY, Qureshi UA, Mynak ML, et al. Thiamine deficiency disorders: a clinical perspective. *Ann N Y Acad Sci*. (2021) 1498:9–28. doi: 10.1111/nyas.14536

16. Tang C, Eshak ES, Shirai K, Tamakoshi A, Iso H. Associations of dietary intakes of vitamins B1 and B3 with risk of mortality from CVD among Japanese men and women: the Japan collaborative cohort study. *Br J Nutr*. (2022) 129:1–8. doi: 10.1017/S0007114522001209

17. Forbang NI, McClelland RL, Remigio-Baker RA, Allison MA, Sandfort V, Michos ED, et al. Associations of cardiovascular disease risk factors with abdominal aortic calcium volume and density: the multi-ethnic study of atherosclerosis (MESA). *Atherosclerosis*. (2016) 255:54–8. doi: 10.1016/j.atherosclerosis.2016.10.036

18. Bartstra JW, Mali WPTM, Spiering W, de Jong PA. Abdominal aortic calcification: from ancient friend to modern foe. *Eur J Prev Cardiol*. (2021) 28(12):1386–91.

19. Quaglino D, Boraldi F, Lofaro FD. The biology of vascular calcification. *Int Rev Cell Mol Biol*. (2020) 354:261–353. doi: 10.1016/bs.ircmb.2020.02.007

20. Lee SJ, Lee I-K, Jeon J-H. Vascular calcification-new insights into its mechanism. *Int J Mol Sci*. (2020) 21:2685. doi: 10.3390/ijms21082685

21. Aikawa E, Nahrendorf M, Figueiredo J-L, Swirski FK, Shtatland T, Kohler RH, et al. Osteogenesis associates with inflammation in early-stage atherosclerosis evaluated by molecular imaging *in vivo*. *Circulation*. (2007) 116:2841–50. doi: 10.1161/CIRCULATIONAHA.107.732867

22. Lee H-L, Woo KM, Ryoo H-M, Baek J-H. Tumor necrosis factor-alpha increases alkaline phosphatase expression in vascular smooth muscle cells via MSX2 induction. *Biochem Biophys Res Commun*. (2010) 391:1087–92. doi: 10.1016/j.bbrc.2009.12.027

23. Ceneri N, Zhao L, Young BD, Healy A, Coskun S, Vasavada H, et al. Rac2 modulates atherosclerotic calcification by regulating macrophage interleukin-1β production. *Arterioscler Thromb Vasc Biol*. (2017) 37:328–40. doi: 10.1161/ATVBAHA.116.308507

24. Al-Aly Z. Medial vascular calcification in diabetes mellitus and chronic kidney disease: the role of inflammation. *Cardiovasc Hematol Disord Drug Targets*. (2007) 7:1–6. doi: 10.2174/187152907780059047

25. Frank LL. Thiamin in clinical practice. *JPEN J Parenter Enteral Nutr*. (2015) 39:503–20. doi: 10.1177/0148607114565245

26. Wooley JA. Characteristics of thiamin and its relevance to the management of heart failure. *Nutr Clin Pract*. (2008) 23:487–93. doi: 10.1177/0884533608323430

27. Chen Q, Okada S, Okeda R. Causality of parenchymal and vascular changes in rats with experimental thiamine deficiency encephalopathy. *Pathol Int*. (1997) 47:748–56. doi: 10.1111/j.1440-1827.1997.tb04452.x

28. Avena R, Arora S, Carmody BJ, Cosby K, Sidawy AN. Thiamine (vitamin B1) protects against glucose- and insulin-mediated proliferation of human infragenicular arterial smooth muscle cells. *Ann Vasc Surg*. (2000) 14:37–43. doi: 10.1007/s100169910007

29. La Selva M, Beltramo E, Pagnozzi F, Bena E, Molinatti PA, Molinatti GM, et al. Thiamine corrects delayed replication and decreases production of lactate and advanced glycation end-products in bovine retinal and human umbilical vein endothelial cells cultured under high glucose conditions. *Diabetologia*. (1996) 39:1263–8. doi: 10.1007/s001250050568

30. Bozic I, Lavrnja I. Thiamine and benfotiamine: focus on their therapeutic potential. *Heliyon*. (2023) 9:e21839. doi: 10.1016/j.heliyon.2023.e21839

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Body Mass Index mediates the associations between dietary approaches to stop hypertension and obstructive sleep apnea among U.S. adults

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Background: The Dietary Approaches to Stop Hypertension (DASH) are associated with reduced cardiovascular, diabetes risk, but the effect on obstructive sleep apnea (OSA) is uncertain.

Methods: This study used data from the National Health and Nutrition Examination Survey (NHANES). DASH score was assessed through 24-h dietary recall interviews, and OSA diagnosis in individuals was based on predefined criteria. Logistic regression analysis was used to assess the association between DASH and OSA. Restricted cubic spline (RCS) analysis was used to investigate the dose–response relationship between DASH score and OSA risk. And comprehensive subgroup and mediation analyses were performed.

Results: Among the 14,978 participants, 27.01% had OSA. DASH scores had a negative association with the risk of OSA (OR = 0.91, 95%CI: 0.88–0.95, $p < 0.01$). Next, we divided DASH scores into quintiles groups. In comparison to the reference group Q1, groups Q5 had adjusted OR values of 0.63 (95%CI: 0.52–0.76, $p < 0.01$). Subgroup analyses revealed that this association was consistent across different groups. Further mediation analyses showed that the associations of DASH with OSA risk parallelly mediated by the above Body Mass Index (BMI) 33.4%,95%CI (20.6–46.2%) (all $p < 0.05$). The restricted cubic spline (RCS) analysis indicated a significant dose–response relationship between DASH diet and OSA risk.

Conclusion: These findings suggested that DASH decreased OSA risk, which was possibly and partly mediated by BMI.

KEYWORDS

OSA (obstructive sleep apnea), DASH (dietary approaches to stop hypertension), NHANES (National Health and Nutrition Examination Survey), BMI - Body Mass Index, mediation

Introduction

Obstructive sleep apnea (OSA) is a medical condition marked by repeated episodes of reduced or paused breathing during sleep, resulting in intermittent low oxygen levels, elevated carbon dioxide, and disturbed sleep (1). Common symptoms include snoring, breathing pauses, and reduced alertness during the day (2). Approximately 1 billion people globally are affected by OSA (3). OSA is recognized as an independent risk factor for hypertension, coronary artery disease, and stroke, and it has a strong link with insulin resistance and diabetes (4, 5). Obesity is the leading risk factor for OSA, while genetic predisposition, anatomical variations in the upper airway, and lifestyle choices like smoking and heavy alcohol use also contribute (6). A balanced diet can mitigate OSA symptoms by supporting weight management and providing benefits such as improved metabolism and reduced inflammation (7).

The DASH diet (Dietary Approaches to Stop Hypertension) focuses on a nutrient-dense approach, including abundant vegetables, fruits, whole grains, and low-fat dairy, while minimizing sodium, saturated fats, and cholesterol. It promotes higher intake of potassium, calcium, and magnesium-rich foods. Research indicates that a healthy diet can improve overall well-being by managing weight and metabolic health, and may also help prevent and manage OSA (8, 9). The DASH diet is recognized for its effectiveness in lowering blood pressure, improving cholesterol levels, and reducing chronic inflammation, making it a potential strategy for targeting OSA's underlying causes. Specifically, its ability to lower blood pressure is key to reducing cardiovascular risks in individuals with OSA.

Hypertension and OSA are closely linked, with hypertension frequently occurring alongside OSA and potentially worsening its symptoms through mechanisms like vascular dysfunction and inflammation (10). The DASH diet, rich in antioxidants and fiber, has been proven to reduce oxidative stress and systemic inflammation, both of which play key roles in the development of OSA (11). Moran et al. found that the DASH diet helps reduce upper airway fat accumulation and decreases airway resistance through weight loss (12). Although the impact of diet on weight management, blood pressure control, and metabolic health is well-established, studies specifically examining its influence on OSA risk are limited. The DASH diet, known for its efficacy in reducing hypertension, is uniquely characterized by its emphasis on key nutrients such as potassium, calcium, magnesium, and low sodium. Recent evidence suggests that this dietary pattern also possesses anti-inflammatory properties and improves metabolic profiles (13).

This study aims to systematically assess how the DASH diet impacts OSA risk, considering BMI as a potential mediator, using the NHANES database, which provides a nationally representative sample of U.S. adults. The large sample size and diverse population enhance the generalizability of our findings, addressing a critical gap in understanding non-pharmacological strategies for OSA management.

Methods

Study design and participants

The NHANES is an ongoing survey conducted by the National Center for Health Statistics (NCHS), under the Centers for Disease Control and Prevention (CDC), aimed at assessing the health and nutritional status of the U.S. population. It collects data on demographics, physical examinations, laboratory tests, and dietary habits. Detailed information on survey design, data collection methods, and access to data files is available at http://www.cdc.gov/nchs/nhanes.html. Participants in NHANES provided written informed consent, with study protocols approved by the NCHS Research Ethics Review Board (14).

The initial sample for this study included 25,161 participants aged 20 years and older, who had completed the sleep questionnaire during the 2005–2008 and 2015–2018 cycles. After excluding participants with missing data for dietary information (n = 2,746), BMI (n = 278), cotinine and systemic immune inflammation index (n = 1,080), waist circumference (n = 480), family income-to-poverty ratio (n = 1,780), albumin levels (n = 241), estimated Glomerular Filtration Rate (n = 1), smoking status (n = 1,849), alcohol consumption (n = 1,413), laboratory examination results (n = 382), hypertension status (n = 1), and education level (n = 4), the final analysis included 14,978 participants (Figure 1).

Dietary information

Two 24-h dietary recall interviews were conducted to gather data on participants' dietary intake. The first recall interview took place face-to-face, while the second was conducted via phone between 3 and 10 days after the initial session. The U.S. Department of Agriculture's Food and Nutrient Database for Dietary Studies (FNDDS) was used to determine the energy and nutrient content of the reported food items (15). Average nutrient intake values were derived from the two recall interviews. The primary aim of these interviews was to collect comprehensive dietary intake data from participants. The collected data provided estimates of the types and amounts of foods and drinks (including all kinds of water) consumed during the 24-h period preceding the interview, and enabled the calculation of the energy, nutrients, and other food components contained in these items.

The DASH diet score for each participant was calculated based on their intake levels of foods and nutrients that are either emphasized or restricted by the DASH diet. This score focuses on nine specific nutrients: saturated fat, total fat, protein, cholesterol, fiber, magnesium, calcium, potassium, and sodium (16). Participants earned 1 point for meeting the set goal for a nutrient, 0.5 points for achieving an intermediate goal, and the score could reach a maximum of 9 points (17). Higher intakes of protein, fiber, magnesium, calcium, and potassium increased the score, while lower intakes of saturated fat, total fat, cholesterol, and sodium also contributed positively to the score. The scoring methodology is summarized in Supplementary Table S1.

Definition of OSA

OSA symptoms were identified based on answers to three binary questions (18): (1) How often do you snore?; (2) How often do you experience snoring with pauses in breathing?; and (3) How often do you feel overly sleepy during the day? Participants who indicated snoring 3 or more times per week, snoring with breathing pauses 3 or more times per week, and feeling excessively sleepy during the

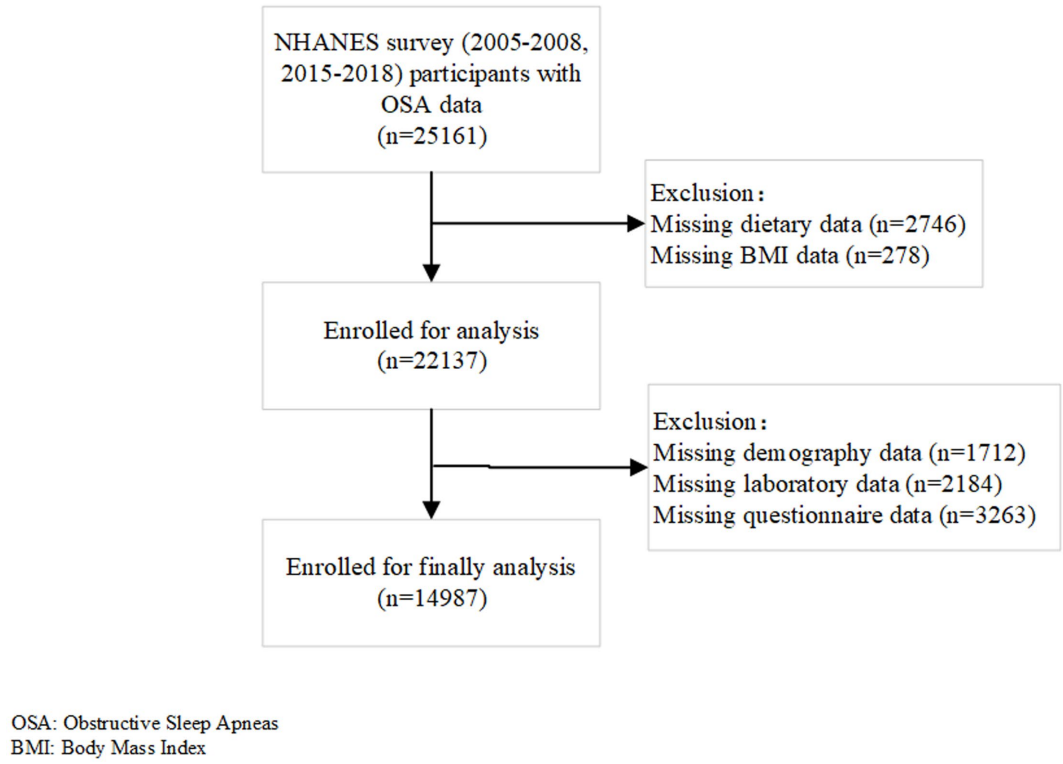

FIGURE 1
Flow chart of the study population.

daytime 16–30 times per month were categorized as having OSA symptoms.

Covariates

Based on their relevance to dietary patterns, OSA, and obesity indicators, the following factors were considered as confounders: age, gender, education level, race, smoking status, alcohol consumption, advanced lung cancer inflammation index (ALI), Family Income-to-Poverty Ratio (PIR), white blood cell count, estimated Glomerular Filtration Rate (eGFR), Body Mass Index (BMI), history of diseases (diabetes, hypertension, and hyperlipidemia) and year (2005–2008, 2015–2018). Education level was categorized as either below college or college and above, while race was classified into non-Hispanic White, non-Hispanic Black, Mexican American, or other races. ALI was calculated using BMI, albumin levels, and the neutrophil-to-lymphocyte ratio (NLR) with the formula: BMI (kg/m^2) × albumin (g/dL) / NLR, where NLR is the ratio of neutrophils to lymphocytes (19). The PIR represents the ratio of family income to the poverty threshold. Data on white blood cell counts were extracted from the NHANES database. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (20). BMI was computed as weight (kg) divided by height squared (m^2). Diabetes was defined according to American Diabetes Association (ADA) criteria and self-reported questionnaires. Participants were considered diabetic if they met any of the following: (1) fasting blood glucose (FBS) ≥7 mmol/L, (2) hemoglobin A1c ≥6.5%, (3) 2-h blood glucose ≥11.1 mmol/L during an oral glucose tolerance test, or (4) a self-reported diagnosis of diabetes and current use of insulin or other glucose-lowering medications (21). Hypertension was defined as having an average systolic blood pressure (SBP) ≥140 mmHg, an average diastolic blood pressure (DBP) ≥90 mmHg, or a self-reported diagnosis of hypertension (22). Hyperlipidemia was defined by total cholesterol ≥200 mg/dL, LDL cholesterol ≥130 mg/dL, HDL <40 mg/dL for men or < 50 mg/dL for women, triglycerides ≥150 mg/dL, or current use of lipid-lowering medications (23).

Statistical analysis

Continuous variables were presented as means with standard deviations, while categorical variables were shown as frequencies and percentages. A *t*-test was used for comparisons of continuous variables, and chi-square tests were utilized for categorical variables. During descriptive analysis, continuous variables were reported as means with standard errors (SE), and categorical variables were displayed as weighted percentages (%). To assess the relationship between DASH scores and OSA, univariate and multivariate logistic regression models were used. Odds ratios (OR) were calculated to determine the strength of the association between DASH scores and OSA symptoms, with 95% confidence intervals (CI) provided for each estimate. The crude model did not include any adjustments for confounding factors. Model 1 adjusted for age, sex, education level, and race, while Model 2 included further adjustments for smoking status, alcohol consumption, ALI, PIR, WBC, BMI, eGFR, year cycle and comorbidities like diabetes, hypertension, and hyperlipidemia, based on Model 1 adjustments. To evaluate if a non-linear relationship between diet scores and OSA risk was present, restricted cubic spline (RCS) curves were used. If non-linearity was detected, a two-piece

linear regression model was applied to determine the threshold effects of the diet score (24). To explore the potential mediator (BMI) between DASH score and OSA, a mediator analysis was conducted. This involved estimating the overall effect of DASH score on OSA (α), the effect of DASH score on BMI ($\beta1$), and the effect of the BMI on OSA ($\beta2$). The direct impact of DASH score on OSA was calculated as $\alpha - \beta1*\beta2$ (25). Subgroup analysis aimed to assess potential effect modifications in the association between DASH scores and OSA, using stratification by age (<60 and ≥ 60), sex, BMI (<30 and ≥ 30), diabetes, hypertension, hyperlipidemia and year cycle. Stratified logistic regression models were used, and likelihood ratio tests assessed differences and interactions across subgroups. Adjustments for each subgroup followed Model 2.

The analyses were performed using R software version 4.2.1 (http://www.R-project.org, R Foundation). Statistical significance was defined as a two-sided p-value <0.05.

Results

Baseline characteristics

The baseline characteristics of the participants revealed significant differences between the OSA and non-OSA groups. Participants with OSA were older and had a higher prevalence of males (54.93%). Clinically, the OSA group exhibited higher BMI, elevated WBC counts, and lower eGFR. Lifestyle factors such as smoking and alcohol use were more prevalent in the OSA group, with higher rates of current smoking (24.10%) and heavy drinking (23.72%). Comorbidities, including hypertension, hyperlipidemia, and diabetes mellitus, were also significantly more common in the OSA group. Temporal trends indicated a higher proportion of OSA cases in the 2015–2018 cycle compared to 2005–2008. Dietary patterns showed that the OSA group had a lower mean DASH score and a higher proportion of individuals in the lowest DASH quintile (all p<0.05) (Table 1).

Analysis of dietary data revealed that individuals with OSA often exhibited dietary patterns with higher energy intake, including increased consumption of fats, proteins, cholesterol, and sodium, alongside reduced intake of dietary fiber. These differences were statistically significant ($p < 0.05$) (Supplementary Table S2).

Association between DASH scores and OSA

Table 2 presents the adjusted correlations between DASH scores and OSA. The results indicate a significant association between the DASH score and the incidence of OSA (Crude model: OR = 0.91, 95% CI: 0.88–0.95, $p < 0.001$; Model 1: OR = 0.92, 95% CI: 0.88–0.95, $p < 0.001$; Model 2: OR = 0.95, 95% CI: 0.92–0.99, $p = 0.02$). When DASH scores were divided into quintiles, participants in the highest quintile (Q5) showed a significantly lower risk of OSA compared to those in the lowest quintile (Q1, reference group). The odds ratios (ORs) for Q5 were: Crude model (OR = 0.63, 95% CI: 0.52–0.76, $p < 0.001$), Model 1 (OR = 0.64, 95% CI: 0.53–0.78, $p < 0.001$), and Model 2 (OR = 0.76, 95% CI: 0.62–0.94, $p = 0.01$) (Table 2).

The restricted cubic spline analyses with multivariable adjustments revealed an inverse L-shaped relationship between the DASH score and OSA incidence. To provide a more detailed analysis of the relationship between DASH adherence and OSA risk, we conducted restricted cubic spline (RCS) analyses using both the crude model and Model 1. The crude model, which adjusts only for DASH adherence, showed an inverse L-shaped relationship (Supplementary Figure S1). When adjusted for gender, age, education level, and race in Model 1, the trend remained consistent but showed slightly adjusted effect estimates (Supplementary Figure S2). As for Model 2, the inflection point of the curve occurred at a DASH score of 1.809. A two-piece linear regression analysis showed that on the right side of the threshold, the odds ratio (OR) was 0.90 (95% CI: 0.86–0.96, $p < 0.001$), meaning that each 1-point increase in the DASH score was associated with a 10% reduction in OSA risk. In contrast, on the left side of the threshold, the OR was 1.04 (95% CI: 0.86–1.22, $p = 0.63$), indicating no statistically significant relationship between the DASH score and OSA incidence (Figure 2).

Subgroup analysis

Subgroup analysis revealed that adherence to the DASH diet was linked to a lower risk of OSA in individuals under 60 years old (OR: 0.952, 95% CI: 0.907–0.998, $p = 0.043$), females (OR: 0.936, 95% CI: 0.880–0.995, $p = 0.035$), time span from 2015 to 2018 (OR: 0.936, 95%CI: 0.880–0.996, p = 0.04), those with hyperlipidemia (OR: 0.941, 95% CI: 0.894–0.990, $p = 0.021$), and individuals without hypertension (OR: 0.933, 95%CI: 0.884–0.983, $p = 0.011$) or without diabetes (OR: 0.942, 95% CI: 0.898–0.987, $p = 0.014$). Adherence to the DASH diet was also significantly associated with a reduced OSA risk, irrespective of BMI status: both in individuals with BMI ≥30 (OR: 0.939, 95% CI: 0.899–0.982, $p = 0.007$) and those with BMI <30 (OR: 0.867, 95% CI: 0.791–0.951, $p = 0.003$). Moreover, no significant differences were found between the subgroups ($p > 0.05$), indicating that the results of the subgroup analysis are consistent and reliable (Figure 3).

Mediation analyses

A mediation analysis was performed to assess the role of BMI in the association between DASH scores and OSA risk. The analysis revealed that BMI significantly mediated this relationship, accounting for an indirect effect of 33.4% (95% CI: 20.6 to 46.2%, $p < 0.05$) (Figure 4).

Discussion

This study revealed a significant inverse association between adherence to the DASH diet and OSA risk. Leveraging the NHANES database, which encompasses a large and representative sample of the U.S. adult population, our findings offer robust evidence supporting the role of dietary interventions in managing OSA risk. The inclusion of a diverse demographic enhances the clinical relevance and applicability of our results to public health strategies.

The DASH diet was originally designed to prevent and manage hypertension. It emphasizes increased intake of vegetables, fruits, whole grains, and low-fat dairy, while limiting sodium, saturated fats, and added sugars. This diet has been shown to effectively reduce blood

TABLE 1 Descriptive baseline characteristics of participants.

Variable	Total (*n* = 14,978)	Non-OSA (*n* = 10,524)	OSA (*n* = 4,454)	*p* value
Age	46.765(0.360)	46.050(0.374)	48.425(0.484)	< 0.0001
Gender				< 0.0001
Female	7,439(51.239)	5,446(53.899)	1993(45.066)	
Male	7,539(48.761)	5,078(46.101)	2,461(54.934)	
Education				0.108
College	7,735(60.396)	5,412(61.111)	2,323(58.736)	
Non-college	7,243(39.604)	5,112(38.889)	2,131(41.264)	
Race				0.576
Mexican American	2,551(8.433)	1817(8.674)	734(7.873)	
Non-Hispanic Black	3,067(10.094)	2,163(10.046)	904(10.205)	
Non-Hispanic White	6,587(69.502)	4,579(69.182)	2008(70.246)	
Other Hispanic	1,345(4.781)	942(4.822)	403(4.685)	
Other Race	1,428(7.191)	1,023(7.277)	405(6.991)	
PIR	3.105(0.044)	3.102(0.047)	3.111(0.058)	0.867
BMI (kg/m^2)	29.024(0.125)	28.088(0.141)	31.196(0.160)	< 0.0001
WBC (1,000 cells/ul)	7.378(0.040)	7.291(0.045)	7.578(0.051)	< 0.0001
Lymphocyte (%)	30.512(0.133)	30.663(0.157)	30.163(0.209)	0.046
Monocyte (%)	8.045(0.032)	8.029(0.030)	8.083(0.062)	0.377
ALI	694.706(5.331)	681.101(6.367)	726.283(8.424)	< 0.0001
NLR	2.134(0.015)	2.120(0.017)	2.167(0.028)	0.155
SII	550.531(4.489)	547.903(5.005)	556.631(7.189)	0.269
eGFR (CKD-EPI)	95.026(0.532)	95.685(0.557)	93.499(0.684)	< 0.001
Smoking status				< 0.0001
Former	3,652(24.499)	2,454(23.424)	1,198(26.993)	
Never	8,242(54.791)	6,061(57.326)	2,181(48.909)	
Now	3,084(20.710)	2009(19.250)	1,075(24.097)	
Alcohol status				< 0.0001
Former	2,258(12.055)	1,553(11.276)	705(13.862)	
Heavy	3,030(22.054)	2072(21.339)	958(23.715)	
Mild	5,141(37.251)	3,538(37.295)	1,603(37.147)	
Moderate	2,417(18.064)	1,692(18.248)	725(17.636)	
Never	2,132(10.576)	1,669(11.842)	463(7.640)	
Hyperlipidemia				< 0.0001
No	4,414(29.704)	3,328(31.877)	1,086(24.660)	
Yes	10,564(70.296)	7,196(68.123)	3,368(75.340)	
Diabetes Mellitus				< 0.0001
Diabetes Mellitus	2,699(13.591)	1,648(11.304)	1,051(18.900)	
No	10,877(77.884)	7,897(80.470)	2,980(71.883)	
PRE-Diabetes Mellitus	1,402(8.524)	979(8.226)	423(9.216)	
Hypertension				< 0.0001
No	8,761(63.132)	6,500(66.897)	2,261(54.394)	
Yes	6,217(36.868)	4,024(33.103)	2,193(45.606)	
Year				< 0.0001
2005–2008	7,790(48.943)	5,679(50.777)	2,111(44.684)	
2015–2018	7,188(51.057)	4,845(49.223)	2,343(55.316)	
DASH score(0–9)	2.331(0.022)	2.392(0.025)	2.189(0.035)	< 0.0001
DASHQ				< 0.0001
Q1	4,099(26.695)	2,754(25.594)	1,345(29.252)	
Q2	2,163(14.486)	1,522(14.337)	641(14.831)	
Q3	3,547(24.340)	2,463(23.828)	1,084(25.528)	
Q4	2,518(17.142)	1809(17.302)	709(16.772)	
Q5	2,651(17.337)	1976(18.939)	675(13.617)	

OSA, Obstructive Sleep Apneas; PIR, Family Income-to-poverty Ratio; BMI, Body Mass Index; WBC, White blood cell; ALI, Advanced Lung cancer Inflammation index; NLR, Neutrophil–Lymphocyte Ratio; SII, Systemic Immune Inflammation Index; eGFR, estimated Glomerular Filtration Rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DASH, Dietary Approaches to Stop Hypertension; DASHQ, DASH score for quintiles.

TABLE 2 Multivariate logistic regression analyses for OSA and DASH score.

Exposure	Crude Model		Model 1		Model 2	
	95%CI	*P*	95%CI	*P*	95%CI	*P*
DASH score	0.91(0.88,0.95)	<0.0001	0.92(0.88,0.95)	<0.0001	0.95(0.92,0.99)	0.02
DASH score for quintiles						
Q1	ref		ref		ref	
Q2	0.91(0.76,1.08)	0.25	0.92(0.77,1.09)	0.34	0.97(0.81,1.17)	0.77
Q3	0.94(0.81,1.09)	0.39	0.96(0.83,1.11)	0.53	1.04(0.87,1.21)	0.65
Q4	0.85(0.71,1.01)	0.06	0.86(0.72,1.03)	0.10	0.95(0.80,1.14)	0.61
Q5	0.63(0.52,0.76)	<0.0001	0.64(0.53,0.78)	<0.0001	0.76(0.62,0.94)	0.01
p for trend		<0.0001		<0.0001		0.03

Crude Model: only DASH score. Model 1: adjusted for included crude model, age, gender, education and race. Model 2: adjusted for all Model 1in addition to smoking status, alcohol status, ALI, diabetes, hyperlipidemia, hypertension, PIR, WBC, BMI and year cycle.

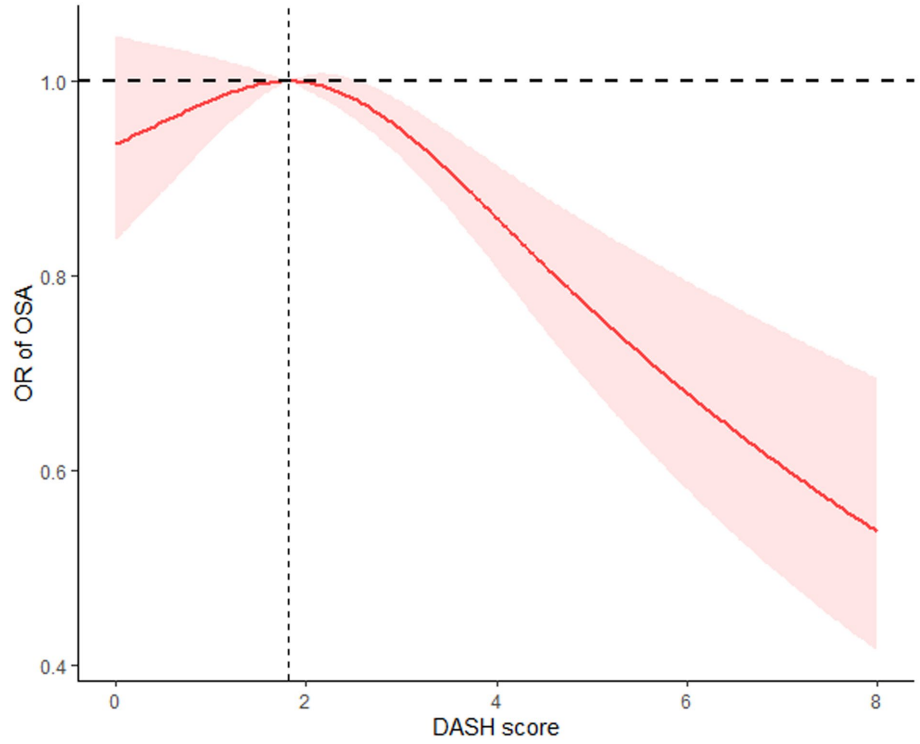

FIGURE 2
The restricted cubic spline (RCS) curve between DASH score and OSA incidence based on Model 2.

pressure and improve cardiovascular health. Excessive sodium intake can cause water and sodium retention, increasing blood volume and exerting more pressure on blood vessel walls, which contributes to hypertension. Studies suggest that reducing sodium intake can significantly decrease blood pressure, particularly in those with existing hypertension (26). Additionally, nutrients such as potassium, calcium, and magnesium are crucial for regulating blood pressure. Potassium lowers blood pressure by aiding in sodium excretion and reducing blood vessel constriction (27). Calcium and magnesium contribute to blood pressure stability by promoting the relaxation of vascular smooth muscles and preventing blood vessel constriction (28). Beyond its cardiovascular and metabolic benefits, the DASH diet also supports improved endothelial function and insulin sensitivity (29, 30). Liu et al. (31) further discovered that the DASH diet, when combined with the Mediterranean diet, effectively reduces the risk of Alzheimer's disease. This finding suggests that the DASH diet may play a role in the prevention of neurodegenerative diseases (31).

The link between metabolic syndrome and OSA is complex. Metabolic syndrome is characterized by conditions like diabetes, hypertension, dyslipidemia, and central obesity (32). These metabolic disturbances contribute to the development and progression of OSA through multiple mechanisms. Obesity, a major risk factor for OSA, leads to excess fat accumulation, especially in the abdominal and neck regions. This buildup around the upper airway increases the risk of airway collapse (33). This mechanical effect aggravates airway obstruction, leading to breathing difficulties and intermittent hypoxia, which in turn worsens insulin resistance and inflammation, forming a self-perpetuating cycle (34).

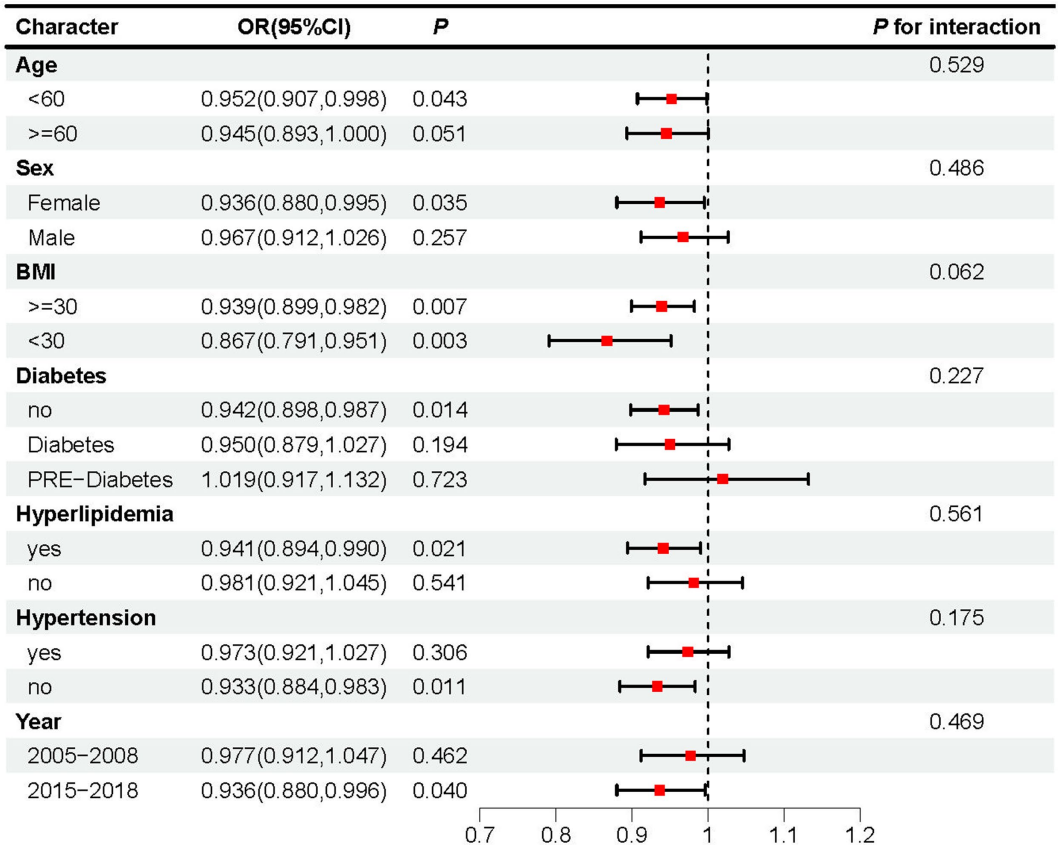

FIGURE 3
Subgroup analyses assessing the effect of DASH score on OSA incidence.

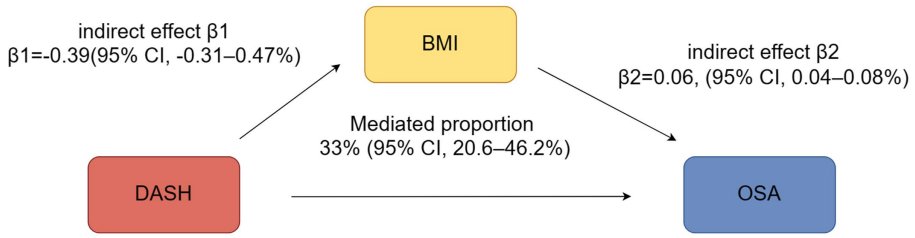

FIGURE 4
The mediating effect analysis of DASH Score and OSA.

In addition to mechanical factors, metabolic syndrome accelerates the progression of OSA through systemic inflammation and oxidative stress. Studies indicate that metabolic imbalances can trigger a prolonged inflammatory response, impairing endothelial function, disrupting blood flow and microcirculation, and decreasing oxygen delivery (35). When oxidative stress is combined with chronic inflammation, it further diminishes upper airway function, worsening the severity of OSA (36). These pathways highlight the significant role of metabolic syndrome in OSA, indicating that managing factors like obesity and insulin resistance is crucial for alleviating OSA symptoms.

BMI serves as a crucial mediator in the relationship between metabolic syndrome and OSA, as higher BMI is strongly linked to increased OSA prevalence. Elevated BMI is associated with various components of metabolic syndrome, including hyperglycemia, hypertension, and dyslipidemia, all of which can exacerbate OSA symptoms (37). Therefore, BMI is not just a result of metabolic disturbances but also a critical factor in the development of OSA (38). BMI plays a significant mediating role in the relationship between DASH diet adherence and OSA risk, likely through multiple physiological pathways. One key mechanism is the reduction of fat accumulation, particularly in the upper airway, which decreases the likelihood of airway obstruction during sleep (39). Furthermore, lower BMI is associated with improved airway resistance, contributing to enhanced airflow and reduced apnea episodes (40). These findings underscore the importance of weight management as a crucial strategy for mitigating OSA risk. The weight gain and heightened metabolic burden due to obesity create a bidirectional feedback loop, where in OSA and metabolic syndrome mutually exacerbate, driving the progression of both conditions.

This study demonstrates that the DASH diet lowers OSA risk by improving metabolic syndrome, with a notably strong protective effect in individuals with hyperlipidemia and those without diabetes. It is proposed that components of the DASH diet may reduce OSA risk by influencing BMI. Our findings support this hypothesis, revealing that the DASH diet significantly reduces OSA risk through BMI reduction. Mediation analysis confirmed that BMI plays a significant mediating role between the DASH diet and OSA risk, highlighting its indirect protective effect. Subgroup analysis further revealed that higher DASH diet scores were associated with reduced OSA risk in both obese and non-obese groups, with no significant differences between them, underscoring the broad applicability and consistency of this protective effect.

Previous research suggests that the dietary fiber in the DASH diet can lower blood glucose levels and enhance insulin sensitivity, thereby reducing the risk of diabetes (41, 42). The DASH diet's emphasis on limiting saturated fats and cholesterol, while favoring unsaturated fats, leads to improved lipid profiles (43). Moreover, the diet's inclusion of antioxidant-rich fruits and vegetables is vital for minimizing oxidative stress and stabilizing blood glucose levels (44, 45). The RCS curve results suggest that adherence to the DASH diet begins to significantly reduce OSA risk when the DASH score exceeds 1.809. This highlights the clinical importance of achieving a DASH score of at least 2, as it marks the point where noticeable protective effects against OSA emerge. Targeted dietary strategies should focus on promoting adherence that reaches or exceeds this level to maximize health benefits. The results of the subgroup analyses for the two NHANES cycles (2005–2008 and 2015–2018) showed consistent associations between DASH adherence and OSA risk across both periods. The interaction *p*-value (0.469) indicated no statistically significant differences between the 2 cycles. These findings suggest that the relationship between DASH adherence and OSA risk is stable over time and is unlikely to be substantially influenced by temporal changes in dietary habits, OSA prevalence, or health status. Thus, to effectively lower blood lipids and glucose, reduce metabolic burden, improve BMI, and manage metabolic conditions through the DASH diet, strict adherence to its principles is crucial. Such comprehensive adherence is essential for realizing significant health benefits and enhancing overall well-being.

In conclusion, the DASH diet exerts a protective effect against OSA through multiple mechanisms, with BMI serving as a central mediator. This study's findings offer further evidence supporting these mechanisms, emphasizing the DASH diet as an effective strategy for both preventing and managing OSA. Although previous research has primarily concentrated on the role of healthy diets in managing OSA-related conditions such as hypertension, cardiovascular disease, and diabetes (46), direct studies on the impact of diet on OSA risk are limited and often involve small sample sizes without support from large-scale data (47). Additionally, the dose–response relationship between diet and OSA risk is not yet fully understood, indicating a need for more research to refine the precision and efficacy of dietary interventions in clinical practice (48).

Limitations

The cross-sectional design of this study limits its ability to assess the long-term effects of the DASH diet on OSA or to perform survival analysis, thus restricting its capacity to determine causal relationships. Dietary data were collected through a 24-h recall method, which may not accurately capture participants' usual dietary habits over time. The assessment of 24-h dietary intake may not accurately capture an individual's lifetime dietary patterns, raising questions about whether the reported intake truly represents their typical daily diet. Future studies, particularly longitudinal designs, are needed to better evaluate long-term adherence to dietary patterns and their impact on health outcomes. The DASH diet score used in this study reflects the dietary intake reported by participants over a 24-h period. While this scoring system provides valuable insights into dietary patterns, it does not directly measure participants' long-term adherence or intent to follow the DASH diet. The assessment of 24-h dietary intake may not accurately capture an individual's habitual diet or lifetime dietary patterns, which is a limitation of the study design. We have acknowledged in the revised manuscript that this study focuses specifically on the association between the DASH diet and OSA, while not accounting for other dietary patterns and their potential impact on OSA. This may limit our ability to fully reflect the dietary behaviors of the target population. Similarly, our study provides valuable initial insights but could benefit from comparisons with other dietary patterns in future research. Nonetheless, dietary intake assessments in NHANES have undergone thorough validation against dietary records and biomarker data (49). Additionally, the OSA diagnosis in this study relied primarily on self-reported questionnaires rather than objective assessments like polysomnography, potentially introducing bias, as symptom reporting may be influenced by participants' subjective perceptions.

Conclusion

This study explored the relationship between the DASH diet and the risk of OSA, revealing that individuals who adhered to DASH dietary recommendations had a lower risk of OSA. The significant role of BMI as a mediator and the identified dose–response relationship between the DASH diet and OSA offer practical implications for non-pharmacological intervention strategies. Further studies are needed to confirm the direct impact of the DASH diet on OSA management, which could provide the scientific foundation for dietary intervention guidelines and support the expanded clinical use of the DASH diet.

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 humans were approved by National Center for Health Statistics Research Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/

next of kin because the studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

SL: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. YY: Data curation, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. ML: Methodology, Writing – review & editing, Conceptualization, Validation, Formal analysis. TL: Data curation, Formal analysis, Software, Writing – original draft. YP: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. JZ: Methodology, Resources, Supervision, Writing – review & editing, Funding acquisition.

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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.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1509711/full#supplementary-material

SUPPLEMENTARY FIGURE S1
The restricted cubic spline (RCS) curve between DASH score and OSA incidence based on Crude Model.

SUPPLEMENTARY FIGURE S2
The restricted cubic spline (RCS) curve between DASH score and OSA incidence based on Model 1.

References

1. Lv R, Liu X, Zhang Y, Dong N, Wang X, He Y, et al. Pathophysiological mechanisms and therapeutic approaches in obstructive sleep apnea syndrome. *Signal Transduct Target Ther*. (2023) 8:218. doi: 10.1038/s41392-023-01496-3

2. Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM. Increased prevalence of sleep-disordered breathing in adults. *Am J Epidemiol*. (2013) 177:1006–14. doi: 10.1093/aje/kws342

3. Benjafield AV, Ayas NT, Eastwood PR, Heinzer R, Ip MSM, Morrell MJ, et al. Estimation of the global prevalence and burden of obstructive sleep Apnoea: a literature-based analysis. *Lancet Respir Med*. (2019) 7:687–98. doi: 10.1016/s2213-2600(19)30198-5

4. Paschou SA, Bletsa E, Saltiki K, Kazakou P, Kantreva K, Katsaounou P, et al. Sleep apnea and cardiovascular risk in patients with prediabetes and type 2 diabetes. *Nutrients*. (2022) 14:989. doi: 10.3390/nu14234989

5. Reutrakul S, Mokhlesi B. Obstructive sleep apnea and diabetes: a state of the art review. *Chest*. (2017) 152:1070–86. doi: 10.1016/j.chest.2017.05.009

6. Yaggi HK, Strohl KP. Adult obstructive sleep apnea/hypopnea syndrome: definitions, risk factors, and pathogenesis. *Clin Chest Med*. (2010) 31:179–86. doi: 10.1016/j.ccm.2010.02.011

7. Ryan S, Taylor CT, McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. *Circulation*. (2005) 112:2660–7. doi: 10.1161/circulationaha.105.556746

8. St-Onge MP, Mikic A, Pietrolungo CE. Effects of diet on sleep quality. *Adv Nutr*. (2016) 7:938–49. doi: 10.3945/an.116.012336

9. Lee CJ, Kim JY, Shim E, Hong SH, Lee M, Jeon JY, et al. The effects of diet alone or in combination with exercise in patients with prehypertension and hypertension: a randomized controlled trial. *Korean Circ J*. (2018) 48:637–51. doi: 10.4070/kcj.2017.0349

10. Logan AG, Perlikowski SM, Mente A, Tisler A, Tkacova R, Niroumand M, et al. High prevalence of unrecognized sleep Apnoea in drug-resistant hypertension. *J Hypertens*. (2001) 19:2271–7. doi: 10.1097/00004872-200112000-00022

11. Chiva-Blanch G, Laake K, Myhre P, Bratseth V, Arnesen H, Solheim S, et al. High adherence to the Nordic diet is associated with lower levels of Total and platelet-derived circulating microvesicles in a Norwegian population. *Nutrients*. (2019) 11:114. doi: 10.3390/nu11051114

12. Wycherley TP, Moran LJ, Clifton PM, Noakes M, Brinkworth GD. Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. (2012) 96:1281–98. doi: 10.3945/ajcn.112.044321

13. Siervo M, Lara J, Chowdhury S, Ashor A, Oggioni C, Mathers JC. Effects of the dietary approach to stop hypertension (Dash) diet on cardiovascular risk factors: a systematic review and Meta-analysis. *Br J Nutr*. (2015) 113:1–15. doi: 10.1017/s0007114514003341

14. Ferguson CC, Knol LL, Ellis AC. Visceral adiposity index and its association with dietary approaches to stop hypertension (Dash) diet scores among older adults: National Health and nutrition examination surveys 2011-2014. *Clin Nutr*. (2021) 40:4085–9. doi: 10.1016/j.clnu.2021.02.008

15. CDC Centers for Disease Control and Prevention [CDC]. Available at: https://www.cdc.gov/nchs/nhanes/ (Accessed September 20, 2024).

16. Mellen PB, Gao SK, Vitolins MZ, Goff DC Jr. Deteriorating dietary habits among adults with hypertension: Dash dietary accordance, NHANES 1988-1994 and 1999-2004. *Arch Intern Med*. (2008) 168:308–14. doi: 10.1001/archinternmed.2007.119

17. Wang JS, Liu WJ, Lee CL. Associations of adherence to the Dash diet and the Mediterranean diet with all-cause mortality in subjects with various glucose regulation states. *Front Nutr*. (2022) 9:828792. doi: 10.3389/fnut.2022.828792

18. Cavallino V, Rankin E, Popescu A, Gopang M, Hale L, Meliker JR. Antimony and sleep health outcomes: NHANES 2009-2016. *Sleep Health*. (2022) 8:373–9. doi: 10.1016/j.sleh.2022.05.005

19. Chen Y, Guan M, Wang R, Wang X. Relationship between advanced lung Cancer inflammation index and long-term all-cause, cardiovascular, and Cancer mortality among type 2 diabetes mellitus patients: NHANES, 1999-2018. *Front Endocrinol*. (2023) 14:1298345. doi: 10.3389/fendo.2023.1298345

20. Kdigo. Clinical practice guideline for the Management of Glomerular Diseases. *Kidney Int*. (2021) 100:S1–s276. doi: 10.1016/j.kint.2021.05.021

21. Gu X, Tang D, Xuan Y, Shen Y, Lu LQ. Association between obstructive sleep apnea symptoms and gout in us population, a cross-sectional study. *Sci Rep*. (2023) 13:10192. doi: 10.1038/s41598-023-36755-4

22. Carey RM, Whelton PK. Prevention, detection, evaluation, and Management of High Blood Pressure in adults: synopsis of the 2017 American College of Cardiology/American Heart Association hypertension guideline. *Ann Intern Med*. (2018) 168:351–8. doi: 10.7326/m17-3203

23. Kuwabara M, Kuwabara R, Niwa K, Hisatome I, Smits G, Roncal-Jimenez CA, et al. Different risk for hypertension, diabetes, dyslipidemia, and hyperuricemia according to level of body mass index in Japanese and American subjects. *Nutrients*. (2018) 10:11. doi: 10.3390/nu10081011

24. Yang Q, Gao J, Zeng X, Chen J, Wen D. Relationship between platelet count and in-hospital mortality in adult patients with Covid-19: a retrospective cohort study. *Front Med*. (2022) 9:802412. doi: 10.3389/fmed.2022.802412

25. Tönnies T, Schlesinger S, Lang A, Kuss O. Mediation analysis in medical research. *Dtsch Arztebl Int*. (2023) 120:681–7. doi: 10.3238/arztebl.m2023.0175

26. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (Dash) diet. Dash-sodium collaborative research group. *N Engl J Med*. (2001) 344:3–10. doi: 10.1056/nejm200101043440101

27. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. Dash collaborative research group. *N Engl J Med*. (1997) 336:1117–24. doi: 10.1056/nejm199704173361601

28. Houston M. The role of magnesium in hypertension and cardiovascular disease. *J Clin Hypertens (Greenwich)*. (2011) 13:843–7. doi: 10.1111/j.1751-7176.2011.00538.x

29. Castro-Barquero S, Ruiz-León AM, Sierra-Pérez M, Estruch R, Casas R. Dietary strategies for metabolic syndrome: a comprehensive review. *Nutrients*. (2020) 12:983. doi: 10.3390/nu12102983

30. Rifai L, Pisano C, Hayden J, Sulo S, Silver MA. Impact of the Dash diet on endothelial function, exercise capacity, and quality of life in patients with heart failure. *Proc Bayl Univ Med Cent*. (2015) 28:151–6. doi: 10.1080/08998280.2015.11929216

31. Liu X, Morris MC, Dhana K, Ventrelle J, Johnson K, Bishop L, et al. Mediterranean-Dash intervention for neurodegenerative delay (mind) study: rationale, design and baseline characteristics of a randomized control trial of the mind diet on cognitive decline. *Contemp Clin Trials*. (2021) 102:106270. doi: 10.1016/j.cct.2021.106270

32. Alkhulaifi F, Darkoh C. Meal timing, meal frequency and metabolic syndrome. *Nutrients*. (2022) 14:719. doi: 10.3390/nu14091719

33. Jullian-Desayes I, Joyeux-Faure M, Tamisier R, Launois S, Borel AL, Levy P, et al. Impact of obstructive sleep apnea treatment by continuous positive airway pressure on Cardiometabolic biomarkers: a systematic review from sham CPAP randomized controlled trials. *Sleep Med Rev*. (2015) 21:23–38. doi: 10.1016/j.smrv.2014.07.004

34. Wyszomirski K, Walędziak M, Różańska-Walędziak A. Obesity, bariatric surgery and obstructive sleep apnea-a narrative literature review. *Medicina (Kaunas)*. (2023) 59:266. doi: 10.3390/medicina59071266

35. Mitra AK, Bhuiyan AR, Jones EA. Association and risk factors for obstructive sleep apnea and cardiovascular diseases: a systematic review. *Diseases*. (2021) 9:88. doi: 10.3390/diseases9040088

36. Giampá SQC, Furlan SF, Freitas LS, Macedo TA, Lebkuchen A, Cardozo KHM, et al. Effects of CPAP on metabolic syndrome in patients with OSA: a randomized trial. *Chest*. (2022) 161:1370–81. doi: 10.1016/j.chest.2021.12.669

37. Bonsignore MR. Obesity and obstructive sleep apnea. *Handb Exp Pharmacol*. (2022) 274:181–201. doi: 10.1007/164_2021_558

38. Rezaie L, Maazinezhad S, Fogelberg DJ, Khazaie H, Sadeghi-Bahmani D, Brand S. Compared to individuals with mild to moderate obstructive sleep apnea (OSA), individuals with severe OSA had higher BMI and respiratory-disturbance scores. *Life*. (2021) 11:368. doi: 10.3390/life11050368

39. Soares CFP, Cahali MB. Upper airway modifications after weight loss: a systematic review. *Braz J Otorhinolaryngol*. (2023) 89:348–57. doi: 10.1016/j.bjorl.2022.10.054

40. Drager LF, Togeiro SM, Polotsky VY, Lorenzi-Filho G. Obstructive sleep apnea: a Cardiometabolic risk in obesity and the metabolic syndrome. *J Am Coll Cardiol*. (2013) 62:569–76. doi: 10.1016/j.jacc.2013.05.045

41. Saslow LR, Jones LM, Sen A, Wolfson JA, Diez HL, O'Brien A, et al. Comparing very low-carbohydrate vs Dash diets for overweight or obese adults with hypertension and prediabetes or type 2 diabetes: a randomized trial. *Ann Fam Med*. (2023) 21:256–63. doi: 10.1370/afm.2968

42. Lari A, Sohouli MH, Fatahi S, Cerqueira HS, Santos HO, Pourrajab B, et al. The effects of the dietary approaches to stop hypertension (Dash) diet on metabolic risk factors in patients with chronic disease: a systematic review and Meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis*. (2021) 31:2766–78. doi: 10.1016/j.numecd.2021.05.030

43. Chiu S, Bergeron N, Williams PT, Bray GA, Sutherland B, Krauss RM. Comparison of the Dash (dietary approaches to stop hypertension) diet and a higher-fat Dash diet on blood pressure and lipids and lipoproteins: a randomized controlled trial. *Am J Clin Nutr*. (2016) 103:341–7. doi: 10.3945/ajcn.115.123281

44. Hussain BM, Deierlein AL, Kanaya AM, Talegawkar SA, O'Connor JA, Gadgil MD, et al. Concordance between Dash diet and hypertension: results from the mediators of atherosclerosis in south Asians living in America (masala) study. *Nutrients*. (2023) 15:611. doi: 10.3390/nu15163611

45. Jayedi A, Soltani S, Motlagh SZ, Emadi A, Shahinfar H, Moosavi H, et al. Anthropometric and adiposity indicators and risk of type 2 diabetes: systematic review and dose-response Meta-analysis of cohort studies. *BMJ*. (2022) 376:e067516. doi: 10.1136/bmj-2021-067516

46. Mao Y, Weng J, Xie Q, Wu L, Xuan Y, Zhang J, et al. Association between dietary inflammatory index and stroke in the us population: evidence from NHANES 1999-2018. *BMC Public Health*. (2024) 24:50. doi: 10.1186/s12889-023-17556-w

47. Ryan CM, Kendzerska T, Wilton K, Lyons OD. The different clinical faces of obstructive sleep apnea (OSA), OSA in older adults as a distinctly different physiological phenotype, and the impact of OSA on cardiovascular events after coronary artery bypass surgery. *Am J Respir Crit Care Med*. (2015) 192:1127–9. doi: 10.1164/rccm.201507-1472RR

48. Tao X, Niu R, Lu W, Zeng X, Sun X, Liu C. Obstructive sleep apnea (OSA) is associated with increased risk of early-onset sarcopenia and Sarcopenic obesity: results from NHANES 2015-2018. *Int J Obes*. (2024) 48:891–9. doi: 10.1038/s41366-024-01493-8

49. Shan Z, Rehm CD, Rogers G, Ruan M, Wang DD, Hu FB, et al. Trends in dietary carbohydrate, protein, and fat intake and diet quality among us adults, 1999-2016. *JAMA*. (2019) 322:1178–87. doi: 10.1001/jama.2019.13771

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The mediating role of vitamin D in the relationship between triglyceride glucose index and mortality in patients with diabetes mellitus: a causal mediation analysis

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Objective: This study investigated the effects of triglyceride glucose index (TyG) and vitamin D levels on all-cause and cardiovascular mortality in diabetic patients and assessed the potential mediating role of vitamin D in the relationship between TyG and mortality.

Methods: The study was based on data from the National Health and Nutrition Examination Survey (NHANES) database from 2001 to 2018, which included 6,318 patients with diabetes. Multivariable Cox proportional risk regression models were employed to assess the association between TyG and vitamin D levels and the risk of death in diabetic patients. The interaction between TyG and vitamin D and its effect on mortality was explored through restricted cubic spline analysis and causal mediation analysis.

Results: The results demonstrated that the TyG index was positively associated with all-cause and cardiovascular mortality in diabetic patients, whereas vitamin D levels were negatively associated with mortality, exhibiting an overall U-shaped association. The results indicated that vitamin D partially mediated the association between TyG and all-cause mortality. Further analysis revealed a significant mediation between vitamin D and TyG, whereby alterations in vitamin D levels influenced the impact of TyG on mortality. Subgroup analyses demonstrated that the correlation between TyG and mortality was more pronounced in diabetic patients with vitamin D insufficiency.

Conclusion: The study demonstrates the mediating influence of vitamin D on the relationship between TyG and mortality in diabetic patients. This finding underscores the necessity of evaluating the influence of vitamin D on survival outcomes in individuals with disparate levels of the TyG index.

KEYWORDS

diabetes mellitus, triglyceride glucose index, vitamin D, mortality, mediation analysis

1 Introduction

Diabetes mellitus (DM) is a global metabolic disease characterized by elevated blood glucose levels. These levels are primarily the result of insufficient insulin secretion or a weakened cellular response to insulin (1). DM not only affects an individual's glucose metabolism but is often accompanied by cardiovascular disease risk factors such as dyslipidemia and hypertension. These factors significantly increase the risk of cardiovascular disease and death in patients with diabetes (2). In recent years, the triglyceride glucose index (TyG), a novel clinical surrogate for insulin resistance, has garnered considerable attention in assessing diabetes risk and prognosis. This is mainly due to its simplicity, accessibility, and reproducibility (3). The TyG index effectively reflects the state of insulin resistance by combining fasting plasma glucose (FPG) and triglyceride (TG) levels. Moreover, it has been confirmed to be closely associated with the risk of developing DM and its cardiovascular complications in numerous studies (4–6).

Vitamin D, a fat-soluble vitamin, plays a role in calcium and phosphorus metabolism within the human body, thereby influencing bone health. Additionally, it affects glucose-lipid metabolism and immune function through various mechanisms (7). The primary form of vitamin D in the human body is 25-hydroxyvitamin D (25 (OH)D). The serum 25 (OH) D level can be utilized as an indicator for evaluating the deficiency, insufficiency, or sufficiency of vitamin D (8). In recent years, an increasing number of studies have demonstrated significant associations between vitamin D levels and glycemic control, dyslipidemia, cardiovascular disease risk, and mortality in diabetic patients (9, 10). In particular, vitamin D deficiency or insufficiency may increase insulin resistance, elevating the risk of diabetes and its associated complications (11, 12). Moreover, vitamin D may influence triglyceride levels by modulating the expression of genes involved in lipid metabolism, thereby indirectly impacting the TyG index (13). Some studies have demonstrated a negative correlation between vitamin D levels and the TyG index, indicating that vitamin D may enhance the prognosis of diabetic patients by influencing insulin resistance and glucolipid metabolism (14). Vitamin D supplementation may improve glycemic control and reduce the risk of diabetes-related complications in diabetic patients (11). Consequently, vitamin D levels may be a mediator in influencing the relationship between the TyG index and mortality in diabetic patients.

While studies have been conducted to investigate the relationship between vitamin D, the TyG index, and the prognosis of diabetic patients separately (15, 16), studies on whether vitamin D levels mediate the relationship between the TyG index and mortality in diabetic patients are scarce. In particular, the predictive value of the TyG index for the risk of mortality in diabetic patients under different vitamin D status and the underlying mechanisms remain to be further elucidated. This knowledge gap limits our comprehensive understanding of the prognostic assessment of diabetic patients and hampers the development of intervention strategies for at-risk populations.

In light of the existing literature and theoretical background, the present study proposes the following hypothesis: vitamin D levels may mediate the relationship between the TyG index and mortality in diabetic patients, with this effect varying across vitamin D levels. To test this hypothesis, this study will investigate the mediating role of vitamin D levels in the relationship between TyG index and mortality in diabetic patients by analyzing data from the National Health and Nutrition Examination Survey (NHANES) database. Furthermore, the predictive value of the TyG index for mortality risk under different vitamin D statuses will be analyzed. This study aims to provide a new perspective on the prognostic assessment of diabetic patients and provide a scientific basis for developing targeted interventions.

2 Materials and methods

2.1 Study population

The data utilized in this study were obtained from the NHANES database, spanning 2001 to 2018. This database contains the results of cross-sectional surveys conducted every 2 years by the Centers for Disease Control and Prevention (CDC). At the study's outset, an extensive sample population was drawn from nine consecutive survey cycles, comprising 91,351 participants. DM was operationalized in this study according to the following criteria: (1) a definitive diagnosis by a healthcare professional, (2) an FPG level of 126 mg/dL or higher, (3) a glycosylated hemoglobin (HbA1c) level of 6.5% or greater, and (4) the individual's current use of diabetic medication or insulin therapy. To ensure the accuracy and relevance of the findings, we excluded ineligible participants, including those under the age of 20, non-diabetic individuals, and those with missing data (specifically missing data on relevant indicators for calculating TyG, vitamin D, survival follow-up, demographic characteristics, chronic disease status, and some biochemical data), as well as pregnant participants. Following applying the above screening criteria, 6,318 participants were identified as eligible for inclusion in the analysis (Figure 1).

2.2 Ethics and STROBE statement

The research protocol of the NHANES project adhered strictly to the guidelines set forth by the Ethics Review Committee of the National Center for Health Statistics (NCHS), and all participants were required to sign an informed consent form. During data analysis, the NIH policy regulations were adhered to. Considering the anonymity and non-direct contact nature of the data, it was utilized directly in the study without requiring additional ethical review. To ensure the highest study design and reporting quality, the study was conducted according to the standards outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

2.3 Assessment of the TyG index

After a minimum 8.5-h fast, TG and FPG were measured using automated biochemical analyzers to ensure accurate data. FPG and TG concentrations were measured by standardized procedures. The following scientifically validated formula was employed to calculate the TyG index (3):

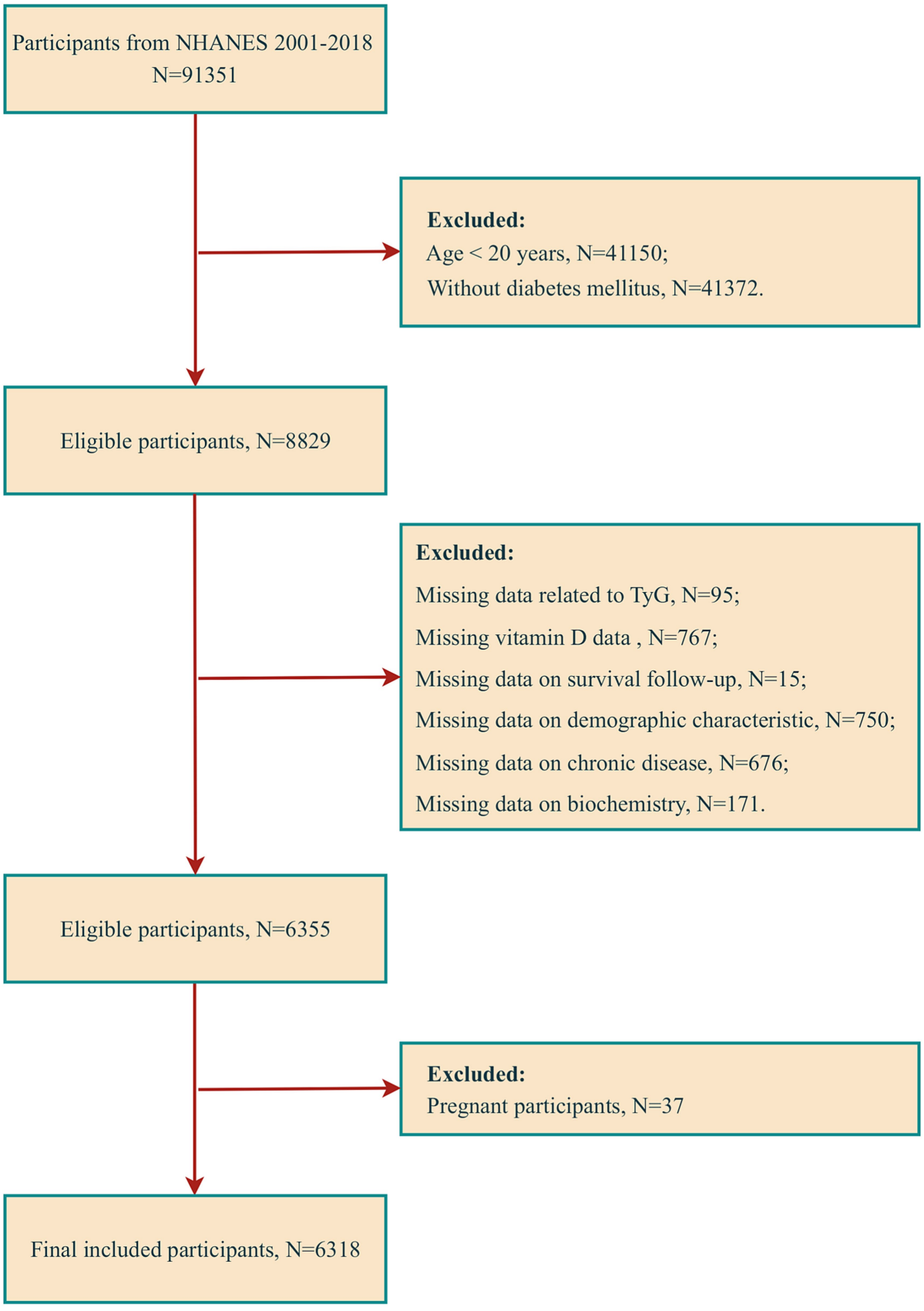

FIGURE 1
Participant screening flowchart.

$$\mathrm{TyG} = \mathrm{Ln}\left[\mathrm{TG}\left(\mathrm{mg}/\mathrm{dL}\right) \times \mathrm{FPG}\left(\mathrm{mg}/\mathrm{dL}\right)/2\right].$$

2.4 Vitamin D assessment

Serum 25(OH)D concentrations from NHANES 2001–2006 were measured using a Dia-Sorin radioimmunoassay kit (Stillwater, MN, United States), and serum 25(OH)D concentrations from NHANES 2007–2018 were calculated using standardized liquid chromatography–tandem mass spectrometry (LC–MS/MS). The serum 25(OH)D data from NHANES 2001–2006 have been converted by regression to equivalent 25(OH)D measurements in standardized LC–MS/MS. The conversion formulae are set forth in Supplementary material. In this study, the variable used for assessing participants' vitamin D status was total 25OHD (sum of 25OHD2 and 25OHD3). By the Endocrine Society Clinical Practice Guidelines, the participants were categorized into two groups according to their serum 25(OH)D concentration: an insufficient group (< 75.0 nmol/L) and an sufficient group (≥75.0 nmol/L) (17).

2.5 Mortality assessment

The principal findings of this study pertain to all-cause mortality and cardiovascular mortality in patients with DM. All-cause mortality was defined as deaths from heart disease, malignant neoplasms, and all other causes. Disease-specific mortality was determined based on the International Statistical Classification of Diseases, 10th edition (ICD-10). Cardiovascular mortality was defined as deaths due to heart disease (ICD-10 codes 100–109, 111, 113, 120–151) and cerebrovascular disease (ICD-10 codes 160–169). The mortality data for the follow-up population were obtained from the NHANES Public Use-Related Mortality File (as of December 31, 2019). This file is correlated to the NCHS and the National Death Index (NDI) through a probabilistic matching algorithm (18). The follow-up period was calculated from the initial interview to the date of the patient's death or December 31, 2019.

2.6 Assessment of covariates

In examining the correlation between TyG and vitamin D levels with all-cause and cardiovascular mortality in patients with DM, we developed multivariable-adjusted models to mitigate the influence of confounding variables on this relationship. The covariates included in this study were gender, age, race, education, marital status, family economic status, alcohol intake, smoking behavior, physical activity level, and history of several important chronic diseases, including hypertension, coronary heart disease, and stroke. For racial categorization, participants were subdivided into the following groups: Mexican American, Non-Hispanic White, Non-Hispanic Black, and Other Race. The sample was divided into three categories based on the years of education completed: less than 9th grade, 9th through 12th grade, and more than 12th grade. Marital status was dichotomized into cohabitation and solitude to ascertain the influence of family structure factors. To categorize family economic status, income was carefully divided into three intervals based on the Poverty-to-Income Ratio (PIR) criterion, as officially defined by the U.S. government. The intervals were designated as low (PIR ≤ 1.3), medium (PIR > 1.3 to ≤3.5), and high (PIR > 3.5). For this study, smoking and drinking habits were assessed using standardized methods. Smoking status was defined by the number of cigarettes smoked by the participant, with a minimum of 100 cigarettes throughout their lifetime, and current smoking status. Alcohol consumption was assessed by asking the participant whether they had consumed at least 12 alcoholic beverages of any type in the past year. Physical activity was classified into three categories: vigorous, moderate, and inactive. A comprehensive medical history was obtained for each participant, encompassing hypertension, coronary heart disease, and stroke. For hypertension, the medical history included whether the participant had ever been diagnosed with high blood pressure or was currently taking medication for high blood pressure. Similarly, for coronary heart disease and stroke, the medical history included whether the participant had ever been diagnosed with these conditions by a medical professional.

2.7 Statistical analysis

In the case of continuous variables, the Kolmogorov–Smirnov test was employed to verify the normality of the data. The mean ± standard deviation or median (25th and 75th percentile) were selected by the test results to characterize the variables in question. The data were presented in the form of frequencies and percentages for categorical variables.

To investigate the relationship between TyG and vitamin D levels with all-cause and cardiovascular mortality in patients with DM, multivariable Cox proportional hazard regression models were constructed to assess the impact of TyG and vitamin D levels and their quartiles on the risk of death in patients with DM, as indicated by the estimation of hazard ratios (HRs) and their 95% confidence intervals (CIs). Furthermore, additional comparisons were conducted between the sufficient and insufficient vitamin D groups. To ensure the accuracy of our assessment, we developed three multivariable adjustment models to control for potential confounding factors. Model 1 served as the baseline and did not incorporate any adjustments. Model 2 incorporated age, gender, and race. Model 3 further introduced education, marital status, family PIR, smoking status, drinking habit, physical activity level, and chronic diseases such as hypertension, coronary heart disease, and stroke.

A restricted cubic spline (RCS) model was employed to elucidate a potential nonlinear dose–response relationship between TyG levels and vitamin D status concerning mortality in patients with DM. In this model, TyG and vitamin D levels were treated as continuous variables, and the 5th, 35th, 65th, and 95th percentiles were selected as critical points for analysis based on their distributional properties. In the event of nonlinear associations, likelihood ratio tests were employed to ascertain the essential effects between TyG and vitamin D levels and mortality in patients with DM. We employed causal mediation analysis (CMA) to evaluate the mediating role of vitamin D in the relationship between TyG and mortality in individuals with diabetes. This approach enabled us to calculate both direct effects (the

direct effect of TyG on mortality in individuals with DM after controlling for the mediating variable, vitamin D) and indirect effects (the impact of TyG on mortality in individuals with DM through vitamin D). Interaction-restricted cubic spline plots were constructed to illustrate potential interactions between TyG and vitamin D levels and mortality in patients with DM. Furthermore, subgroup analyses were conducted to stratify participants based on vitamin D levels and to explore the heterogeneity of the patterns of association between TyG and DM mortality risk in vitamin D insufficient and vitamin D sufficient subgroups.

All data analysis was conducted with the assistance of R 4.4.0 software (provided by the R Foundation at http://www.R-project.org) and SPSS version 23.0 (IBM Corporation, Armonk, New York, USA). Graphic presentations were generated using GraphPad Prism version 9.0 (GraphPad Software, United States).

3 Results

3.1 Baseline characteristics of patients with DM

A total of 6,318 diabetic patients were included in this study, of whom 4,775 were identified as survivors and 1,543 were identified as non-survivors. The non-survivor group exhibited a higher proportion of males (58.59%) than the survivor group (51.12%), and the mean age was higher as well (71.00 vs. 60.00 years). The racial distribution revealed a higher proportion of Non-Hispanic White individuals among non-survivors (54.05%) than among survivors (33.63%). Compared to survivors, non-survivors exhibited a lower educational level, a higher proportion of individuals living alone, a lower family income, and a lower prevalence of physical activity. The prevalence of smoking was significantly higher among non-survivors (61.50% vs. 48.08%). Additionally, the prevalence of hypertension, coronary heart disease, and stroke was higher in non-survivors. The mean body mass index (BMI), total cholesterol (TC), and vitamin D levels were lower in non-survivors. In contrast, the mean blood creatinine and uric acid levels were higher in non-survivors (Table 1).

3.2 Relationship between TyG and mortality in diabetic patients

The association between the TyG index and all-cause and cardiovascular mortality was evaluated using three distinct models. In the unadjusted model (Model 1), there was no significant association between TyG and all-cause mortality (HR = 0.99, 95% CI: 0.93, 1.05). After adjustment for gender, age, and race (Model 2), the association of TyG with all-cause mortality was strengthened (HR = 1.16, 95% CI: 1.08, 1.24). After further adjustment for education level, marital status, family PIR, smoking, alcohol consumption, physical activity, hypertension, coronary heart disease, and stroke (Model 3), the association of TyG with all-cause mortality was slightly attenuated (HR = 1.11, 95% CI: 1.04, 1.19). Regarding cardiovascular mortality, a correlation was observed between TyG and cardiovascular mortality in model 2 (HR = 1.15, 95% CI: 1.02, 1.30). Further quartile-stratified analyses revealed a significantly elevated risk of all-cause mortality and cardiovascular mortality in the highest TyG quartile group in Models 2 and 3, adjusted for confounding factors (Table 2).

3.3 Relationship between vitamin D and mortality in diabetic patients

This study aimed to assess the association between vitamin D levels and all-cause and cardiovascular mortality in patients with diabetes. The unadjusted model 1 revealed a positive association between vitamin D levels and all-cause mortality (HR = 1.01, 95% CI: 1.01, 1.01). However, this association was reversed in both Model 2 and Model 3 after adjusting for multiple confounders (Model 2: HR = 0.99, 95% CI: 0.99, 0.99; Model 3: HR = 0.99, 95% CI: 0.99, 0.99). After adjusting for potential confounding variables (model 3), all-cause mortality was lower in patients with sufficient vitamin D levels than those with insufficient (HR = 0.89, 95% CI: 0.79, 0.99) (Table 3). Quartile-stratified analyses demonstrated that in both Model 2 and Model 3, adjusted for confounders, the risk of all-cause mortality was lower in the higher vitamin D quartile compared with the lowest quartile group (Supplementary Table S1). Furthermore, vitamin D levels were inversely correlated with cardiovascular mortality following adjustment for potential confounding variables (Model 2: HR = 0.99, 95% CI: 0.99, 0.99; Model 3: HR = 0.99, 95% CI: 0.99, 0.99) (Table 3). Similarly, the risk of cardiovascular mortality was lower in all quartiles with higher vitamin D levels compared with the lowest quartile group (Supplementary Table S1).

3.4 RCS analysis

The results of the analysis indicated the existence of significant nonlinear associations between TyG and vitamin D levels and mortality. Figures 2A,B illustrate the correlation between TyG and vitamin D levels and all-cause mortality. Figure 2A demonstrates that the *p*-value for the overall association between TyG and all-cause mortality was less than 0.001, the nonlinear *p*-value was 0.003, and the inflection point was 9.04. This suggests that the risk of all-cause mortality initially decreased and then significantly increased with increasing TyG values. Figure 2B illustrates the overall association between vitamin D and all-cause mortality, with a *p*-value of less than 0.001, a nonlinear *p*-value of less than 0.001, and an inflection point of 75 nmol/L. This indicates a negative association between vitamin D levels and all-cause mortality at vitamin D levels below 75 nmol/L, suggesting that the risk of all-cause mortality gradually decreases with increasing vitamin D levels. Figures 2C,D illustrate the correlation between TyG and vitamin D levels and cardiovascular mortality. Figure 2C demonstrates that the p-value for the overall association between TyG and cardiovascular mortality was 0.010, the nonlinear p-value was 0.010, and the inflection point was 8.86. This suggests that the risk ratio for cardiovascular mortality increases with increasing TyG levels above 8.86. Figure 2D illustrates that the overall association between vitamin D and cardiovascular mortality was less than 0.001, the nonlinear *p*-value was less than 0.001, and the inflection point was 50 nmol/L. This suggests a significantly negative correlation between the risk ratio for cardiovascular mortality and the level of vitamin D when the level of vitamin D is below 50 nmol/L.

TABLE 1 Baseline characteristics of participants with diabetes mellitus.

Variables	Total (*n* = 6,318)	Survivors (*n* = 4,775)	Non-survivors (*n* = 1,543)
Gender, *n* (%)			
Male	3,345 (52.94)	2,441 (51.12)	904 (58.59)
Female	2,973 (47.06)	2,334 (48.88)	639 (41.41)
Age (years)	62.00 (51.00, 71.00)	60.00 (49.00, 68.00)	71.00 (63.00, 80.00)
Race, *n* (%)			
Mexican American	1,222 (19.34)	997 (20.88)	225 (14.58)
Non-Hispanic White	2,440 (38.62)	1,606 (33.63)	834 (54.05)
Non-Hispanic Black	1,522 (24.09)	1,176 (24.63)	346 (22.42)
Other Race	1,134 (17.95)	996 (20.86)	138 (8.94)
Education Level, *n* (%)			
Less than 9th grade	1,094 (17.32)	736 (15.41)	358 (23.20)
9–12th grade	2,508 (39.70)	1832 (38.37)	676 (43.81)
More than 12th grade	2,716 (42.99)	2,207 (46.22)	509 (32.99)
Marital Status, *n* (%)			
Cohabitation	3,817 (60.41)	3,010 (63.04)	807 (52.30)
Solitude	2,501 (39.59)	1765 (36.96)	736 (47.70)
Family PIR, *n* (%)			
Low (≤1.3)	2,192 (34.69)	1,619 (33.91)	573 (37.14)
Medium (1.3–3.5)	2,562 (40.55)	1870 (39.16)	692 (44.85)
High (>3.5)	1,564 (24.75)	1,286 (26.93)	278 (18.02)
Smoke, *n* (%)			
Yes	3,245 (51.36)	2,296 (48.08)	949 (61.50)
No	3,073 (48.64)	2,479 (51.92)	594 (38.50)
Alcohol, *n* (%)			
Yes	3,803 (60.19)	2,868 (60.06)	935 (60.60)
No	2,515 (39.81)	1907 (39.94)	608 (39.40)
Physical activity, *n* (%)			
Inactive	2,513 (39.78)	1,669 (34.95)	844 (54.70)
Moderate	2,537 (40.16)	1972 (41.30)	565 (36.62)
Vigorous	1,268 (20.07)	1,134 (23.75)	134 (8.68)
Hypertension, *n* (%)			
Yes	4,014 (63.53)	2,894 (60.61)	1,120 (72.59)
No	2,304 (36.47)	1881 (39.39)	423 (27.41)
Coronary heart disease, *n* (%)			
Yes	649 (10.27)	350 (7.33)	299 (19.38)
No	5,669 (89.73)	4,425 (92.67)	1,244 (80.62)
Stroke, *n* (%)			
Yes	498 (7.88)	289 (6.05)	209 (13.55)
No	5,820 (92.12)	4,486 (93.95)	1,334 (86.45)
BMI (kg/m^2)	31.00 (27.00, 36.12)	31.43 (27.46, 36.51)	29.66 (26.07, 34.49)
FPG (mg/dL)	132.00 (107.00, 170.00)	132.00 (107.00, 169.00)	133.00 (107.00, 174.00)
HbA1c (%)	6.70 (6.10, 7.80)	6.70 (6.00, 7.80)	6.70 (6.10, 7.70)
TC (mg/dL)	184.00 (156.00, 216.00)	184.00 (156.00, 217.00)	182.00 (154.00, 215.00)
TG (mg/dL)	156.00 (105.00, 235.00)	156.00 (105.00, 235.00)	154.00 (104.00, 233.00)

(Continued)

TABLE 1 (Continued)

Variables	Total (*n* = 6,318)	Survivors (*n* = 4,775)	Non-survivors (*n* = 1,543)
HDL-c (mg/dL)	45.00 (38.00, 56.00)	45.00 (38.00, 55.00)	45.00 (38.00, 56.00)
Creatinine (mg/dL)	0.90 (0.74, 1.10)	0.86 (0.71, 1.04)	1.01 (0.83, 1.30)
Uric acid (mg/dL)	5.60 (4.70, 6.70)	5.50 (4.60, 6.60)	5.90 (4.90, 7.20)
Vitamin D (nmol/L)	59.12 (43.31, 77.54)	59.75 (44.23, 77.90)	57.75 (41.95, 75.94)
TyG	9.24 (8.77, 9.81)	9.24 (8.77, 9.82)	9.26 (8.77, 9.80)

PIR, Poverty-to-income ratio; BMI, Body mass index; FPG, Fasting plasma-glucose; HbA1c, Hemoglobin A1c; TC, Total cholesterol; TG, Triglyceride; HDL-c, High density lipoprotein cholesterol; TyG, Triglyceride-glucose.

TABLE 2 Relationships between TyG and mortality in participants with diabetes mellitus.

Variables	Model 1	Model 2	Model 3
	HR (95% CI)	HR (95% CI)	HR (95% CI)
All-cause mortality			
TyG	0.99 (0.93 ~ 1.05)	1.16 (1.08 ~ 1.24)	1.11 (1.04 ~ 1.19)
Categories			
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Quartile 2	0.95 (0.82 ~ 1.09)	0.94 (0.81 ~ 1.08)	0.95 (0.82 ~ 1.09)
Quartile 3	1.03 (0.89 ~ 1.18)	1.04 (0.91 ~ 1.20)	0.99 (0.86 ~ 1.14)
Quartile 4	0.97 (0.85 ~ 1.12)	1.29 (1.11 ~ 1.49)	1.20 (1.04 ~ 1.39)
Cardiovascular mortality			
TyG	0.96 (0.86 ~ 1.07)	1.15 (1.02 ~ 1.30)	1.10 (0.98 ~ 1.25)
Categories			
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Quartile 2	0.93 (0.73 ~ 1.19)	0.94 (0.73 ~ 1.20)	0.96 (0.75 ~ 1.23)
Quartile 3	1.03 (0.81 ~ 1.31)	1.05 (0.83 ~ 1.34)	1.01 (0.79 ~ 1.29)
Quartile 4	1.00 (0.79 ~ 1.28)	1.39 (1.08 ~ 1.78)	1.31 (1.02 ~ 1.68)

Model 1: crude.
Model 2: adjusted for Gender, Age, Race.
Model 3: adjusted for Gender, Age, Race, Education Level, Marital Status, Family PIR, Smoke, Alcohol, Physical Activity, Hypertension, Coronary heart disease, Stroke.
TyG, Triglyceride-glucose; HR, Hazard ratio; CI, Confidence interval.

TABLE 3 Relationships between vitamin D and mortality in participants with diabetes mellitus.

Variables	Model 1	Model 2	Model 3
	HR (95% CI)	HR (95% CI)	HR (95% CI)
All-cause mortality			
Vitamin D	1.01 (1.01 ~ 1.01)	0.99 (0.99 ~ 0.99)	0.99 (0.99 ~ 0.99)
Insufficient	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Sufficient	1.27 (1.14 ~ 1.43)	0.85 (0.75 ~ 0.95)	0.89 (0.79 ~ 0.99)
Cardiovascular mortality			
Vitamin D	1.00 (1.00 ~ 1.01)	0.99 (0.99 ~ 0.99)	0.99 (0.99 ~ 0.99)
Insufficient	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Sufficient	1.35 (1.11 ~ 1.64)	0.86 (0.71 ~ 1.05)	0.92 (0.76 ~ 1.13)

Model 1: crude.
Model 2: adjusted for Gender, Age, Race.
Model 3: adjusted for Gender, Age, Race, Education Level, Marital Status, Family PIR, Smoke, Alcohol, Physical Activity, Hypertension, Coronary heart disease, Stroke.
HR, Hazard ratio; CI, Confidence interval.

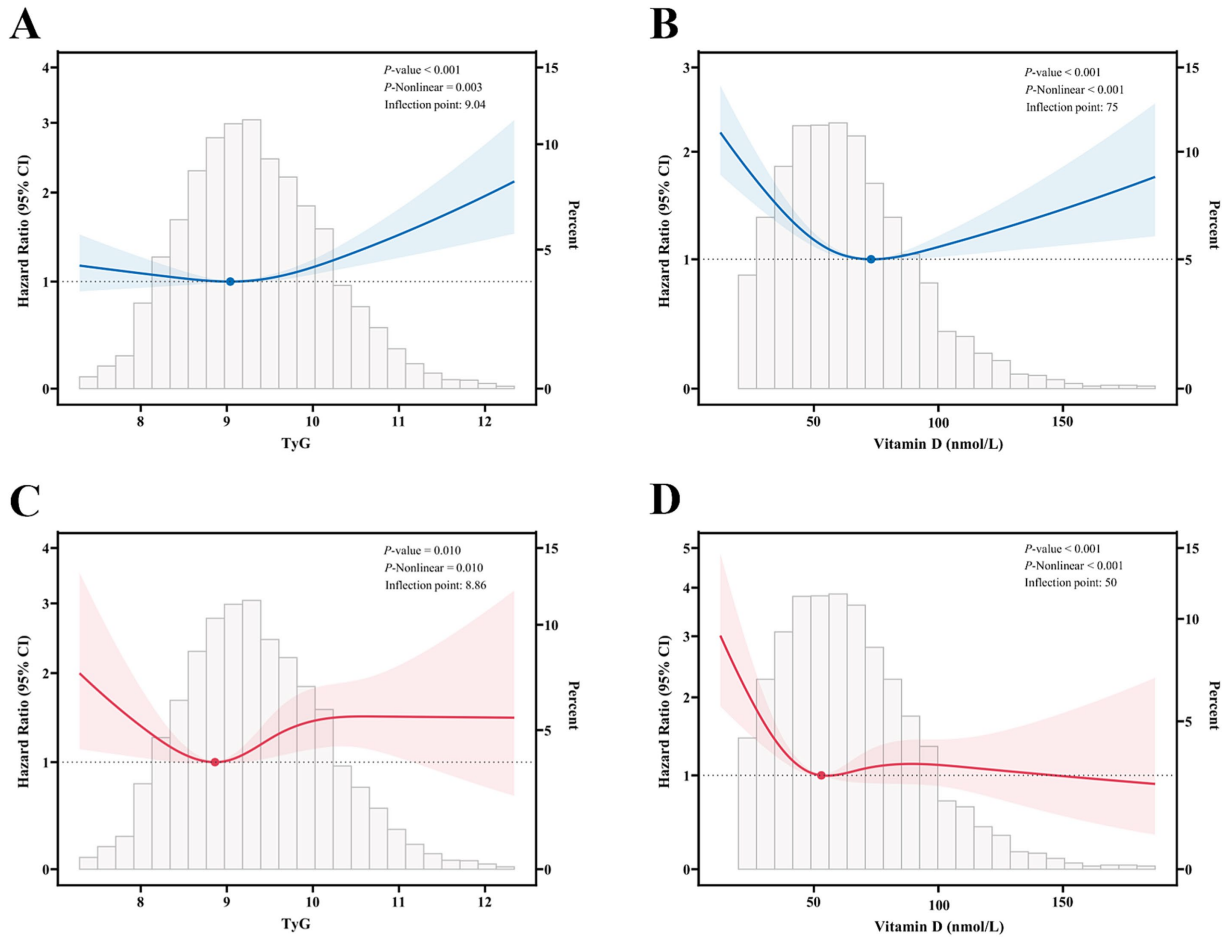

FIGURE 2
Restricted cubic spline fitting for the association between mortality and the TyG and vitamin D. **(A,B)** All-cause mortality according to the TyG and vitamin D; **(C,D)** cardiovascular mortality according to the TyG and vitamin D. Solid lines indicate HR, and shaded areas indicate 95% CI. These analyses were adjusted according to Model 3.

TABLE 4 **Mediation of vitamin D for the association between TyG and mortality.**

Mortality	Indirect effect	Direct effect	Proportion mediated, % (95% CI)
	Coefficient (95% CI)	Coefficient (95% CI)	
All-cause	−5.82 (−12.55, −1.71)	−58.24 (−152.26, −7.33)	9.1 (2.3, 30.5)
Cardiovascular	−14.76 (−38.02, −2.12)	−146.88 (−471.30, 15.57)	9.6 (−34.8, 84.1)

The mediation analyses were adjusted for Gender, Age, Race, Education Level, Marital Status, Family PIR, Smoke, Alcohol, Physical Activity, Hypertension, Coronary heart disease, and Stroke. TyG, Triglyceride-glucose; CI, Confidence interval.

3.5 Mediating role of vitamin D in the relationship between TyG and mortality

The present study also sought to ascertain the mediating role of vitamin D in the association between TyG and mortality. The results demonstrated that vitamin D partially mediated the association between TyG and all-cause mortality, with an indirect effect of −5.82 (95% CI: −12.55, −1.71), and the proportion of mediating impact was 9.1% (95% CI: 2.3, 30.5%). However, the mediating role of vitamin D in the association between TyG and cardiovascular mortality was insignificant, with an indirect effect of −14.76 (95% CI: −38.02, −2.12). Nevertheless, the proportion of mediating effect remains uncertain (95% CI: −34.8, 84.1%) (Table 4). The analyses above were conducted after adjusting for gender, age, race, education level, marital status, family PIR, smoking, alcohol consumption, physical activity, hypertension, coronary heart disease, and stroke. These findings suggest that vitamin D may play a role in the association of TyG with all-cause mortality.

3.6 RCS analysis of the interaction between vitamin D and TyG

Figure 3A illustrates the nonlinear relationship between TyG and all-cause mortality at varying levels of vitamin D. The

analyses demonstrated that alterations in vitamin D levels significantly moderated the impact of TyG on all-cause mortality. At vitamin D levels below 75 nmol/L, the HRs and 95% CIs of TyG on all-cause mortality were more significant than 1, indicating that the positive correlation between TyG and all-cause mortality was significant in this interval and gradually diminished with increasing vitamin D levels. However, when vitamin D levels were greater than or equal to 75 nmol/L, although the HR of TyG on all-cause mortality exhibited a gradual increase, the lower limit of the 95% confidence interval was markedly distant from 1, indicating that the effect of TyG on all-cause mortality was no longer statistically significant under conditions of sufficient vitamin D. Figure 3B illustrates the interaction between vitamin D and TyG levels on cardiovascular mortality. The results were analogous to those observed for all-cause mortality. When vitamin D levels were less than 75 nmol/L, the HR for cardiovascular mortality from TyG decreased progressively with increasing vitamin D levels; however, the lower limit of the 95% confidence interval was lower. The effect of TyG on cardiovascular mortality was no longer statistically significant when vitamin D levels were greater than or equal to 75 nmol/L. These findings indicate that the interaction between vitamin D and TyG may significantly prevent and treat cardiovascular disease, particularly in all-cause and cardiovascular mortality.

3.7 Subgroup analysis of vitamin D levels

The participants were divided into two subgroups based on their vitamin D levels, with those with levels below 75 nmol/L classified as the first subgroup and those with levels above 75 nmol/L classified as the second subgroup. The associations between TyG and all-cause and cardiovascular mortality were analyzed within each subgroup. All analyses were adjusted for potential confounding variables using a multivariable model. As illustrated in Figure 4, in the subgroup with vitamin D levels below 75 nmol/L, there was a notable elevation in the risk of all-cause mortality with each unit increase in TyG (HR = 1.13, 95% CI: 1.05, 1.22, $p = 0.002$). However, in the subgroup with vitamin D levels above 75 nmol/L, the association between TyG and all-cause mortality was attenuated. It did not reach statistical significance (HR = 1.02, 95% CI: 0.88, 1.19, $p = 0.791$). Figure 5 illustrates the correlation between TyG and cardiovascular mortality within distinct vitamin D subgroups. In the subgroup with vitamin D levels below 75 nmol/L, a significant positive correlation was observed between TyG and cardiovascular mortality (HR = 1.14, 95% CI: 1.01, 1.31, $p = 0.047$). This indicates that an increase in TyG is associated with a notable elevation in the risk of cardiovascular mortality at this vitamin D level. Nevertheless, the correlation between TyG and cardiovascular mortality was diminished and did not attain statistical significance in the subgroup with vitamin D levels above 75 nmol/L (HR = 0.97, 95% CI: 0.75, 1.26, $p = 0.834$). These findings indicate that the correlation

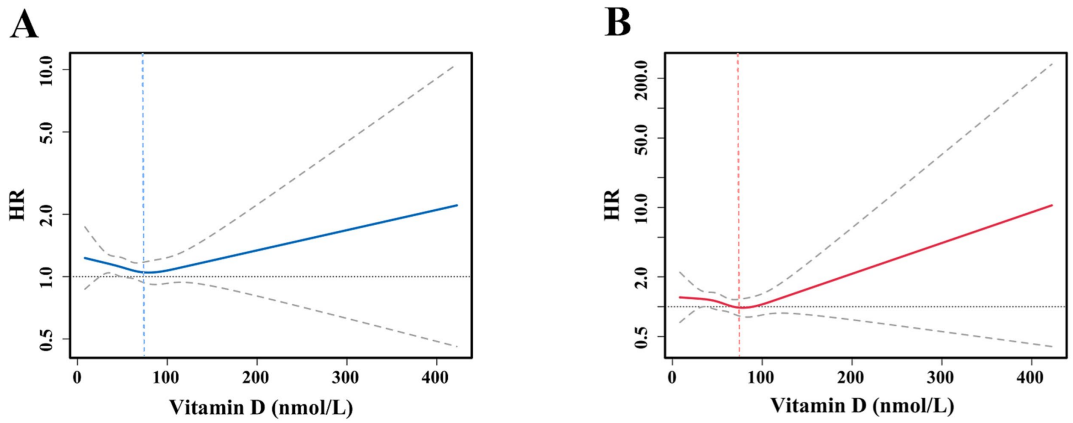

FIGURE 3
Interaction restricted cubic spline fitting of vitamin D for the association between mortality and the TyG. **(A)** All-cause mortality according to the TyG; **(B)** cardiovascular mortality according to the TyG. Solid lines indicate HR, and areas between the two dotted lines indicate 95% CI. These analyses were adjusted according to Model 3.

Subgroup	N	Adjusted HR (95% CI)	*P* value	*P* for interaction
Overall	6318	1.11 (1.04, 1.19)	0.003	
Vitamin D				0.075
< 75	4551	1.13 (1.05, 1.22)	0.002	
≥ 75	1767	1.02 (0.88, 1.19)	0.791	

FIGURE 4
Subgroup analysis of vitamin D for the association between TyG and all-cause mortality. These analyses were adjusted according to Model 3.

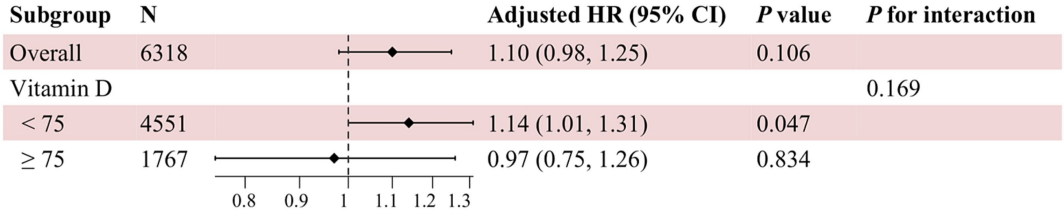

FIGURE 5
Subgroup analysis of vitamin D for the association between TyG and cardiovascular mortality. These analyses were adjusted according to Model 3.

between TyG and all-cause and cardiovascular mortality is more pronounced in individuals with insufficient vitamin D levels. Furthermore, sufficient vitamin D levels may mitigate the adverse effects of TyG on all-cause and cardiovascular mortality in diabetic patients.

4 Discussion

This study examined the correlation between the TyG index and all-cause mortality and cardiovascular mortality in individuals with DM across varying vitamin D levels. The analysis was conducted using data from the NHANES database. The study's results demonstrated a statistically significant positive correlation between the TyG index and all-cause mortality and cardiovascular mortality after adjusting for potential confounding factors such as gender, age, and race. Conversely, the relationship between vitamin D level and mortality exhibited a statistically significant negative correlation, with an overall U-shaped trend. The findings indicated that the TyG index and vitamin D level were significant independent prognostic factors in diabetic patients. Further analysis of the interaction between TyG and vitamin D levels revealed that a significant positive association between TyG and all-cause mortality and cardiovascular mortality persisted in diabetic patients with insufficient vitamin D levels. In contrast, this association was significantly diminished in patients with sufficient vitamin D levels. The findings of this study offer a novel perspective on the clinical management of diabetes. Clinicians should consider both the TyG index and vitamin D levels when assessing the prognosis of diabetic patients to develop a more personalized management strategy.

Insulin resistance represents a pivotal pathophysiologic feature of diabetes and serves as a risk factor for many cardiovascular diseases and metabolic syndromes. The relationship between the TyG index, a marker for insulin resistance, and mortality in diabetic patients may be influenced by various biological mechanisms. Firstly, insulin resistance reduces the body's sensitivity to insulin, which promotes the overproduction of glucose by the liver and increases insulin secretion, leading to hyperinsulinemia (19, 20). Chronic hyperinsulinemia may contribute to the development of atherosclerosis and increase the risk of cardiovascular disease, which in turn affects the survival of diabetic patients (20, 21). Secondly, there is an association between insulin resistance, chronic inflammation, and oxidative stress (22, 23). Increases in inflammatory factors, such as tumor necrosis factor-alpha and interleukin 6, can further exacerbate insulin resistance, promoting thrombosis and atherosclerosis. Furthermore, insulin resistance may influence the functionality and structure of microvessels, potentially leading to microvascular complications such as diabetic retinopathy and diabetic nephropathy, which could indirectly elevate the risk of mortality in patients (24, 25). Furthermore, insulin resistance frequently occurs alongside hypertriglyceridemia and low high-density lipoprotein cholesterol levels. These dyslipidemias represent significant risk factors for atherosclerosis, thereby exacerbating cardiovascular complications (26, 27). The results of the present study demonstrated a significant positive correlation between TyG and all-cause mortality and cardiovascular mortality in diabetic patients. This finding underscores the pivotal role of insulin resistance in the prognosis of diabetic patients.

It is hypothesized that vitamin D may act on mortality in diabetic patients through several mechanisms. Firstly, vitamin D may increase insulin secretion and improve insulin resistance by directly acting on pancreatic beta cells, promoting their function and proliferation (28, 29). Secondly, vitamin D has been demonstrated to inhibit the proliferation and migration of vascular smooth muscle cells, thereby reducing the formation of atherosclerosis (30, 31). Furthermore, it regulates the production of vasodilatory factors, improves vascular endothelial function, and protects the cardiovascular system from damage (32). Moreover, vitamin D exerts notable anti-inflammatory and immunomodulatory effects, inhibiting the synthesis of pro-inflammatory cytokines and mitigating the detrimental effects of inflammatory responses on the body (33, 34). Moreover, vitamin D regulates the renin-angiotensin system (RAS), which benefits blood pressure control and cardiovascular health (35–37). The present study revealed a significant negative correlation between vitamin D levels, all-cause mortality, and cardiovascular mortality in diabetic patients. These findings indicate that vitamin D levels may benefit from reducing the mortality risk in diabetic patients.

Vitamin D may influence the state of insulin resistance, and thus the risk of diabetes and its complications, through some mechanisms. First, vitamin D affects gene expression by binding to the vitamin D receptor (VDR) through its active form, 1,25-dihydroxyvitamin D. The VDR is expressed in several tissues, including muscle, fat, and liver, which play a pivotal role in insulin action and glucose metabolism. Vitamin D regulates insulin signaling in these tissues via the VDR, which may result in enhanced insulin sensitivity (38, 39). Secondly, vitamin D has been demonstrated to promote pancreatic β-cell proliferation and insulin synthesis and secretion, thereby contributing to normal blood glucose levels. In a state of insulin resistance, the function of pancreatic β-cells may be compromised. Vitamin D supplementation may help protect and improve these cells' function (28, 29). Furthermore, insulin resistance is linked to a sustained, low-grade inflammatory condition. Vitamin D has anti-inflammatory and antioxidant properties that reduce inflammatory

factors and oxidative stress products in the body, thereby protecting the vascular endothelium and other target organs from damage (33, 34). Furthermore, it has been demonstrated to attenuate the adverse effects of insulin resistance on mortality (40, 41). Moreover, vitamin D may impact the distribution and metabolism of adipose tissue by regulating adipocyte differentiation and function. In states of insulin resistance, the abnormal distribution and function of adipose tissue may result in metabolic disturbances. Vitamin D supplementation may prove an effective means of alleviating these conditions. Furthermore, vitamin D plays a role in regulating lipid metabolism, including triglyceride and cholesterol metabolism (41, 42). Vitamin D supplementation may facilitate improvements in blood lipid levels and a reduction in the risk of atherosclerosis, which is crucial for mitigating the risk of insulin resistance and diabetic complications (31).

The present study employed RCS analysis and subgroup analysis to ascertain whether vitamin D levels significantly modulate the effect of the TyG index on mortality in diabetic patients. The results indicated that this was indeed the case. This finding suggests vitamin D may reduce mortality risk in diabetic patients by improving insulin resistance status, thereby underscoring the synergistic role of vitamin D and insulin resistance in diabetes prognosis. The interaction between vitamin D and insulin resistance collectively influences the risk of developing diabetes and its associated complications, as well as mortality in diabetic patients. It is, therefore, of particular importance in clinical practice to develop individualized management strategies that consider the patient's vitamin D level and insulin resistance status.

In this study, the confidence intervals observed for the mediation effect analysis for direct effects, indirect effects, and mediation proportions were notably wide. The primary potential cause for this phenomenon is the presence of errors in the measurement of the variables, which may impact the precision of the effect estimates, resulting in wider confidence intervals. The presence of unrecognized or uncontrolled confounding factors (e.g., genetic factors, dietary habits, etc.) may affect the accurate estimate of the relationship between the variables, leading to wider confidence intervals. The distribution of the variables included in the study is abnormal, which may affect the stability of the effect estimates and lead to wider confidence intervals. Additionally, the sample size included in the study may still be insufficient, which may also affect the confidence intervals. These factors may act individually or in combination to lead to the emergence of broader confidence intervals. Therefore, caution and consideration of possible limitations and uncertainties are needed when interpreting these results.

While this study offers valuable insights, its limitations may impact the interpretation and generalizability of the results. First, this study did not account for certain important confounding factors, which may have affected the accuracy of the observed results. In particular, an individual's socioeconomic status, dietary habits, and genetic predispositions may exert an influence on the mortality rate among patients with diabetes. Socioeconomic status represents a significant confounding factor, as it is strongly associated with the complications of diabetes and may also influence an individual's capacity to access healthcare resources. It is also possible that dietary habits, genetic factors, and other such variables may significantly impact the study outcome. However, these factors were not considered in this study. The failure to include these confounding factors may have resulted in an inaccurate estimation of the relationship between exposure and outcome, thereby limiting the reliability of the study findings. Secondly, some participants were excluded from the final analysis due to the absence of pertinent exposure variable data (e.g., vitamin D levels). This situation may have impacted the accuracy and generalizability of the study results. In particular, excluding these participants may have resulted in a biased sample selection, which may have introduced random error. If these excluded participants differed significantly from those included in the analysis regarding key characteristics such as age, gender, and lifestyle, such differences could result in a biased representation of the findings, limiting the broad applicability of the conclusions. Furthermore, missing data may introduce a systematic error, particularly if the pattern of missing data is associated with specific identifiable factors (e.g., socioeconomic status, disease severity). For instance, if participants with incomplete vitamin D data are predominantly from a lower socioeconomic status population, which may exhibit disparate health outcomes compared to the overall population, the study results may be susceptible to systematic bias. Such bias may compromise the reliability of the study findings and limit their applicability to populations from different socioeconomic backgrounds. Consequently, the potential errors associated with missing data should be fully considered when interpreting the results of this study. Future studies should prioritize the completeness and representativeness of data collection to validate further and extend the findings of this study. Furthermore, information bias represents a potential limitation of this study. In this study, information bias emerged due to measurement errors in the TyG index and vitamin D levels and inaccurate reporting of mortality data. For instance, it is conceivable that serum vitamin D measurements may not accurately reflect lifetime exposure or exposure during critical or sensitive periods. Furthermore, the mortality data collection relies on follow-up surveys, which may be subject to incomplete or inaccurate follow-up, potentially introducing additional bias. Such biases may obscure or amplify the relationship between exposure and outcome, further compromising the precision of study conclusions. To mitigate the influence of these biases, future studies should implement more rigorous sampling techniques to guarantee sample representativeness and utilize more precise measurement instruments and data collection methods to enhance data quality. Concurrently, incorporating additional confounding variables and applying advanced statistical techniques to regulate the impact of these variables is essential to attain more precise and reliable research conclusions.

5 Conclusion

This study examined the impact of the TyG index and vitamin D levels and their interaction on all-cause mortality and cardiovascular mortality in individuals with diabetes. The analysis was conducted using data from the NHANES database. The study's findings indicated that TyG, as a marker of insulin resistance, was positively correlated with mortality in diabetic patients, particularly in low vitamin D levels. The beneficial effects of vitamin D on mortality in diabetic patients may be mediated by its ability to improve insulin sensitivity, exert anti-inflammatory effects, and provide cardiovascular protection, thereby attenuating the adverse impact of TyG (insulin resistance) on mortality. Moreover, the mediating effect of vitamin D levels on the relationship between TyG and mortality indicates that vitamin D

may serve as a pivotal protective factor in the pathophysiological process of diabetes. These findings underscore the necessity of monitoring and adjusting TyG and vitamin D levels in diabetes management and offer novel insights for future clinical research on improving the prognosis of diabetic patients by intervening in these biomarkers. While this study is limited in scope, the results offer insight into the potential for personalized treatment and prevention strategies for diabetes. Further investigation into the interactions between these biomarkers and their potential application in diabetes management is warranted.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

FZ: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. WL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing.

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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.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1492647/full#supplementary-material

References

1. American Diabetes Association Professional Practice Committee. 2. Diagnosis and classification of diabetes: standards of care in diabetes—2024. *Diabetes Care*. (2024) 47:S20–S42. doi: 10.2337/dc24-S002

2. Chan JCN, Lim LL, Wareham NJ, Shaw JE, Orchard TJ, Zhang P, et al. The lancet commission on diabetes: using data to transform diabetes care and patient lives. *Lancet*. (2021) 396:2019–82. doi: 10.1016/S0140-6736(20)32374-6

3. Guerrero-Romero F, Simental-Mendía LE, González-Ortiz M, Martínez-Abundis E, Ramos-Zavala MG, Hernández-González SO, et al. The product of triglycerides and glucose, a simple measure of insulin sensitivity. Comparison with the euglycemic-hyperinsulinemic clamp. *J Clin Endocrinol Metab*. (2010) 95:3347–51. doi: 10.1210/jc.2010-0288

4. Ma X, Dong L, Shao Q, Cheng Y, Lv S, Sun Y, et al. Triglyceride glucose index for predicting cardiovascular outcomes after percutaneous coronary intervention in patients with type 2 diabetes mellitus and acute coronary syndrome. *Cardiovasc Diabetol*. (2020) 19:31. doi: 10.1186/s12933-020-01006-7

5. Liu L, Xia R, Song X, Zhang B, He W, Zhou X, et al. Association between the triglyceride-glucose index and diabetic nephropathy in patients with type 2 diabetes: a cross-sectional study. *J Diabetes Investig*. (2021) 12:557–65. doi: 10.1111/jdi.13371

6. Yao H, Sun Z, Yuan W, Shao C, Cai H, Li L, et al. Relationship between the triglyceride-glucose index and type 2 diabetic Macroangiopathy: a single-center retrospective analysis. *Diabetes Metab Syndr Obes*. (2022) 15:3483–97. doi: 10.2147/DMSO.S387040

7. Bouillon R, Marcocci C, Carmeliet G, Bikle D, White JH, Dawson-Hughes B, et al. Skeletal and Extraskeletal actions of vitamin D: current evidence and outstanding questions. *Endocr Rev*. (2019) 40:1109–51. doi: 10.1210/er.2018-00126

8. Giustina A, Bilezikian JP, Adler RA, Banfi G, Bikle DD, Binkley NC, et al. Consensus statement on vitamin D status assessment and supplementation: whys, whens, and hows. *Endocr Rev*. (2024) 45:625–54. doi: 10.1210/endrev/bnae009

9. Dominguez LJ, Veronese N, Marrone E, Di Palermo C, Iommi C, Ruggirello R, et al. Vitamin D and risk of incident type 2 diabetes in older adults: An updated systematic review and Meta-analysis. *Nutrients*. (2024) 16:1561. doi: 10.3390/nu16111561

10. Li C, Fu J, Ye Y, Li J, He Y, Fang T. The impact of vitamin D on the etiopathogenesis and the progression of type 1 and type 2 diabetes in children and adults. *Front Endocrinol (Lausanne)*. (2024) 15:1360525. doi: 10.3389/fendo.2024.1360525

11. Niroomand M, Fotouhi A, Irannejad N, Hosseinpanah F. Does high-dose vitamin D supplementation impact insulin resistance and risk of development of diabetes in patients with pre-diabetes? A double-blind randomized clinical trial. *Diabetes Res Clin Pract*. (2019) 148:1–9. doi: 10.1016/j.diabres.2018.12.008

12. Szymczak-Pajor I, Śliwińska A. Analysis of association between vitamin D deficiency and insulin resistance. *Nutrients*. (2019) 11:794. doi: 10.3390/nu11040794

13. Martínez-Sena T, Soluyanova P, Guzmán C, Valdivielso JM, Castell JV, Jover R. The vitamin D receptor regulates Glycerolipid and phospholipid metabolism in human hepatocytes. *Biomol Ther*. (2020) 10:493. doi: 10.3390/biom10030493

14. Li Z, Liu S, Li X, Liu J, Li X, Zhao Y, et al. The association between the triglyceride-glucose index and vitamin D status: a systematic review and meta-analysis. *BMC Endocr Disord*. (2024) 24:222. doi: 10.1186/s12902-024-01743-w

15. Fan Y, Ding L, Zhang Y, Shu H, He Q, Cui J, et al. Vitamin D status and all-cause mortality in patients with type 2 diabetes in China. *Front Endocrinol (Lausanne)*. (2022) 13:794947. doi: 10.3389/fendo.2022.794947

16. He HM, Xie YY, Chen Q, Li YK, Li XX, Mu YK, et al. The additive effect of the triglyceride-glucose index and estimated glucose disposal rate on long-term mortality among individuals with and without diabetes: a population-based study. *Cardiovasc Diabetol*. (2024) 23:307. doi: 10.1186/s12933-024-02396-8

17. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. (2011) 96:1911–30. doi: 10.1210/jc.2011-0385

18. National Center for Health Statistics. The linkage of National Center for Health Statistics survey data to the National Death Index-2019 linked mortality file (LMF): Linkage methodology and analytic considerations; Hyattsville, MD, USA (2021).

19. Rachdaoui N. Insulin: the friend and the foe in the development of type 2 diabetes mellitus. *Int J Mol Sci*. (2020) 21:1770. doi: 10.3390/ijms21051770

20. Kosmas CE, Bousvarou MD, Kostara CE, Papakonstantinou EJ, Salamou E, Guzman E. Insulin resistance and cardiovascular disease. *J Int Med Res*. (2023) 51:3000605231164548. doi: 10.1177/03000605231164548

21. Jia G, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat Rev Endocrinol*. (2016) 12:144–53. doi: 10.1038/nrendo.2015.216

22. Molina MN, Ferder L, Manucha W. Emerging role of nitric oxide and heat shock proteins in insulin resistance. *Curr Hypertens Rep*. (2016) 18:1. doi: 10.1007/s11906-015-0615-4

23. Lee SH, Park SY, Choi CS. Insulin resistance: from mechanisms to therapeutic strategies. *Diabetes Metab J*. (2022) 46:15–37. doi: 10.4093/dmj.2021.0280

24. Yuan SY, Breslin JW, Perrin R, Gaudreault N, Guo M, Kargozaran H, et al. Microvascular permeability in diabetes and insulin resistance. *Microcirculation*. (2007) 14:363–73. doi: 10.1080/10739680701283091

25. An Y, Xu BT, Wan SR, Ma XM, Long Y, Xu Y, et al. The role of oxidative stress in diabetes mellitus-induced vascular endothelial dysfunction. *Cardiovasc Diabetol*. (2023) 22:237. doi: 10.1186/s12933-023-01965-7

26. Vergès B. Pathophysiology of diabetic dyslipidaemia: where are we? *Diabetologia*. (2015) 58:886–99. doi: 10.1007/s00125-015-3525-8

27. Yang Q, Vijayakumar A, Kahn BB. Metabolites as regulators of insulin sensitivity and metabolism. *Nat Rev Mol Cell Biol*. (2018) 19:654–72. doi: 10.1038/s41580-018-0044-8

28. Wei Z, Yoshihara E, He N, Hah N, Fan W, Pinto AFM, et al. Vitamin D switches BAF complexes to protect β cells. *Cell*. (2018) 173:1135–49.e15. doi: 10.1016/j.cell.2018.04.013

29. Lee YJ, Kim GH, Park SI, Lim JH. Vitamin D rescues pancreatic β cell dysfunction due to iron overload via elevation of the vitamin D receptor and maintenance of Ca2+homeostasis. *Mol Nutr Food Res*. (2021) 65:e2000772. doi: 10.1002/mnfr.202000772

30. Valcheva P, Cardus A, Panizo S, Parisi E, Bozic M, Lopez Novoa JM, et al. Lack of vitamin D receptor causes stress-induced premature senescence in vascular smooth muscle cells through enhanced local angiotensin-II signals. *Atherosclerosis*. (2014) 235:247–55. doi: 10.1016/j.atherosclerosis.2014.05.911

31. Surdu AM, Pînzariu O, Ciobanu DM, Negru AG, Căinap SS, Lazea C, et al. Vitamin D and its role in the lipid metabolism and the development of atherosclerosis. *Biomedicines*. (2021) 9:172. doi: 10.3390/biomedicines9020172

32. Kim DH, Meza CA, Clarke H, Kim JS, Hickner RC. Vitamin D and endothelial function. *Nutrients*. (2020) 12:2. doi: 10.3390/nu12020575

33. Marcotorchino J, Gouranton E, Romier B, Tourniaire F, Astier J, Malezet C, et al. Vitamin D reduces the inflammatory response and restores glucose uptake in adipocytes. *Mol Nutr Food Res*. (2012) 56:1771–82. doi: 10.1002/mnfr.201200383

34. Wenclewska S, Szymczak-Pajor I, Drzewoski J, Bunk M, Śliwińska A. Vitamin D supplementation reduces both oxidative DNA damage and insulin resistance in the elderly with metabolic disorders. *Int J Mol Sci*. (2019) 20:2891. doi: 10.3390/ijms20122891

35. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *J Steroid Biochem Mol Biol*. (2004) 89-90:387–92. doi: 10.1016/j.jsbmb.2004.03.004

36. Luchi WM, Crajoinas RO, Martins FL, Castro PC, Venturini G, Seguro AC, et al. High blood pressure induced by vitamin D deficiency is associated with renal overexpression and hyperphosphorylation of Na+-K+-2Cl- cotransporter type 2. *J Hypertens*. (2021) 39:880–91. doi: 10.1097/HJH.0000000000002745

37. Jia J, Tao X, Tian Z, Liu J, Ye X, Zhan Y. Vitamin D receptor deficiency increases systolic blood pressure by upregulating the renin-angiotensin system and autophagy. *Exp Ther Med*. (2022) 23:314. doi: 10.3892/etm.2022.11243

38. Leung PS. The potential protective action of vitamin D in hepatic insulin resistance and pancreatic islet dysfunction in type 2 diabetes mellitus. *Nutrients*. (2016) 8:147. doi: 10.3390/nu8030147

39. Wimalawansa SJ. Associations of vitamin D with insulin resistance, obesity, type 2 diabetes, and metabolic syndrome. *J Steroid Biochem Mol Biol*. (2018) 175:177–89. doi: 10.1016/j.jsbmb.2016.09.017

40. Abbas MA. Physiological functions of vitamin D in adipose tissue. *J Steroid Biochem Mol Biol*. (2017) 165:369–81. doi: 10.1016/j.jsbmb.2016.08.004

41. Lu S, Cao ZB. Interplay between vitamin D and adipose tissue: implications for Adipogenesis and adipose tissue function. *Nutrients*. (2023) 15:15 (22). doi: 10.3390/nu15224832

42. Asano L, Watanabe M, Ryoden Y, Usuda K, Yamaguchi T, Khambu B, et al. Vitamin D metabolite, 25-Hydroxyvitamin D, regulates lipid metabolism by inducing degradation of SREBP/SCAP. *Cell Chem Biol*. (2017) 24:207–17. doi: 10.1016/j.chembiol.2016.12.017

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Age-dependent interaction between serum zinc and triglyceride-glucose index among American adults: National Health and Nutrition Examination Survey

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Introduction: Zinc plays a crucial role in glucose metabolism. The association between serum zinc and insulin resistance has recently been investigated as well, but the findings are inconsistent. The triglyceride-glucose index (TyG) is frequently utilized in epidemiological research to assess insulin resistance. The association between serum zinc levels and TyG has not yet been explored. Therefore, we designed this cross-sectional study to assess the relationship between serum zinc and TyG in adults using data from the National Health and Nutrition Examination Survey (NHANES).

Methods: A cross-sectional analysis was performed on 1,610 adults aged ≥20 years who participated in the National Health and Nutrition Examination Survey (NHANES) 2011–2016. The participants were stratified by age, and the differences in log-transformed serum zinc quartiles and TyG were further evaluated in age groups <60 years and ≥60 years using multivariable linear regression with an interaction test. Additionally, a restricted cubic spline (RCS) model was employed to examine the dose-response relationships between log-transformed serum zinc and TyG.

Results: In this cross-sectional study, a significant interaction was observed between log-transformed serum zinc and TyG in individuals aged <60 years and those aged ≥60 years when log-transformed serum zinc was transformed into a categorical variable (*P*-value for the likelihood ratio test for the interaction was $P = 0.017$). Additionally, in the fully adjusted analyses, the association between log-transformed serum zinc and TyG in the age <60 years group demonstrated a J-shaped nonlinear pattern (*P* for nonlinearity = 0.014), with an inflection point at ~1.94 μg/dL. While in the age ≥60 years group, it exhibited an inverted-L shaped nonlinear pattern (*P* for nonlinearity < 0.001***).

Conclusion: There is a significant relationship between log-transformed serum zinc and TyG in adults in the United States, with age potentially influencing this association. Further prospective studies are needed to offer additional evidence and insights into these findings.

KEYWORDS

serum zinc, triglyceride-glucose index, age, NHANES, cross-sectional analysis

1 Introduction

Zinc, the second most prevalent trace metal in the human body, is a vital micronutrient essential for growth and development (1). It is a component of numerous enzymes (2) and may play a protective role by regulating inflammation, reducing oxidative stress, and participating in lipid and glucose metabolism (3). Additionally, zinc is crucial in the biochemistry of insulin and glucagon within pancreatic β- and α-cells (4), playing a key role in the synthesis, storage, and release of insulin, and is linked to diabetes and metabolic syndrome (3, 5). Over recent decades, zinc has been extensively studied for its antioxidative and anti-inflammatory properties. Mild or moderate zinc deficiency in humans can result in stunted growth, delayed puberty in adolescents, hypogonadism in males, dermatitis, decreased appetite, mental lethargy, and delayed wound healing (6). However, several studies showed high doses of zinc-based biomaterials may have adverse effects, including liver, spleen, and pancreas damage in mice, disruption of energy metabolism, and impairment of mitochondrial and cell membrane function in rat kidneys (7–9). Assessing zinc status is challenging due to tightly regulated zinc homeostasis. The Biomarkers of Nutrition for Development Zinc Expert Panel and the International Zinc Nutrition Consultative Group recommend using plasma or serum zinc concentration as a biomarker for zinc status (10).

Insulin resistance is closely linked to risk factors for cardiovascular and metabolic diseases, including coronary heart disease, stroke, hypertension, atherosclerosis, diabetes, and atrial fibrillation (11–13). It significantly contributes to the morbidity and mortality rates associated with these conditions, as well as imposing a substantial economic burden (14). Currently, the hyperinsulinemic-euglycemic clamp (HEC) is considered the gold standard for evaluating insulin sensitivity in peripheral tissues (15). However, this invasive method is complex, time-consuming, and technically challenging, which has led to a preference for simpler indicators of insulin resistance. Traditional measures like the homeostatic model assessment for insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI), both of which rely on fasting insulin levels, are limited by practical constraints and variability (16). The triglyceride-glucose index (TyG) is a reliable and easily acquired indicator which is derived from fasting plasma glucose and triglyceride (TG) levels, serves as an indicator for assessing insulin resistance in epidemiological research (17). TyG has emerged as a novel tool that demonstrates superiority over HOMA-IR in evaluating insulin resistance, particularly in individuals with diabetes undergoing insulin therapy or those lacking functional beta cells (18–21). A 12-year longitudinal study from the Korean Genome and Epidemiology Study cohort found that a higher TyG index precedes and significantly predicts type 2 diabetes in community-dwelling, middle-aged, and elderly lean Koreans (22). Several studies have provided evidences linking TyG to the onset and prognosis of cardiovascular diseases, including stable coronary artery disease, carotid plaque, coronary artery calcification, and acute coronary syndrome (13, 23–25). Moreover, TyG is closely associated with cardiovascular disease risk factors such as arterial stiffness and hypertension (11, 12).

The association between serum zinc and insulin resistance has recently been investigated as well, but the findings are inconsistent. Some studies have documented that zinc deficiency may predispose glucose intolerance and insulin resistance, diabetes mellitus, and coronary artery disease (26–29). While previous studies suggest that higher serum zinc concentrations may be associated with an increased risk of metabolic syndrome (5, 30, 31), and serum zinc concentration was significantly higher in both abnormal glucose tolerance and the presence of diabetes individuals (32). Animal research shows that the administration of zinc in small doses has been demonstrated to confer protection against type 2 diabetes; however, a high concentration of the element has been shown to exert a toxic effect on the beta cells within the islets of Langerhans (33). Meanwhile, a study found statistically significant positive association between zinc and HOMA-IR in men aged 50–75 years without diabetes, and the men with metabolic syndrome showed statistically significant higher zinc (34). But a study report that there is no statistically significant association between the concentration of zinc and metabolic syndrome with its individual components in adults from Lebanon aged 18–65 years (35).

Our study aims to examine the association between serum zinc levels and TyG, which has not yet been explored. To fill this knowledge gap, we evaluated the relationship between serum zinc and TyG in adults using data from the National Health and Nutrition Examination Survey (NHANES). Our hypothesis was that individuals with elevated TyG levels would have higher serum zinc levels, based on observed nutritional patterns in this population. Additionally, we assessed the dose-response relationship between serum zinc and TyG.

2 Materials and methods

2.1 Data sources and study population

The National Health and Nutrition Examination Survey (NHANES) is a series of health-related research aimed at determining non-institutionalized Americans' health and nutritional status. As a representative sample, a multistage, stratified probability strategy was used to select survey participants (36). This cross-sectional study used the data from 2011–2012, 2013–2014, and 2015–2016 cycles from the NHANES, as the interesting trace metal was only examined in these three survey waves. Demographic, socioeconomic and health-related information were collected through questionnaires, physical examinations, and laboratory tests. Health interviews were conducted at participants' homes, while thorough physical examinations, including blood sample collection, were carried out at the Mobile Examination Center (MEC). The collected serum specimens were then tested at the National Center for Environmental Health's Division of Laboratory Sciences of the Centers for Disease Control and Prevention (37).

The NHANES was authorized by the National Center for Health Statistics Ethics Review Board (https://www.cdc.gov/nchs/nhanes/irba98.htm). Before participating, all participants completed written informed consent forms. The secondary analysis did not require additional Institutional Review Board approval

(38). The NHANES data are accessible through the NHANES website (http://www.cdc.gov/nchs/nhanes.htm; accessed on 19 Oct 2023).

2.2 Inclusion criteria

Our study's participants were above the age of 20 and had completed an interview and evaluation at a MEC.

2.3 Exclusion criteria

We excluded pregnant women or individuals with missing data on serum zinc, fasting plasma glucose (FPG), triglyceride (TG) or covariates. And we excluded participants with extreme energy intake, consuming <500 or >5,000 kcal per day.

2.4 Serum zinc

Serum zinc was detected at the Environmental Health Sciences Laboratory of the CDC National Center for Environmental Health using the inductively coupled plasma dynamic reaction cell mass spectrometry following extensive quality control procedures. The lower limit of detection (LLOD) for serum zinc was 2.9 μg/dL, and all the data was above the LLOD for all tests. In the multivariable linear models, log-transformed serum zinc was categorized into quartiles: Q1 (1.69–1.89 μg/dL; $n = 397$), Q2 (1.90–1.93 μg/dL; $n = 408$), Q3 (1.94–1.97 μg/dL; $n = 401$), Q4 (1.98–2.37 μg/dL; $n = 404$).

2.5 Triglyceride-glucose index

Triglyceride-glucose index (TyG) was calculated using the formula Ln [fasting TG (mg/dL) × fasting plasma glucose (FPG; mg/dL)/2] (18). Blood samples were taken in the morning after fasting overnight to measure the levels of TG and glucose in the blood. The concentration of TG and FPG was measured using an automatic biochemistry analyzer. The serum TG levels were determined using a Roche Cobas 6000 chemistry analyzer and a Roche Modular P chemistry analyzer. A Roche/Hitachi Cobas C 501 chemistry analyzer was used to measure FPG using the hexokinase-mediated reaction.

2.6 Covariates

The covariates considered in this study consisted of sociodemographic, behavioral, health characteristics and laboratory data deemed a priori as potential confounders.

Sociodemographic variables consisted of age groups (20–59 years and ≥60 years) (39–41), gender (female and male), race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, or other races), education level (<9, 9–12, or >12 years), marital status (married, living with a partner, or living alone). According to a US government report (42), family income was categorized into three groups by the poverty income ratio (PIR): low (PIR ≤ 1.3), medium (PIR > 1.3–3.5), and high (PIR > 3.5).

Behavioral characteristics comprised smoking status, drinking status, and physical activity. According to previous literature definitions (43), smoking status was classified into three categories: never smokers (participants who had smoked fewer than 100 cigarettes), current smokers, and former smokers (those who had quit smoking after smoking more than 100 cigarettes). Furthermore, individuals who consumed at least 12 alcoholic drinks per year throughout their lifetime were classified as drinkers (37). Physical activity was categorized as sedentary, moderate (involving at least 10 min of movement within the past 30 days, resulting in light sweating or a mild to moderate increase in breathing or heart rate), and vigorous (involving at least 10 min of activity within the past 30 days, resulting in profuse sweating or a significant increase in breathing or heart rate) (43).

Health factors included body mass index (BMI), trouble sleeping, hypertension (no or yes) (44), diabetes (no or yes) (45) and failing kidneys (no or yes) (46). BMI was computed using a standardized technique which is weight (kg) divided by height (m) and divided into four categories with cut-off values of 18.5, 25, and 30 kg/m^2 (underweight, normal, overweight, and obese) (47). Hypertension was diagnosed based on a self-reported physician diagnosis (a positive response to "Have you been diagnosed with hypertension?"), and/or recent use of an antihypertensive agent (a positive response to "Are you currently taking any antihypertensive drugs to treat or control your blood pressure?"), and/or a systolic blood pressure/diastolic blood pressure ≥140/90 mmHg (44). Diabetes cases were defined as participants who fulfilled the inclusion criteria: (1) FPG ≥126 mg/dL, (2) 2-h plasma glucose ≥200 mg/dL on an oral glucose tolerance test (OGTT), (3) HbA1c ≥6.5%, and (4) current use of insulin or diabetes pills to lower blood glucose levels, or a self-report questionnaire that indicates a previously diagnosed of T2DM by a physician (45). Failing kidneys was determined for participants who positively responded to the question has he/she ever been told by a doctor or other health professional that had weak or failing kidneys (excluding kidney stones, bladder infections, or incontinence) (46). A dietary recall interview preceded and interview including total energy intake.

Laboratory data including HbA1c, high density lipoprotein cholesterol (HDL-C), triglyceride, creatinine, total cholesterol, FPG and uric acid.

2.7 Statistical analysis

Statistical analyses were performed using the statistical software programs R (The R Foundation) and Free Statistics software version 1.9.2 (Beijing Free Clinical Medical Technology Co., Ltd.) (48). All statistical tests were two-sided, and significance was considered at $P < 0.05$. Analyses were conducted according to the Centers for Disease Control and Prevention (CDC) guidelines for the analysis of NHANES data. As the sample size was determined based solely on the available data, no a priori statistical power estimates were conducted. We used fasting subsample MEC weights for the weighted analysis. For the combined analyses

of NHANES 2011–2016 data, a 6-year fasting subsample MEC weights (WTSAF2YR) set was used, stratum (SDMVSTRA), and primary sampling units (SDMVPSU) were taken into account for the complex survey design (48).

Categorical data were expressed as unweighted numbers (weighted percentages), whereas continuous data were expressed as means (standard deviation, SD). One-way analyses of variance (continuous variables) and chi-square tests (categorical variables) were used to compare differences between the groups. To analyze the association between serum zinc and TyG, we used univariate and multivariable linear regression models. The models integrated regression coefficients (β) and 95% confidence intervals (CI) while controlling for significant covariates. Log-transformed serum zinc was considered a continuous variable after undergoing a logarithm 10 transformation. The selection of confounding variables was guided by clinical relevance, existing scientific literature, the significance of covariates in univariate analysis, their correlation with the outcomes of interest, or a change in effect estimate exceeding 10%. In multivariable linear regression, we showed (1) unadjusted models, (2) model 1 adjusted covariates with a change in effect estimate exceeding 10%, including sex, BMI, HDL-C, TC, uric acid, diabetes and trouble sleeping, (3) model 2 adjusted for variables from model 1 plus covariates that P values were <0.05 in the univariate analysis, including age, race and ethnicity, educational level, physical activity, smoking status, HbA1c, failing kidneys, hypertension, and (4) model 3 adjusted for variables from model 2 plus covariates that on the basis of previous findings and clinical constraints, including marital status, PIR, drinking status, creatinine, total energy intake (17, 49).

In addition, we examined possible dose-response relationships between log-transformed serum zinc and TyG after adjusting variables in model 3, restricted cubic spline (RCS) regression was performed with 4 knots at the 5th, 35th, 65th, and 95th percentiles of the distribution (43).

We used a two-piece-wise linear regression model with smoothing to analyze the association threshold between log-transformed serum zinc and TyG after adjusting the variables in model 3, the likelihood-ratio test and the bootstrap resampling method were used in determining inflection points, in addition to conducting separate analyses for the age groups <60 years and age ≥60 years.

Furthermore, we compared potential modifications of the relationship between log-transformed serum zinc and TyG in the groups with age <60 years and age ≥60 years. The heterogeneity in the subgroup were assessed using multivariable linear regression and interactions between the subgroup and log-transformed serum zinc were examined through likelihood ratio testing.

3 Results

3.1 Study population

In total, 29 902 participants completed the interview, of whom 12,854 participants were <20 years old. We excluded pregnant women (n = 192), those missing data on TG (n = 9,680), those missing data on FPG (n = 9), those missing data on serum zinc (n = 4,841), or those with covariates (n = 636). And we excluded participants with extreme energy intake, consuming <500 or >5,000 kcal per day (n = 80). Ultimately, this cross-sectional study included 1 610 participants from the NHANES between 2011 and 2016 in the analysis. The detailed inclusion and exclusion process is shown in Figure 1. The figure delineates the study's design, sampling, and exclusion procedures. This study included American adults (aged ≥20 years) who participated in the 2011–2012, 2013–2014, and 2015–2016 cycles of NHANES, as the serum zinc is only assessed during these survey waves.

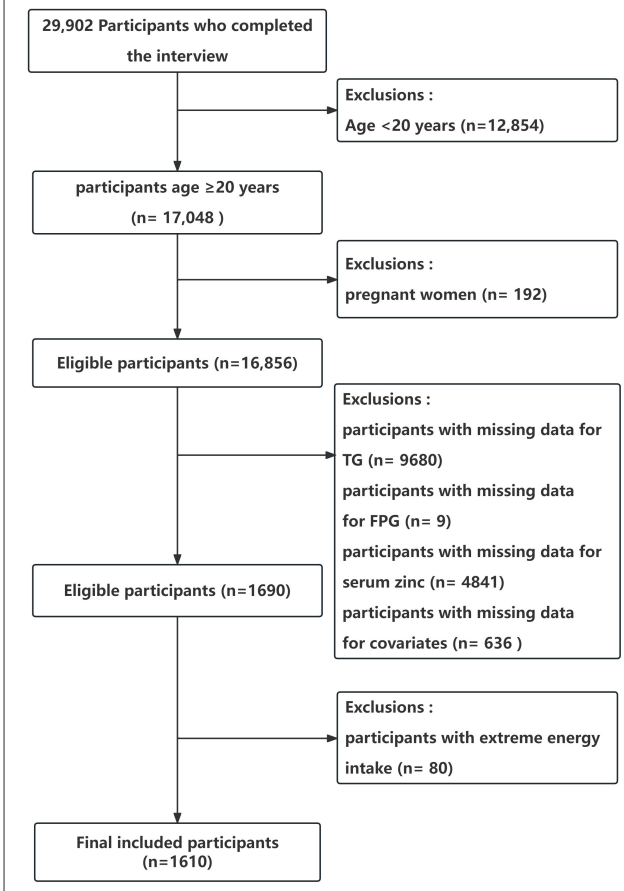

FIGURE 1
Flow chart of the study population enrollment. TG, triglyceride; FPG, fasting blood glucose.

3.2 Baseline characteristics

The Supplementary Table S1 describes the baseline characteristics of the excluded and included participants. Table 1 illustrates the baseline characteristics of all subjects based on their age, categorized into the age <60 group and age ≥60 group. The average age of the study participants was 49.6 (17.6) years, and 839 (52.1%) individuals were male. In comparison to the age <60 group, the age ≥60 group exhibited elevated levels of HbA1c, HDL-C, creatinine, FPG, uric acid, and TyG. Furthermore, they demonstrated a higher prevalence of hypertension, diabetes, renal impairment, and sedentary physical activity, along with a lower

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prevalence of current smoking, current alcohol use, and lower educational attainment. The log-transformed serum zinc levels in the age ≥60 group were comparable to those in the age <60 group ($P = 0.29$).

3.3 Relationship between serum zinc level and TyG

The univariate analysis demonstrated that age, sex, race/ethnicity, education level, BMI, physical activity, smoking status, trouble sleeping, HbA1c, HDL-C, TC, uric acid, failing kidneys, hypertension, diabetes, and log-transformed serum zinc were associated with TyG (Table 2).

The findings of the multivariable linear regression analysis are shown in Table 3. In the unadjusted model, there was a positive association of log-transformed serum zinc with TyG ($\beta = 1.16$, 95% CI = 0.55–1.78). Results were similar after adjusting for sex, BMI, HDL-C, TC, uric acid, diabetes and trouble sleeping ($\beta = 0.50$, 95% CI = 0.04–0.96). After adjusting for other possible confounders, including age, race and ethnicity, educational level, physical activity, smoking status, HbA1c, failing kidneys, hypertension, marital status, PIR, alcohol, creatinine, and total daily energy intake, the positive association remained significant ($\beta = 0.50$, 95% CI = 0.08–0.93; $P < 0.05$). When log-transformed serum zinc was analyzed using quartiles, the association between TyG was consistent across all models, indicating their robustness (Table 3). The individuals with quartile 3 (Q3) group of log-transformed serum zinc (1.94–1.97 μg/dL) were used as the baseline reference, those with Q4 group of log-transformed serum zinc (1.98–2.37 μg/dL) had an adjusted β for TyG of 0.092 (95% CI 0.013–0.17, $P < 0.05$; Table 3) after adjusting for the variables in Model 3.

Accordingly, in the fully adjusted analyses, the restricted cubic spline (RCS) regression (Figure 2) indicated a non-linear relationship between log-transformed serum zinc and TyG levels in a J-shaped manner (P for nonlinearity = 0.019) (A). A segmented regression model was employed to delineate the intervals and calculate threshold effects, with an inflection point at ~1.94 μg/dL. The results are presented in Table 4. When the log-transformed serum zinc was <1.94 μg/dL, the estimated dose-response curve exhibited a consistent horizontal trend, and the relationships between the log-transformed serum zinc and TyG was not significant ($P > 0.05$). Likewise, the TyG exhibited an increase with rising log-transformed serum zinc after the inflection point, with a correlation coefficient (β) of 0.96 (95% CI: 0.25–1.66) after adjusting for the variables in Model 3 (Table 4). Furthermore, within the age < 60 years group, the TyG demonstrated an increase with escalating log-transformed serum zinc after the inflection point, with a correlation coefficient (β) of 1.21 (95% CI: 0.49–1.94) after adjusting for the variables in Model 3. Conversely, within the age ≥60 years group, the TyG exhibited an increase with increasing log-transformed serum zinc prior to the inflection point, with a correlation coefficient (β) of 1.98 (95% CI: 0.62–3.35) after adjusting for the variables in Model 3 (Table 5).

And the RCS analysis was also applied to investigated the dose-response association between log-transformed serum zinc level and TyG with age ≥60 years and age <60 years. Figure 2 shows that in

TABLE 1 Baseline characteristics of participants.

Covariates	Total (n = 1,610)	Age < 60 (n = 1,065)	Age ≥ 60 (n = 545)	P-value
Age (mean ± SD, years)	49.6 ± 17.6	39.4 ±11.7	69.6 ± 6.6	<0.001
Gender, *n* (%)				
Male	839 (52.1)	550 (51.6)	289 (53.0)	0.60
Female	771 (47.9)	515 (48.4)	256 (47.0)	
Race/Ethnicity, *n* (%)				
Non-Hispanic White	696 (43.2)	415 (39.0)	281 (51.6)	<0.001
Non-Hispanic Black	303 (18.8)	209 (19.6)	94 (17.2)	
Mexican American	219 (13.6)	157 (14.7)	62 (11.4)	
Others	392 (24.4)	284 (26.7)	108 (19.82)	
Education level, *n* (%)				
<9	137 (8.5)	59 (5.5)	78 (14.3)	<0.001
9–12	556 (34.5)	357 (33.5)	199 (36.5)	
>12	917 (57.0)	649 (60.9)	268 (49.2)	
Marital status, *n* (%)				
Living alone	969 (60.2)	634 (59.5)	335 (61.5)	0.45
Married or living with a partner	641 (39.8)	431 (40.5)	210 (38.5)	
PIR, *n* (%)				
Low (PIR ≤1.3)	526 (32.7)	349 (32.8)	177 (32.5)	0.37
Medium (PIR >1.3–3.5)	604 (37.5)	388 (36.4)	216 (39.6)	
High (PIR >3.5)	480 (29.8)	328 (30.8)	152 (27.9)	
BMI (kg/m^2), mean ± SD	29.3 ± 7.1	29.3 ± 7.4	29.3 ± 6.4	0.93
BMI, *n* (%)				
<18.5 kg/m^2	30 (1.9)	23 (2.2)	7 (1.3)	0.33
18.5–24.9 kg/m^2	442 (27.4)	301 (28.3)	141 (25.9)	
25–29.9 kg/m^2	526 (32.7)	336 (31.6)	190 (34.9)	
≥30 kg/m^2	612 (38.0)	405 (38.0)	207 (38.0)	
Physical activity, *n* (%)				
Sedentary	743 (46.2)	452 (42.4)	291 (53.4)	<0.001
Moderate	537 (33.4)	354 (33.2)	183 (33.6)	
Vigorous	330 (20.5)	259 (24.3)	71 (13.0)	
Smoking status, *n* (%)				
Never	890 (55.3)	625 (587)	265 (48.6)	<0.001
Former	408 (25.3)	192 (18.0)	216 (39.6)	
Current	312 (19.4)	248 (23.3)	64 (11.7)	
Drinking status, *n* (%)				
≥12 alcohol drinks a year	1,189 (73.8)	816 (76.6)	373 (68.4)	<0.001

(Continued)

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TABLE 1 (Continued)

Covariates	Total (n = 1,610)	Age < 60 (n = 1,065)	Age ≥ 60 (n = 545)	P-value
Trouble sleeping, n (%)	431 (26.8)	260 (24.4)	171 (31.4)	0.0029
HbA1c (%), mean ± SD	5.77 ± 1.08	5.62 ±1.05	6.07 ± 1.08	<0.001
HDL-C (mg/dL), mean ± SD	54.0 ± 15.8	52.7 ±14.9	56.5 ±17.3	<0.001
TG (mg/dL), mean ± SD	114.1 ± 65.4	112.4 ± 67.1	117.4 ±61.8	0.14
Creatinine (mg/dL), mean ± SD	0.89 ± 0.45	0.84 ± 0.36	1.00 ± 0.57	<0.001
TC (mg/dL), mean ± SD	191 ± 41	191 ± 40	190 ± 41	0.62
Energy (kcal), mean ± SD	2,119 ± 873	2,233 ± 912	1,897 ±744	<0.001
FPG (mg/dL), mean ± SD	109 ±34	105 ±32	116 ±36	<0.001
Uric acid (mg/dL), mean ± SD	5.53 ± 1.37	5.45 ± 1.38	5.68 ± 1.35	0.0014
TyG, mean ± SD	8.55 ±0.64	8.49 ± 0.66	8.67 ±0.60	<0.001
log-transformed serum zinc (μg/dL), mean ± SD	1.94 ±0.07	1.94 ±0.07	1.94 ±0.07	0.29
Hypertension, n (%)	748 ±46	386 ±36	362 ±66	<0.001
Diabetes, n (%)	365 (22.7)	144 (13.5)	221 (40.6)	<0.001
Failing kidneys, n (%)	60 (3.7)	22 (2.1)	38 (7.0)	<0.001

Data presented are mean ± SD or N (%).
SD, standard deviation; PIR, family poverty income ratio; BMI, body mass index; HbA1c, glycated hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; FPG, fasting blood glucose; TyG, triglyceride-glucose index.

the fully adjusted analyses log-transformed serum zinc was related to level of TyG in a J shaped nonlinear manner (P for nonlinearity = 0.014) in age <60 years group (B), but in a inverted-L shaped nonlinear manner (P for nonlinearity < 0.001) in age ≥60 years group (C).

3.4 Stratified analyses

A stratified analysis was conducted according to age, gender, BMI and diabetes to determine whether there were differential effects in the association between log-transformed serum zinc and TyG in American adults (aged ≥20 years). The results demonstrated that no statistically significant interactions were identified in any of the subgroups after stratification by gender, BMI and diabetes in model 3 (Supplementary Table S2). However, when log-transformed serum zinc was transformed into a categorical

TABLE 2 Association of convariates and triglyceride-glucose index.

Variable	β (95% CI)	P-value
Age (years)	0.01 (0.01 to 0.01)	<0.001
Gender		
Male	0 (reference)	<0.001
Female	−0.19 (−0.27 to −0.12)	
Race/Ethnicity		
Non-Hispanic White	0 (reference)	
Non-Hispanic Black	−0.26 (−0.36 to −0.15)	<0.001
Mexican American	0.02 (−0.09 to 0.13)	0.710
Others	−0.05 (−0.13 to 0.03)	0.219
Education level		
<9	0 (reference)	
9–12	−0.09 (−0.23 to 0.05)	0.199
>12	−0.22 (−0.34 to −0.11)	<0.001
Married or living with a partner	−0.05 (−0.13 to 0.02)	0.154
BMI kg/m^2	0.03 (0.02 to 0.03)	<0.001
BMI		
<18.5 kg/m^2	0 (reference)	
18.5–24.9 kg/m^2	−0.02 (−0.19 to 0.16)	0.845
25–29.9 kg/m^2	0.41 (0.22 to 0.59)	<0.001
≥30 kg/m^2	0.52 (0.34 to 0.70)	<0.001
Physical activity		
Sedentary	0 (reference)	
Moderate	−0.16 (−0.27 to −0.05)	0.005
Vigorous	−0.10 (−0.21 to 0)	0.053
Smoking status		
Never	0 (reference)	
Former	0.13 (0.02 to 0.23)	0.02
Current	0.17 (0.04 to 0.31)	0.014
Drinking status	0 (reference)	
≥12 alcohol drinks a year	0.04 (−0.06 to 0.14)	0.384
Trouble sleeping	0.14 (0.05 to 0.24)	0.004
HbA1c (%)	0.28 (0.23 to 0.34)	<0.001
HDL (mg/dL)	−0.02 (−0.02 to −0.02)	<0.001
TC (mg/dL)	0.01 (0 to 0.01)	<0.001
Creatinine (mg/dL)	0.20 (0.04 to 0.36)	0.107
Uric acid (mg/dL)	0.14 (0.11 to 0.18)	<0.001
Failing kidneys	0.25 (0.09 to 0.41)	0.003
Hypertension	0.32 (0.25 to 0.40)	<0.001
Diabetes	0.60 (0.51 to 0.69)	<0.001
Log-transformed serum zinc (μg/dL)	1.16 (0.55–1.78)	<0.001

Data presented are β and 95% CI.
BMI, body mass index; HbA1c, glycated hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; 95% CI, 95% confidence interval.

TABLE 3 Multivariable linear regression was used to determine the relationship between log-transformed serum zinc and triglyceride-glucose index, weighted.

Variable	No.	Crude model		Model 1		Model 2		Model 3	
		β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
log-transformed serum zinc (μg/dL)	1,624	1.16 (0.55 to 1.78)	<0.001	0.50 (0.04 to 0.96)	0.033	0.50 (0.08 to 0.92)	0.022	0.50 (0.08 to 0.93)	0.023
Quartiles [log-transformed serum zinc (μg/dL)]									
Q1 (1.69–1.89)	397	−0.07 (−0.19 to 0.06)	0.28	0.004 (−0.08 to 0.09)	0.92	0.004 (−0.08 to 0.09)	0.93	0.01 (−0.08 to 0.09)	0.89
Q2 (1.90–1.93)	408	−0.08 (−0.20 to 0.05)	0.28	0.01 (−0.06 to 0.08)	0.76	0.01 (−0.05 to 0.07)	0.75	0.01 (−0.06 to 0.07)	0.83
Q3 (1.94–1.97)	401	0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)	
Q4 (1.98–2.37)	404	0.13 (0.02 to 0.23)	0.016	0.09 (0.02 to 0.17)	0.015	0.087 (0.012 to 0.16)	0.025	0.09 (0.01 to 0.17)	0.025
P for trend			0.031		0.011		0.018		0.020

Data presented are β and 95% CI.
Model 1: Adjusted for gender, BMI, HDL-C, TC, uric acid, diabetes, trouble sleeping.
Model 2: Adjusted for Model 1 + age, race and ethnicity, educational level, physical activity, smoke, HbA1c, failing kidneys, hypertension.
Model 3: Adjusted for Model 2+ marital status, PIR, alcohol, creatinine, energy.
BMI, body mass index; HbA1c, glycated hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; PIR, family poverty income ratio; 95% CI, 95% confidence interval.

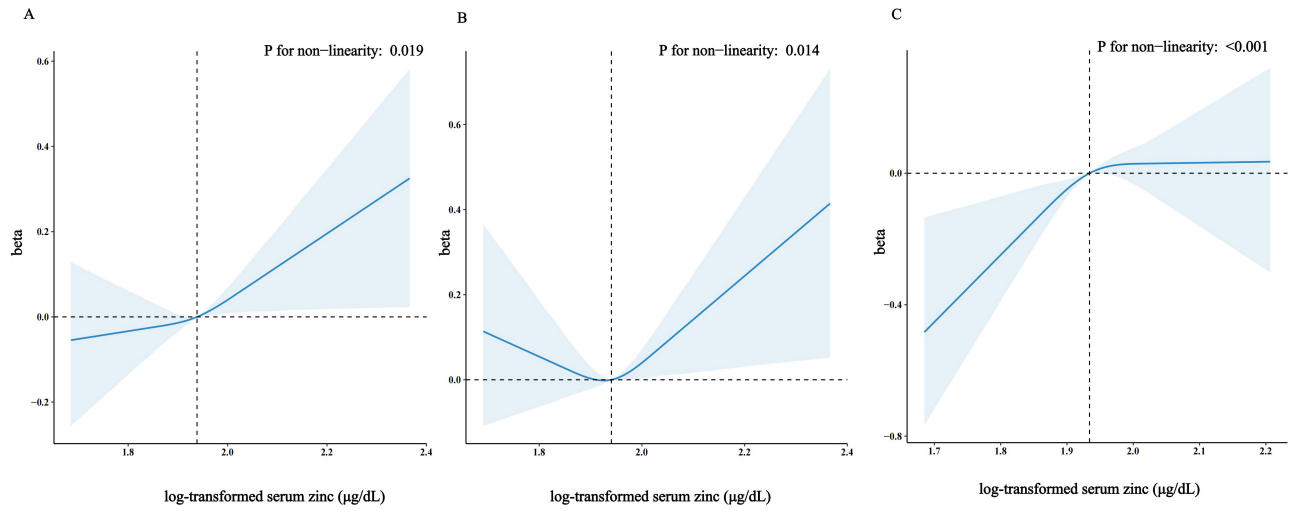

FIGURE 2
Restricted cubic spline model of the β coefficient of log-transformed serum zinc level with TyG with age ≥20 years **(A)** or with age <60 years **(B)** or with age ≥60 years **(C)**. Adjusted for gender, body mass index (BMI), high-density lipoproteins (HDL), total cholesterol (TC), uric acid, diabetes, trouble sleeping, age, race and ethnicity, educational level, physical activity, smoke, HbA1c, failing kidneys, hypertension, marital status, poverty to income ratio (PIR), drinking status, creatinine, total energy intake. The dashed lines represent the 95% confidence intervals. Heavy central lines represent the estimated adjusted correlation coefficient (β), with LightSkyBlue shaded ribbons denoting 95% confidence intervals. The horizontal dotted lines represent the correlation coefficient (β) of 0 (Reference point). The reference point was set at the median level of log-transformed serum zinc (1.94 μg/dL), and the vertical dotted lines indicate the threshold value of log-transformed serum zinc at 1.94 μg/dL.

variable, an interaction between log-transformed serum zinc and TyG was observed in individuals aged <60 years and those aged ≥60 years (*P* value for the likelihood ratio test for the interaction was $P = 0.017$; Table 6). The individuals with the log-transformed serum zinc quartile 3 (Q3) group (1.94–1.97 μg/dL) were used as the baseline reference. In the group of individuals aged <60 years, those with the Q4 group of log-transformed serum zinc (1.98–2.37 μg/dL) exhibited an adjusted β for TyG of 0.096 (95% CI 0.009–0.18; $P < 0.05$) in comparison to the Q3 group, after adjusting for the variables in Model 3. In the group of individuals aged ≥60 years, those with the Q1 group of log-transformed serum zinc levels (1.69–1.89 μg/dL) exhibited an adjusted β for TyG of −0.169 (95% CI −0.280 to −0.058; $P < 0.05$) in comparison to the Q3 group.

4 Discussion

In this cross-sectional analysis of US adults aged ≥ 20 years, using NHANES data from 2011 to 2016, we identified a positive association between log-transformed serum zinc levels and TyG. Across all models, the effect size for log-transformed serum zinc with TyG (β = 0.50) remains relatively consistent in Models 1, 2, and 3. Notably, we observed a J-shaped non-linear relationship between log-transformed serum zinc levels and TyG, with an inflection point at ~1.94 μg/dL. Furthermore, a statistically significant interaction was identified between log-transformed serum zinc levels and TyG in individuals aged ≥60 years and those < 60 years (P < 0.05). These findings have significant clinical implications.

Insulin resistance has been suggested to play a noteworthy role in the pathogenesis of metabolic syndrome (30, 50, 51), and some evidences (51–54) suggests a direct association between serum zinc and insulin resistance which is consistent with our studies. For example, an 11-year prospective follow-up investigation was carried out among 683 male participants from the Kuopio Ischaemic Heart Disease Risk Factor Study (51) who were aged 42–60 years at baseline between 1984 and 1989. Teymoor Yary et al. (51) revealed that elevated serum zinc levels were linked to increased Homeostatic Model Assessment (HOMA) of insulin resistance and HOMA of beta cell. Additionally, a positive correlation was observed between higher serum zinc levels and the development of metabolic syndrome, as well as three of its constituent features, namely increased waist circumference, hypertension, and low serum HDL cholesterol (30). And a cross-sectional observational study (55) using NHANES data from

TABLE 4 Association between log-transformed serum zinc level and triglyceride-glucose index using two-piece-wise regression models.

Variable log-transformed serum zinc (μg/dL)	Crude model		Adjusted model	
	β (95% CI)	P value	β (95% CI)	P value
<1.94	0.33 (−0.85 to 1.50)	0.58	0.26 (−0.65 to 1.16)	0.56
≥1.94	1.52 (0.34 to 2.70)	0.013	0.96 (0.25 to 1.66)	0.01

Data presented are β and 95% CI.
Adjusted for gender, BMI, HDL-C, TC, uric acid, diabetes, trouble sleeping, age, race and ethnicity, educational level, physical activity, smoke, HbA1c, failing kidneys, hypertension, marital status, PIR, drinking status, creatinine, total energy intake.
BMI, body mass index; HbA1c, glycated hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; PIR, family poverty income ratio; 95% CI, 95% confidence interval.

TABLE 5 Association between log-transformed serum zinc level and triglyceride-glucose index using two-piece-wise regression models within the age < 60 years group and the age ≥ 60 years group (All participants).

Variable	<60 years (n = 1,065)				≥60 years (n = 545)			
	Crude model		Adjusted model		Crude model		Adjusted model	
Log-transformed serum zinc (μg/dL)	β (95%CI)	P-value	β (95%CI)	P-value	β (95%CI)	P-value	β (95%CI)	P-value
<1.94	0.39 (−1.23~2.00)	0.63	−0.41 (−1.65~0.84)	0.50	0.20 (−1.71~2.10)	0.83	1.98 (0.62~3.35)	0.01
≥1.94	1.85 (0.54~3.16)	0.01	1.21 (0.49~1.94)	0.002	0.38 (−1.58~2.34)	0.70	−0.05 (−1.98~1.89)	0.95

Adjusted model: gender, BMI, HDL, TC, uric acid, diabetes, trouble sleeping, age, race and ethnicity, educational level, physical activity, smoke, HbA1c, failing kidneys, hypertension, marital status, PIR, drinking status, creatinine, total energy intake.
BMI, body mass index; HbA1c, glycated hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TyG, Triglyceride-glucose index; PIR, family poverty income ratio; 95% CI, 95% confidence interval.

TABLE 6 Interactive effect of log-transformed serum zinc and triglyceride-glucose index in patients within the age < 60 years group and the age ≥ 60 years group (All participants).

Variable	<60 years (n = 1,065)		≥60 years (n = 545)		P for interaction
	β (95%CI)	P-value	β (95%CI)	P-value	
Log-transformed serum zinc (μg/dL)	0.301 (−0.190, 0.791)	0.215	0.968 (0.297, 1.639)	0.007	0.166
Quartiles (log-transformed serum zinc (μg/dL))					
Q1(1.69-1.89)	0.070 (−0.028, 0.169)	0.151	−0.169 (−0.280,−0.058)	0.005	
Q2(1.90-1.93)	0.010 (−0.070, 0.089)	0.801	−0.001 (−0.111, 0.109)	0.985	
Q3(1.94-1.97)	0 (reference)		0 (reference)		0.017
Q4(1.98-2.37)	0.096 (0.009, 0.184)	0.033	0.049 (−0.069, 0.167)	0.39	

Adjusted model: gender, BMI, HDL, TC, uric acid, diabetes, trouble sleeping, age, race and ethnicity, educational level, physical activity, smoke, HbA1c, failing kidneys, hypertension, marital status, PIR, drinking status, creatinine, total energy intake.
BMI, body mass index; HbA1c, glycated hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TyG, Triglyceride-glucose index; PIR, family poverty income ratio; 95% CI, 95% confidence interval.

2011 to 2016 also revealed that serum zinc concentration was significantly higher in both abnormal glucose tolerance and diabetes mellitus groups when compared to the normal glucose tolerance group.

However, other studies have produced contrasting results. According to a cross-sectional study (56), it was found that the prevalence of insulin resistance (HOMA-IR; categorized according to the 75th percentile of the sample distribution) was elevated among Brazilian adolescents falling within the lower quartiles of zinc intake (<7.5 mg), with a prevalence ratios (PR; 95% CI) of 1.23 (1.10–1.38) compared to those in the higher quartiles of zinc intake (>16.3 mg; P <0.05).

One randomized, placebo-control study (57) found that zinc supplementation at 30 mg daily for 4 weeks significantly decreased fasting insulin and HOMA values in Brazilian obese women aged 25–45 years, but that plasma zinc, BMI, fasting glucose, and leptin levels were unaffected by zinc supplementation.

However, a study conducted in Korea (58) revealed that a daily zinc supplementation of 30 mg over an 8-week period enhanced serum zinc and urinary zinc concentrations in obese Korean women (BMI ≥25 kg/m^2) aged 19–28 years. Nevertheless, the study found that zinc supplementation did not lead to improvements in insulin resistance (HOMA-IR) or in any other metabolic risk factors. Similarly, Beletate et al. (59) found that a 4-week zinc supplementation did not result in any significant improvements in insulin resistance, fasting glucose, or lipid levels in women who were obese and aged 25–45 years with normal glucose tolerance. Regina El Dib et al. (29) included three randomized controlled studies in their review. The duration of zinc supplementation ranged between four and 12 weeks. The trials' primary outcome measure was insulin resistance, which was assessed using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). The comparative analysis of this parameter between the zinc supplemented cohort and the control group revealed no statistically significant disparities across two trials, which collectively enrolled 114 participants.

Table 3 indicates a significant association between the highest quartile of serum zinc and TyG. However, the underlying mechanisms remain unclear, with several potential mechanisms having been proposed. First, elevated zinc concentrations may influence hormonal homeostasis, including leptin. Such hormonal imbalances could potentially lead to an increase in BMI, which in turn may precipitate insulin resistance (53). Second, it has been demonstrated that zinc plays a significant role in the function of β-cells and the secretion of insulin (60). Consequently, it has been proposed that the activity of β-cells should be enhanced in order to facilitate the management of glucose levels among individuals with type 2 diabetes. However, excessive zinc intake may result in hyperactivity of β-cells and insulin production, potentially leading to insulin resistance through receptor exhaustion or prolonged zinc stimulation, which could have adverse effects on β-cells. Third, excessive zinc intake can also result in adverse effects, including altered copper and iron homeostasis, decreased concentrations of HDL cholesterol and serum lipoprotein, and impairment of liver function (61, 62). As previous studies have demonstrated that patients with type 2 diabetes have lower serum zinc concentrations and higher urinary zinc excretion compared to healthy controls (63, 64), this might be a protective mechanism aimed at eliminating surplus zinc to avert the onset of zinc-induced toxicity.

Very few studies have focused on relationship between serum zinc and TyG combined with age. In this study, the associations between serum zinc levels and TyG in participants aged ≥60 and <60 years were evaluated, adjusting for relevant variables. In Table 6, The results indicate a significant association between serum zinc and TyG in the ≥60 years group (β = 0.968, P = 0.007), but not in the <60 years group (β = 0.301, P = 0.215). However, the underlying mechanisms remain unclear. On the one hand, zinc plays a crucial role in immune function, and its deficiency is more prevalent in older adults, documented by a decline in serum or plasma zinc levels with age. Low zinc status is associated with a weakened immune system, but long-term and high-dose zinc supplementation may lead to some potential adverse effects, such as copper deficiency (65) and immunosuppressive, especially suppress T cell mediated events which will have a significant impact on the immunological outcome (66). This process may be associated with insulin resistance. On the other hand, in contrast to younger individuals, elderly patients experience a decline in physiological functions, making them more prone to various metabolic disorders (3). Abnormal bioelement levels can contribute to metabolic syndrome, especially in aging men. Zinc plays a crucial role in the function of beta cells within the islets of Langerhans. Animal studies indicate that low doses of zinc can protect against type 2 diabetes, whereas high concentrations can be toxic to these beta cells. This toxicity may lead to insulin resistance (34).

This study has several strengths. Firstly, the study encompasses a large, nationally representative sample of US adults. Secondly, the investigation modeled the associations between serum zinc and TyG while accounting for established and potential covariates. Thirdly, the study explored associations stratified by age groups of ≥60 and <60 years. Furthermore, a dose-response analysis was conducted to assess the relationship between serum zinc and TyG, as well as with age groups of ≥60 and <60 years.

Despite the strengths of the study, several limitations should be noted. First, this study was conducted with a US population, additional research is required to confirm whether our results can be generalized to other populations. Second, residual confounding effects could not be excluded. We constructed multivariable linear regression models and performed subgroup and sensitivity analyses to control for the effects of potential confounders on the relationship between serum zinc and TyG. Third, the study is in the lack of data on zinc intake in the population under investigation. High-quality, interventional and prospective studies are required to clarify the effects of zinc intake on TyG. Four, we recognize that nonrandom missing data could influence our findings due to baseline differences between included and excluded participants. To address this, we adopted a rigorous methodological approach. Following NHANES guidelines, we conducted a weighted analysis to account for survey design intricacies, including stratification and weighting, ensuring our results are representative of the broader U.S. population. Additionally, we performed model adjustments to enhance the reliability and robustness of our outcomes. Finally, because this was a cross-sectional observation study, the associations found in this study may not result in

direct causality (67). Our study, a secondary analysis of publicly available data, explores the association between serum zinc and TyG index in adult Americans. While the evidence level from such secondary analyses is lower than that from primary studies, they effectively utilize existing data and can lay the groundwork for future research. Therefore, longitudinal studies are required to determine whether the observed relationship between the serum zinc and TyG is causal, as well as to explore the interactive effect of age on serum zinc and TyG. There may be a mechanistic association between age and TyG, which requires further investigation due to the biological distinctions it creates.

5 Conclusions

A J-shaped, non-linear positive correlation was observed between serum zinc levels and TyG, with an inflection point at ~1.94 μg/dL. Additionally, a statistically significant interaction was noted between serum zinc levels and TyG in individuals aged ≥60 years and those <60 years. In the age <60 years group, serum zinc exhibited a J-shaped non-linear association with TyG, while in the age ≥60 years group, the relationship followed an inverted-L shaped non-linear pattern. These outcomes suggest that there are potential adverse effects of high serum zinc levels on glucose metabolism by levels of TyG. Although this study offers valuable clinical insights, further prospective research is warranted to substantiate these findings, and to delve into the underlying mechanisms.

Data availability statement

NHANES data used in this work is publicly available. All raw data are available on the NHANES website (https://www.cdc.gov/nchs/nhanes/).

Ethics statement

The studies involving humans were approved by the US National Center for Health Statistics Research Ethics Review Board granted ethical approval for NHANES (Protocol No. 2011-17, Continuation of Protocol No. 2011-17; available at: https://www.cdc.gov/nchs/nhanes/irba98.htm). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JL: Conceptualization, Formal analysis, Writing – original draft. X-QL: Data curation, Writing – review & editing. YZ: Data curation, Writing – review & editing. ZL: Data curation, Writing – review & editing. QW: Data curation, Writing – review & editing. YC: Conceptualization, Project administration, Writing – review & editing.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1475204/full#supplementary-material

References

1. Seo JA, Song SW, Han K, Lee KJ, Kim HN. The associations between serum zinc levels and metabolic syndrome in the Korean population: findings from the 2010 Korean National Health and Nutrition Examination Survey. *PLoS ONE.* (2014) 9:e105990. doi: 10.1371/journal.pone.0105990

2. Dubey P, Thakur V, Chattopadhyay M. Role of minerals and trace elements in diabetes and insulin resistance. *Nutrients.* (2020) 12:1864. doi: 10.3390/nu12061864

3. Olechnowicz J, Tinkov A, Skalny A, Suliburska J. Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. *J Physiol Sci.* (2018) 68:19–31. doi: 10.1007/s12576-017-0571-7

4. Maret W. Zinc in pancreatic islet biology, insulin sensitivity, and diabetes. *Prev Nutr Food Sci.* (2017) 22:1–8. doi: 10.3746/pnf.2017.22.1.1

5. Lu CW, Lee YC, Kuo CS, Chiang CH, Chang HH, Huang KC, et al. Association of serum levels of zinc, copper, and iron with risk of metabolic syndrome. *Nutrients.* (2021) 13:548. doi: 10.3390/nu13020548

6. Prasad AS. Clinical, endocrinological and biochemical effects of zinc deficiency. *Clin Endocrinol Metab.* (1985) 14:567–89. doi: 10.1016/S0300-595X(85)80007-4

7. Hong JS, Park MK, Kim MS, Lim JH, Park GJ, Maeng EH, et al. Effect of zinc oxide nanoparticles on dams and embryo-fetal development in rats. *Int J Nanomed.* (2014) 9:145–57. doi: 10.2147/IJN.S57931

8. Wang B, Feng W, Wang M, Wang T, Gu Y, Zhu M, et al. Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice. *J Nanoparticle Res.* (2008) 10:263–76. doi: 10.1007/s11051-007-9245-3

9. Yan G, Huang Y, Bu Q, Lv L, Deng P, Zhou J, et al. Zinc oxide nanoparticles cause nephrotoxicity and kidney metabolism alterations in rats. *J Environ Sci Health A Tox Hazard Subst Environ Eng.* (2012) 47:577–88. doi: 10.1080/10934529.2012.650576

10. King JC, Brown KH, Gibson RS, Krebs NF, Lowe NM, Siekmann JH, et al. Biomarkers of Nutrition for Development (BOND)-zinc review. *J Nutr.* (2016) 146:858S–85S. doi: 10.3945/jn.115.220079

11. Di Pino A, DeFronzo RA. Insulin resistance and atherosclerosis: implications for insulin-sensitizing agents. *Endocr Rev.* (2019) 40:1447–67. doi: 10.1210/er.2018-00141

12. Beverly JK, Budoff MJ. Atherosclerosis: pathophysiology of insulin resistance, hyperglycemia, hyperlipidemia, and inflammation. *J Diabetes.* (2020) 12:102–4. doi: 10.1111/1753-0407.12970

13. Ding X, Wang X, Wu J, Zhang M, Cui M. Triglyceride-glucose index and the incidence of atherosclerotic cardiovascular diseases: a meta-analysis of cohort studies. *Cardiovasc Diabetol.* (2021) 20:76. doi: 10.1186/s12933-021-01268-9

14. Zhang Q, Xiao S, Jiao X, Shen Y. The triglyceride-glucose index is a predictor for cardiovascular and all-cause mortality in CVD patients with diabetes or pre-diabetes: evidence from NHANES 2001–2018. *Cardiovasc Diabetol.* (2023) 22:279. doi: 10.1186/s12933-023-02030-z

15. Bello-Chavolla OY, Almeda-Valdes P, Gomez-Velasco D, Viveros-Ruiz T, Cruz-Bautista I, Romo-Romo A, et al. METS-IR, a novel score to evaluate insulin sensitivity, is predictive of visceral adiposity and incident type 2 diabetes. *Eur J Endocrinol.* (2018) 178:533–44. doi: 10.1530/EJE-17-0883

16. Borai A, Livingstone C, Ferns GA. The biochemical assessment of insulin resistance. *Ann Clin Biochem.* (2007) 44:324–42. doi: 10.1258/000456307780945778

17. Li S, Ding J, Sun X, Feng L, Zhou W, Gui Z, et al. Selenium concentration is positively associated with triglyceride-glucose index and triglyceride glucose-body mass index in adults: data from NHANES 2011–2018. *Biol Trace Elem Res.* (2024) 202:401–9. doi: 10.1007/s12011-023-03684-2

18. Simental-Mendía LE, Rodríguez-Morán M, Guerrero-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metab Syndr Relat Disord.* (2008) 6:299–304. doi: 10.1089/met.2008.0034

19. Tahapary DL, Pratisthita LB, Fitri NA, Marcella C, Wafa S, Kurniawan F, et al. Challenges in the diagnosis of insulin resistance: focusing on the role of HOMA-IR and tryglyceride/glucose index. *Diabetes Metab Syndr.* (2022) 16:102581. doi: 10.1016/j.dsx.2022.102581

20. Liu D, Yang K, Gu H, Li Z, Wang Y, Wang Y. Predictive effect of triglyceride-glucose index on clinical events in patients with acute ischemic stroke and type 2 diabetes mellitus. *Cardiovasc Diabetol.* (2022) 21:280. doi: 10.1186/s12933-022-01704-4

21. Minh HV, Tien HA, Sinh CT, Thang DC, Chen CH, Tay JC, et al. Assessment of preferred methods to measure insulin resistance in Asian patients with hypertension. *J Clin Hypertens.* (2021) 23:529–37. doi: 10.1111/jch.14155

22. Park B, Lee HS, Lee YJ. Triglyceride glucose (TyG) index as a predictor of incident type 2 diabetes among nonobese adults: a 12-year longitudinal study of the Korean Genome and Epidemiology Study cohort. *Transl Res.* (2021) 228:42–51. doi: 10.1016/j.trsl.2020.08.003

23. Cui C, Liu L, Zhang T, Fang L, Mo Z, Qi Y, et al. Triglyceride-glucose index, renal function and cardiovascular disease: a national cohort study. *Cardiovasc Diabetol.* (2023) 22:325. doi: 10.1186/s12933-023-02055-4

24. Gao S, Ma W, Huang S, Lin X, Yu M. Impact of triglyceride-glucose index on long-term cardiovascular outcomes in patients with Myocardial Infarction with nonobstructive coronary arteries. *Nutr Metabolism Cardiovasc Dis.* (2021) 31:3184–92. doi: 10.1016/j.numecd.2021.07.027

25. Tao L, Xu J, Wang T, Hua F, Li J. Triglyceride-glucose index as a marker in cardiovascular diseases: landscape and limitations. *Cardiovasc Diabetol.* (2022) 21:1–17. doi: 10.1186/s12933-022-01511-x

26. Fernández-Cao JC, Warthon-Medina M, Hall Moran V, Arija V, Doepking C, Lowe NM. Dietary zinc intake and whole blood zinc concentration in subjects with type 2 diabetes versus healthy subjects: a systematic review, meta-analysis and meta-regression. *J Trace Elem Med Biol.* (2018) 49:241–51. doi: 10.1016/j.jtemb.2018.02.008

27. Li YV. Zinc and insulin in pancreatic beta-cells. *Endocrine.* (2014) 45:178–89. doi: 10.1007/s12020-013-0032-x

28. Jansen J, Karges W, Rink L. Zinc and diabetes-clinical links and molecular mechanisms. *J Nutr Biochem.* (2009) 20:399–417. doi: 10.1016/j.jnutbio.2009.01.009

29. El Dib R, Gameiro OL, Ogata MS, Módolo NS, Braz LG, Jorge EC, et al. Zinc supplementation for the prevention of type 2 diabetes mellitus in adults with insulin resistance. *Cochrane Database Syst Rev.* (2015) 2015:CD005525. doi: 10.1002/14651858.CD005525.pub3

30. Ghasemi A, Zahediasl S, Hosseini-Esfahani F, Azizi F. Gender differences in the relationship between serum zinc concentration and metabolic syndrome. *Ann Hum Biol.* (2014) 41:436–42. doi: 10.3109/03014460.2013.870228

31. Yu Y, Cai Z, Zheng J, Chen J, Zhang X, Huang X, et al. Serum levels of polyunsaturated fatty acids are low in Chinese men with metabolic syndrome, whereas serum levels of saturated fatty acids, zinc, and magnesium are high. *Nutr Res.* (2012) 32:71–7. doi: 10.1016/j.nutres.2011.12.004

32. Pattan V, Chang Villacreses MM, Karnchanasorn R, Chiu KC, Samoa R. Daily intake and serum levels of copper, selenium and zinc according to glucose metabolism: cross-sectional and comparative study. *Nutrients.* (2021) 13:4044. doi: 10.3390/nu13114044

33. Pushparani DS, Anandan SN, Theagarayan P. Serum zinc and magnesium concentrations in type 2 diabetes mellitus with periodontitis. *J Indian Soc Periodontol.* (2014) 18:187–93. doi: 10.4103/0972-124X.131322

34. Rotter I, Kosik-Bogacka D, Dołęgowska B, Safranow K, Lubkowska A, Laszczyńska M. Relationship between the concentrations of heavy metals and bioelements in aging men with metabolic syndrome. *Int J Environ Res Public Health.* (2015) 12:3944–61. doi: 10.3390/ijerph120403944

35. Obeid O, Elfakhani M, Hlais S, Iskandar M, Batal M, Mouneimne Y, et al. Plasma copper, zinc, and selenium levels and correlates with metabolic syndrome components of lebanese adults. *Biol Trace Elem Res.* (2008) 123:58–65. doi: 10.1007/s12011-008-8112-0

36. Mao Y, Li X, Zhu S, Ma J, Geng Y, Zhao Y. Associations between urea nitrogen and risk of depression among subjects with and without type 2 diabetes: a nationwide population-based study. *Front Endocrinol.* (2022) 13:985167. doi: 10.3389/fendo.2022.985167

37. Mao Y, Li X, Zhu S, Geng Y. Association between dietary fiber intake and risk of depression in patients with or without type 2 diabetes. *Front Neurosci.* (2022) 16:920845. doi: 10.3389/fnins.2022.920845

38. Yalcin G, Ozsoy E, Karabag T. The relationship of body composition indices with the significance, extension and severity of coronary artery disease. *Nutr Metab Cardiovasc Dis.* (2020) 30:2279–85. doi: 10.1016/j.numecd.2020.07.014

39. Matsunaga M, Lim E, Davis J, Chen JJ. Dietary quality associated with self-reported diabetes, osteoarthritis, and rheumatoid arthritis among younger and older US adults: a cross-sectional study using NHANES 2011-2016. *Nutrients.* (2021) 13:545. doi: 10.3390/nu13020545

40. Deng M, Liu F, Liang Y, Chen Y, Nie J, Chai C, et al. Associations of serum zinc, copper, and selenium with sleep disorders in the American adults: data from NHANES 2011-2016. *J Affect Disord.* (2023) 323:378–85. doi: 10.1016/j.jad.2022.11.088

41. Cheng Y, Fang Z, Zhang X, Wen Y, Lu J, He S, et al. Association between triglyceride glucose-body mass index and cardiovascular outcomes in patients undergoing percutaneous coronary intervention: a retrospective study. *Cardiovasc Diabetol.* (2023) 22:75. doi: 10.1186/s12933-023-01794-8

42. Agricultural Research Service, US Department of Agriculture. *What we eat in America: data tables.* Available at: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/wweia-data-tables/ (accessed August 3, 2022).

43. Liu H, Wang L, Chen C, Dong Z, Yu S. Association between dietary niacin intake and migraine among american adults: national health and nutrition examination survey. *Nutrients.* (2022) 14:3052. doi: 10.3390/nu14153052

44. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *JAMA.* (2003) 289:2560–72. doi: 10.1001/jama.289.19.2560

45. Huang W, Ma X, Liang H, Li H, Chen J, Fang L, et al. Dietary magnesium intake affects the association between serum vitamin D and type 2 diabetes: a cross-sectional study. *Front Nutr.* (2021) 8:763076. doi: 10.3389/fnut.2021.763076

46. Ostojic SM. Dietary creatine and kidney function in adult population: NHANES 2017-2018. *Food Sci Nutr.* (2021) 9:2257–9. doi: 10.1002/fsn3.2200

47. Zhu L, Zhou B, Zhu X, Cheng F, Pan Y, Zhou Y, et al. Association between body mass index and female infertility in the United States: data from National Health and Nutrition Examination Survey 2013-2018. *Int J Gen Med.* (2022) 15:1821–31. doi: 10.2147/IJGM.S349874

48. Liu H, Wang Q, Dong Z, Yu S. Dietary zinc intake and migraine in adults: a cross-sectional analysis of the National Health and Nutrition Examination Survey 1999-2004. *Headache.* (2023) 63:127–35. doi: 10.1111/head.14431

49. Kant R, Verma V, Patel S, Chandra R, Chaudhary R, Shuldiner AR, et al. Effect of serum zinc and copper levels on insulin secretion, insulin resistance and pancreatic β cell dysfunction in US adults: findings from the National Health and

Nutrition Examination Survey (NHANES) 2011-2012. *Diabetes Res Clin Pract.* (2021) 172:108627. doi: 10.1016/j.diabres.2020.108627

50. Gill H, Mugo M, Whaley-Connell A, Stump C, Sowers J. The key role of insulin resistance in the cardiometabolic syndrome. *Am J Med Sci.* (2005) 330:290–4. doi: 10.1097/00000441-200512000-00006

51. Yary T, Virtanen JK, Ruusunen A, Tuomainen TP, Voutilainen S. Association between serum zinc and later development of metabolic syndrome in middle aged and older men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Nutrition.* (2017) 37:43–7. doi: 10.1016/j.nut.2016.09.004

52. Yary T, Virtanen JK, Ruusunen A, Tuomainen TP, Voutilainen S. Serum zinc and risk of type 2 diabetes incidence in men: the Kuopio ischaemic heart disease risk factor study. *J Trace Elem Med Biol.* (2016) 33:120–4. doi: 10.1016/j.jtemb.2015.11.001

53. Taneja SK, Jain M, Mandal R, Megha K. Excessive zinc in diet induces leptin resistance in wistar rat through increased uptake of nutrients at intestinal level. *J Trace Elem Med Biol.* (2012) 26:267–72. doi: 10.1016/j.jtemb.2012.03.002

54. Qu X, Yang H, Yu Z, Jia B, Qiao H, Zheng Y, et al. Serum zinc levels and multiple health outcomes: implications for zinc-based biomaterials. *Bioact Mater.* (2020) 5:410–22. doi: 10.1016/j.bioactmat.2020.03.006

55. Zhang J, Hu J, Zhao J, Li J, Cai X. Serum zinc concentrations and prediabetes and diabetes in the general population. *Biol Trace Elem Res.* (2022) 200:1071–7. doi: 10.1007/s12011-021-02739-6

56. Siqueira de Andrade MI, Oliveira JS, Leal VS, Cabral PC, Lira PIC. Independent predictors of insulin resistance in Brazilian adolescents: results of the study of cardiovascular risk in adolescents–Brazil. *PLoS ONE.* (2021) 16:e0246445. doi: 10.1371/journal.pone.0246445

57. Marreiro DN, Geloneze B, Tambascia MA, Lerário AC, Halpern A, Cozzolino SM. Effect of zinc supplementation on serum leptin levels and insulin resistance of obese women. *Biol Trace Elem Res.* (2006) 112:109–18. doi: 10.1385/BTER:112:2:109

58. Kim J, Lee S. Effect of zinc supplementation on insulin resistance and metabolic risk factors in obese Korean women. *Nutr Res Pract.* (2012) 6:221–25. doi: 10.4162/nrp.2012.6.3.221

59. Beletate V, El Dib RP, Atallah AN. Zinc supplementation for the prevention of type 2 diabetes mellitus. *Cochrane Database Syst Rev.* (2007) 24:CD005525. doi: 10.1002/14651858.CD005525.pub2

60. Rink L, Gabriel P. Extracellular and immunological actions of zinc. *Biometals.* (2001) 14:367–83. doi: 10.1023/A:1012986225203

61. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *J Am Diet Assoc.* (2001) 101:294–301. doi: 10.1016/S0002-8223(01)00078-5

62. Hu J, Cai X, Li J, Zheng N, Zhang J. Associations between serum zinc levels and alanine aminotransferase elevation in adults. *Biol Trace Elem Res.* (2021) 199:2077–84. doi: 10.1007/s12011-020-02318-1

63. Al-Maroof RA, Al-Sharbatti SS. Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type 2 diabetics. *Saudi Med J.* (2006) 27:344–50.

64. Carneiro G, Laferrère B, Zanella MT. Vitamin and mineral deficiency and glucose metabolism-a review. *E-SPEN J.* (2013) 8:e73–9. doi: 10.1016/j.clnme.2013.03.003

65. Calder PC, Ortega EF, Meydani SN, Adkins Y, Stephensen CB, Thompson B, et al. Nutrition, immunosenescence, and infectious disease: an overview of the scientific evidence on micronutrients and on modulation of the gut microbiota. *Adv Nutr.* (2022) 13:S1–26. doi: 10.1093/advances/nmac052

66. Haase H, Mocchegiani E, Rink L. Correlation between zinc status and immune function in the elderly. *Biogerontology.* (2006) 7:421–8. doi: 10.1007/s10522-006-9057-3

67. Xiao Y, Xiao Z. Association between serum Klotho and kidney stones in US middle-aged and older individuals with diabetes mellitus: results from 2007-2016 National Health and Nutrition Survey. *Am J Nephrol.* (2023) 54:224–33. doi: 10.1159/000531045

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Evaluating the effects of seed oils on lipid profile, inflammatory and oxidative markers, and glycemic control of diabetic and dyslipidemic patients: a systematic review of clinical studies

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Diabetes mellitus and dyslipidemia are significant health concerns that elevate the risk of cardiovascular disease and other metabolic disorders, necessitating effective management strategies. Recent research has highlighted the potential role of dietary fats, particularly seed oils, in influencing health outcomes in these conditions. This systematic review evaluates the impact of seed oils on lipid profiles, inflammatory and oxidative markers, and glycemic control in patients with diabetes and dyslipidemia. A comprehensive search across databases, including PubMed, Scopus, Web of Science, Cochrane Library, and Google Scholar, identified studies focusing on the effects of seed oils. The studies include randomized controlled, parallel-design, double-blind, placebo-controlled, and open-label studies published in English. The quality of the studies was assessed through a detailed review process, and data were extracted to evaluate the effects of seed oils on key metabolic markers. The review included 11 studies demonstrating that seed oils derived from canola, flaxseed, and sesame seeds can positively influence lipid profiles and glycemic control while potentially modulating oxidative stress markers. The findings suggest that seed

oils may benefit in managing diabetes and dyslipidemia, although the results are sometimes inconsistent. This review provides valuable insights for dietary recommendations and therapeutic strategies, highlighting the need for further research to clarify the role of seed oils in metabolic health.

KEYWORDS

seed oils, dyslipidemia, type 2 diabetes, lipid profiles, inflammatory markers, glycemic control, clinical trials, cardiometabolic health

1 Introduction

Diabetes mellitus and dyslipidemia are significant health concerns that contribute to an increased risk of cardiovascular disease and other metabolic disorders (1, 2). Both conditions require effective management strategies to mitigate their adverse effects on health (3, 4). In recent years, dietary interventions have emerged as a pivotal component of treatment plans for these conditions, focusing on the types of fats consumed (5–7). Seed oils have attracted considerable attention among various dietary fats due to their distinct fatty acid profiles and potential impact on health outcomes (8–10).

Seed oils, such as those derived from sunflower (11), safflower (12), and canola seeds (13), are commonly used in cooking and food preparation. They are often touted for their favorable fatty acid composition (14), including high levels of Polyunsaturated Fatty Acids (PUFAs) (15), which are believed to influence lipid profiles and other metabolic markers positively (16). However, the reports of the effects of these oils on lipid levels, inflammation, oxidative stress, and glycemic control in diabetic and dyslipidemic patients are complex and sometimes conflicting.

The detrimental effects of chronic inflammation and oxidative stress on health are well-documented (17, 18). Chronic inflammation has been linked to the progression of insulin resistance (19) and diabetes (20), contributing to the development of cardiovascular diseases and other serious complications (21, 22). Oxidative stress, resulting from an imbalance between reactive oxygen species and the body's ability to neutralize them, exacerbates inflammatory responses and damages cellular structures (23, 24), further impairing metabolic health and increasing disease risk (25). These processes play a crucial role in the pathophysiology of diabetes and dyslipidemia (26–29), underscoring the importance of dietary factors that can modulate these harmful effects (30, 31).

Despite the growing body of research on seed oils, there has yet to be a comprehensive systematic review that consolidates the evidence explicitly focusing on their impact on diabetic and dyslipidemic patients. Existing reviews often address broader dietary fat topics or focus on single health outcomes, lacking a focused analysis of seed oils across multiple metabolic markers. Additionally, there is a limited synthesis of how seed oils influence inflammatory and oxidative stress pathways in these conditions. Tian et al. (32) discussed the health-promoting effects of vegetable oils, highlighting their chemical compositions and pharmacological potential. However, their comprehensive analysis relied majorly on chemical compositions and nutritional values and did not evaluate the health benefits systematically and holistically. In other words, they did not focus on the analysis of the included studies but on the oil's characteristics, with prospecting results mainly based on their bioactive components. In addition, they did not solely focus on diabetes and dyslipidemia patients but on cancer and individuals suffering from cardiovascular disease. On the other hand, Schwingshackl et al. (33) published a systematic review focusing on the effects of oils and solid fats on blood lipids. Although their analysis was comprehensive, they did not focus solely on dyslipidemic patients and lacked comparisons on the effects of vegetable oils in patients suffering from diabetes and dyslipidemia together. In addition, they did not focus only on seed oils but also on solid fats. Finally, none of the studies mentioned above analyzed the effects of seed oils on inflammatory and oxidative markers during their interventions with diabetic and dyslipidemic patients. Since inflammation and oxidative stress are paramount components of diabetes and dyslipidemia pathophysiologies and related pathologies, our study is of utmost importance since we analyzed patients suffering from these two conditions and their respective markers.

To address the existing knowledge gap, this systematic review comprehensively evaluates clinical studies that assess the impact of seed oils on key health markers in patients with diabetes and dyslipidemia. By synthesizing data from diverse studies, this review aims to elucidate how seed oils influence various health parameters, including lipid profiles, inflammatory and oxidative markers, and glycemic control. The insights derived from this analysis are expected to inform dietary recommendations and therapeutic strategies, thereby contributing to improved management and outcomes for individuals affected by these common conditions. It also highlights the limitations of the included studies, such as variability in study designs, sample sizes, and methods of assessing health outcomes. By identifying these limitations, the review underscores the need for more robust, longitudinal, and methodologically sound research to clarify the relationship between seed oils and health markers. Addressing these research gaps will be crucial for developing more accurate and evidence-based dietary guidelines and therapeutic approaches.

2 Literature search methodology

In this section, we outline the systematic approach taken to identify and analyze relevant studies concerning the impact of seed oils on dyslipidemia and type 2 diabetes. The comprehensive search strategy was designed to ensure a thorough and unbiased review of the existing literature.

2.1 Databases searched

To identify relevant studies, a comprehensive search was conducted across the following electronic databases: PubMed, Scopus, Web of Science, Cochrane Library, and Google Scholar. These databases were chosen to ensure broad coverage and include peer-reviewed journals and gray literature.

2.2 Search strategy

The search strategy involved using specific keywords and their combinations to capture studies on seed oils' impact on dyslipidemia and type 2 diabetes. The primary keywords included "seed oils," "dyslipidemia," "hyperlipidemia," "type 2 diabetes," "lipid profiles," "inflammatory markers," "glycemic control," and "clinical trials." These keywords were combined using Boolean operators (AND, OR) to refine the search results. For instance, combinations like "seed oils AND dyslipidemia," "seed oils AND type 2 diabetes," and "seed oils AND lipid profiles" were used.

2.3 PRISMA guidelines

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (34) guidelines were followed to ensure a systematic and transparent approach to the literature search and selection process. The PRISMA flow diagram was used to document the number of studies identified, screened, assessed for eligibility, and included in the review, along with reasons for exclusion at each stage.

2.4 Inclusion and exclusion criteria

Studies were included if they were randomized controlled trials, cohort studies, or case-control studies examining the effects of seed oils on lipid profiles, inflammatory markers, or glycemic control in patients with dyslipidemia or type 2 diabetes. Studies had to be published in English and involve human participants. Exclusion criteria included review articles, animal studies, studies unrelated to the specified seed oils, and studies without relevant outcome measures.

2.5 Quality assessment

The quality of the included studies was assessed through a comprehensive review process following the Cochrane Handbook for Systematic Reviews of Interventions (35). A detailed evaluation was conducted for randomized and non-randomized controlled trials to identify potential biases such as selection, performance, detection, attrition, and reporting. This involved carefully examining how participants were selected, how interventions were administered, and how outcomes were measured and reported. This assessment aimed to ensure that the studies appropriately selected participants, matched them to relevant variables and accurately reported outcomes. Two researchers (Lucas. F.L. and S.M.B.) independently reviewed each study to ensure a rigorous evaluation process. In cases where disagreements arose between the reviewers, these were resolved through discussion or consulting a third reviewer (R.D.) to achieve consensus. The selection bias involves evaluating studies with unclear or high selection bias for individual inclusions in each interventional or non-interventional group. Randomization and allocation methods are determined and discussed based on each study design to ensure the utmost accuracy of the included findings. Secondly, the performance and detection bias report involves studies lacking blinding, which might impact the studies' results based on their specific begins. Thirdly, attrition and reporting bias involve attrition rates or selective reporting of results in the included studies. Studies missing data or skewing results must be excluded. For a study to be included besides bias recognition, it must not have had critical bias reporting. This approach aimed to enhance the reliability and validity of the quality assessment studies included in the review.

2.6 Data extraction and synthesis

Two experienced researchers (Lucas. F.L. and S.M.B.) extracted data from the included studies. Following data collection, two additional researchers (Lívia. F.L. and R.D) verified the data's significance, reliability, and correctness. Relevant studies were screened based on titles and abstracts, followed by a full-text review. Data extracted included study design, sample size, participant characteristics, type and dosage of seed oil used, duration of intervention, and outcomes related to lipid profiles, inflammatory markers, and glycemic control. The extracted data were synthesized to evaluate the overall impact of seed oils on dyslipidemia and type 2 diabetes, highlighting the most significant findings and trends. The results were presented in a narrative format, supported by tables and figures where appropriate.

By employing this methodology, the review aims to provide a comprehensive understanding of the therapeutic potential of various seed oils in managing dyslipidemia and type 2 diabetes. This systematic approach ensures the reliability and validity of the findings, contributing valuable insights to the field of nutritional therapy.

3 Results of literature search methodology and overview of the included studies

This section outlines the results of our literature search methodology and provides an overview of the studies included in the review. It presents a comprehensive evaluation of the effects of seed oils on dyslipidemia and type 2 diabetes.

3.1 Literature search results

The initial search process produced a comprehensive dataset of 258 records from various databases and an additional 78 records

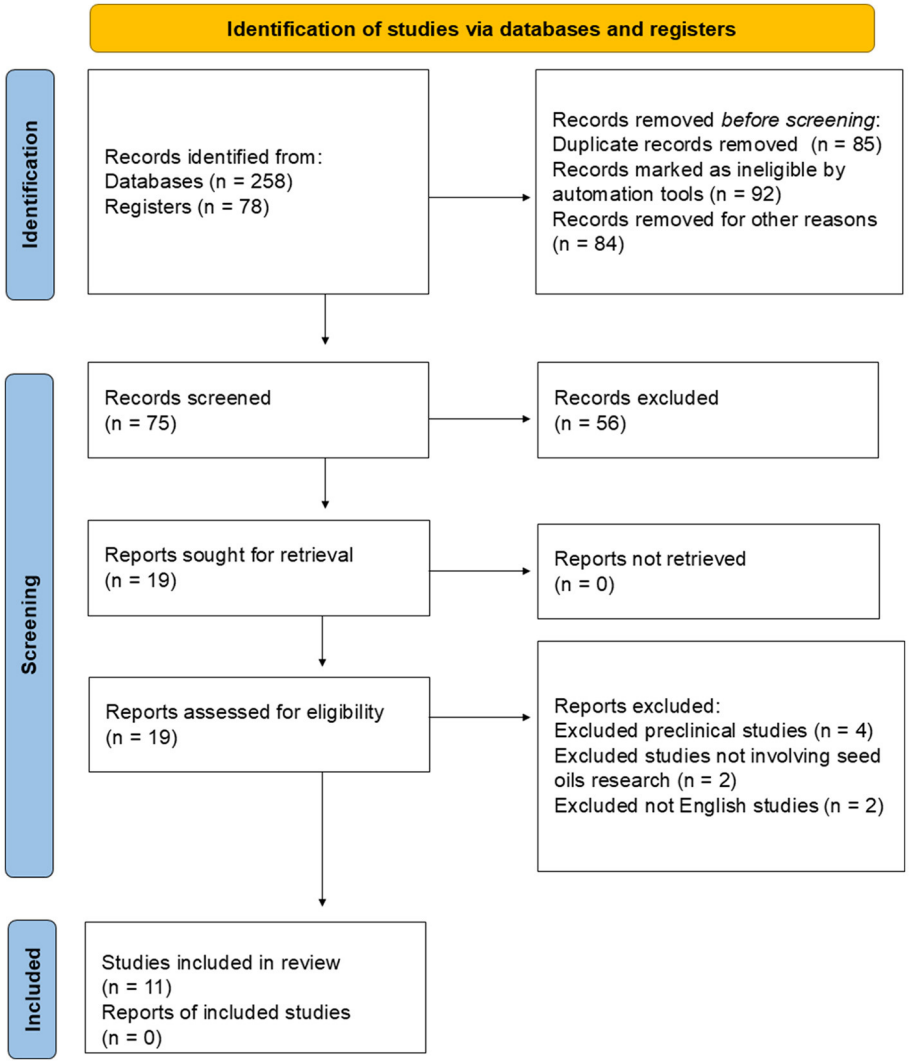

FIGURE 1
Process flow for record selection and inclusion in the review following PRISMA.

from registries. Following this initial collection, duplicate records were identified and removed, totaling 85 duplicates. In addition to eliminating these duplicates, ineligible records flagged by automation tools (Rayyan online application) were also excluded, accounting for 92 records. Moreover, 84 records were excluded for other reasons unrelated to eligibility criteria, 70 still duplicate publications, and 14 studies of non-peer-reviewed sources). After applying these exclusions, 75 records remained for the screening phase. During the screening process, 56 records were excluded based on their content, which did not meet the criteria for inclusion in the review. Subsequently, 19 reports were selected for retrieval and thorough assessment. Each of these 19 reports was successfully retrieved. However, upon evaluation for eligibility, 8 of these reports were excluded: 4 were preclinical studies not relevant to the research question, two did not focus on seed oils as required, and two were in languages other than English, which were not feasible for inclusion. As a result of this assessment, 11 studies were deemed suitable and were included in the final review. No additional reports from the included studies were missing from the final assessment process. The PRISMA (Figure 1) flow diagram illustrates the study selection process, including the reasons for exclusion at each stage. All studies underwent a rigorous quality assessment using the COCHRANE handbook for intervention evaluations. This assessment evaluated selection, performance, detection, attrition, and reporting bias. It is worth noting that, following our quality assessment, no studies were excluded for bias reporting, meaning that all included studies achieved minimum quality standards set out in our inclusion criteria.

3.2 Overview of the included studies

Table 1 provides a comprehensive overview of clinical studies evaluating the impact of various oil supplements on glycaemic control, lipid profiles, and inflammatory and oxidative markers. The table summarizes key aspects of each study, including

population characteristics, intervention details, comparisons, primary outcome measures, and notable results. Table 2 provides the COCHRANE assessment for the included interventions. Selection, performance, detection, attrition, and reporting biases were reported using "critical," "serious," "moderate," or "low" bias risk stamps.

The publication date range from the included studies is from 2010 to 2023. Regarding country examination, Iran possesses most of the included studies ($n = 6$), followed by China ($n = 2$), Pakistan ($n = 1$), Canada ($n = 1$), and India ($n = 1$). The most common seed oils utilized were canola oil, studied by Nikooyeh et al. (36) and Jenkins et al. (37), sesame oil, studied by Aslam et al. (38), Haldar et al. (39), Alipoor et al. (40), and Sankar et al. (41), perilla oil studied by Khajebishak et al. (42), Wei et al. (43), and Asghari et al. (44), Flaxseed Oil (FO) studied by Haldar et al. (39), rice bran oil studied by Haldar et al. (39), and Sunflower Oil (SO) studied by Nikooyeh et al. (36). The studies collectively explore how various oils and dietary modifications affect health outcomes such as glycemic control, lipid profiles, and oxidative stress markers. In this overview, we have grouped the results by outcomes following PRISMA guidelines recommendations.

The studies included in this research were conducted across various countries, each focusing on different health conditions and populations. Nikooyeh et al. (36) investigated 92 adults with type 2 diabetes from Iran. Haldar et al. (39) examined 143 borderline hypercholesterolemic Chinese volunteers. Aslam et al. (38) focused on 46 adults with type 2 diabetes from Pakistan. Khajebishak et al. (42) studied 60 obese type 2 diabetic patients in Iran. Akrami et al. (45) looked at 60 adults with Metabolic Syndrome (MetSyn), also from Iran. Jenkins et al. (37) conducted their trial with 141 adults with type 2 diabetes in Canada. Wei et al. (43) included 36 individuals with elevated blood lipids from China. Alipoor et al. (40) researched 38 hyperlipidemic patients from Iran. Asghari et al. (44) studied 51 individuals with specific lipid profiles from Iran. Sankar et al. (41) worked with 60 type 2 diabetes patients in India, and Mirmiran et al. (46) examined 51 hyperlipidemic subjects from Iran.

The participants' ages varied significantly in the studies. Nikooyeh et al. (36) included adults aged 20–65, while Haldar et al. (36) focused on individuals aged 50–70. Aslam et al. (38) studied adults between 18 and 60 years old. Khajebishak et al. (42) investigated patients aged 30–50 years. Akrami et al. (45) included adults aged 30–60 years. Jenkins et al. (37) worked with adults, although their age range was not detailed. Wei et al. (43) examined individuals from 18 to 75 years old. Alipoor et al. (40) studied participants aged 50–70 years. Asghari et al. (44) involved adults aged 20 years and older. Sankar et al. (41) had a mean participant age of 57–58 years, and Mirmiran et al. (46) included subjects over 20 years old.

3.2.1 Glycemic and lipid profile controls

Nikooyeh et al. (36) found that γ-Oryzanol (ORZ)-fortified canola oil significantly improved fasting blood glucose and triglycerides. Haldar et al. (39) observed notable cholesterol and blood glucose reductions with a blend of rice bran, flaxseed, and sesame oils. Aslam et al. (38) reported that white sesame seed oil improved glycemic control, while Khajebishak et al. (42) found that Pomegranate Seed Oil (PSO) enhanced Glucose Transporter Type 4 (GLUT-4) gene expression and reduced fasting blood glucose. Akrami et al. (45) demonstrated reduced cholesterol and triglycerides with FO. Wei et al. (43) highlighted significant improvements in lipid profiles with perilla oil combined with exercise. Jenkins et al. (37) showed that a low-Glycemic Load (GL) diet with canola oil led to more significant reductions in Glycated Hemoglobin (HbA1c) and improvements in lipid profiles compared to a whole-grain diet. Alipoor et al. (40) found that white sesame seeds and dietary modifications significantly reduced total cholesterol and Low-Density Lipoprotein Cholesterol (LDL-C). Asghari et al. (44) observed reductions in triglycerides and the triglyceride/High-Density Lipoprotein Cholesterol (HDL-C) ratio with PSO. Sankar et al. (41) noted that combining sesame oil and glibenclamide substantially improved glycemic control and lipid profiles. Mirmiran et al. (46) reported that PSO reduced triglycerides and improved HDL-C levels.

3.2.2 Inflammatory and oxidative markers

Aslam et al. (38) reported that sesame seed oil improves the body's antioxidant activity, while Wei et al. (43) highlighted significant improvements in inflammatory markers with perilla oil combined with exercise. Asghari et al. (44) observed insignificant reductions in inflammatory markers with PSO. Sankar et al. (41) noted that combining sesame oil and glibenclamide substantially improved the body's antioxidant activity.

4 Comparative efficacy of dietary oils and supplementation in managing glycaemic control, lipid profiles, inflammatory, and oxidative markers: insights from recent clinical trials

Nikooyeh et al. (36) examined the effects of ORZ-fortified canola oil, unfortified canola oil, and SO on various cardiometabolic markers. The ORZ-fortified canola oil group experienced significant reductions in fasting blood glucose, HbA1c, and triglycerides compared to the other oils, highlighting its potential for improving glycaemic control in type 2 diabetes. The positive impact on triglycerides also suggests potential benefits for lipid management and cardiovascular risk reduction. This study is notable for its focus on ORZ and its potential benefits beyond traditional oils. The 12-week intervention period, while longer than some studies, may still be insufficient to evaluate long-term benefits or potential side effects. Finally, although a decrease in triglycerides was observed in all groups, this was not significant for unfortified canola oil.

Haldar et al. (39) assessed the effects of a blend of rice bran, flaxseed, and sesame oils compared to refined olive oil on various cardiometabolic markers in borderline hypercholesterolemic individuals. The study reported significant reductions in total cholesterol, LDL-C, triglycerides, and cardiovascular risk ratios and improved blood pressure and blood glucose. However, there was a small but significant increase in body weight, and no

TABLE 1 Summary of clinical studies on oil supplementation and its effects on glycemic control, lipid profiles, and inflammatory and oxidative markers.

Study	Population	Intervention	Comparison	Outcome measures	Glycemic control	Lipid profiles	Inflammatory markers	Oxidative stress markers
Nikooyeh et al. (36)	92 adults with T2DM, aged 20–65 years, not receiving insulin, recruited from Iran Diabetes Society and the general population, with BMI 28.4 ± 0.79 for Group 1, 29.7 ± 0.72 for Group 2, and 29.2 ± 0.68 for Group 3	ORZ-fortified canola oil (Group 1, n = 30) for 12 weeks; unfortified canola oil (Group 2, n = 32) for 12 weeks; SO (Group 3, n = 30) for 12 weeks; Randomized, double-blind clinical trial	Unfortified canola oil (n = 32) and SO (n = 30) for 12 weeks	**Primary:** WC, BP, FBG, HbA1c, TG	Significant reductions in FBG (134.0 ± 6.2 mg/dL → 126.4 ± 5.8 mg/dL) and HbA1c (6.1% ± 0.12% → 5.4% ± 0.12%) in ORZ-fortified canola oil group only.	There was a significant decrease in TG (134.7 ± 9.8 mg/dL → 116.8 ± 7.8 mg/dL) only in the ORZ-fortified canola oil group. A reduction in TG was observed in all groups but not substantial for unfortified canola oil (147.1 ± 11.8 mg/dL → 129.8 ± 10.5 mg/dL) or SO (131.1 ± 10.5 mg/dL → 115.7 ± 7.7 mg/dL).	Not reported.	Not reported.
Haldar et al. (39)	143 borderline hypercholesterolemic Chinese volunteers, aged 50–70 years, with BMI ≤27.5 kg/m²	30 g of refined rice bran, flaxseed, and sesame oil blends per day for 8 weeks (n = 88); Parallel-design, randomized controlled trial	Refined olive oil (n = 47)	**Primary:** Serum TC, LDL-C, TG, apoB, TC to HDL-C ratio, apoB to apoA1 ratio; **Secondary:** Systolic and diastolic BP, serum glucose, body weight	Significant reductions in FBG levels (−1.51%).	Significant reductions in serum TC (−3.47%), LDL-c (−4.16%), TG (−10.3%), apoB (−3.93%), TC to HDL-C ratio (−3.44%), apoB to apoA1 ratio (−3.99%), systolic and diastolic BP (−3.32% and −3.16%); Small but significant increase in body weight; No significant effects on HDL-C or apoA1 concentration.	Not reported.	Not reported.
Aslam et al. (38)	46 adults (18–60 years) with T2DM from Pakistan	30 ml of white sesame seed oil daily for 90 days; Randomized controlled trial	30 ml of soybean oil daily for 90 days (control group)	**Primary:** Blood glucose, insulin, HbA1c, TBARS, SOD, CAT, GPx	Blood glucose decreased in the DSO group (189.09 ± 4.42 mg/dL → 137.8 3 ± 3.16 mg/dL) and increased in the DCON (185.04 ± 6.84 mg/dL → 218.14 ± 5.92 mg/dL) group. Insulin was higher in the DSO group (12.26 ± 1.24 μU/mL → 23.13 ± 1.15 μU/ml) than in the DCON group (11.97 ± 0.81 μU/mL → 7.93 ± 0.38 μU/mL). HbA1c was lower in the DSO (6.96% ± 0.26%) group at 90 days compared to the DCON group (8.02% ± 0.37%).	Not reported.	Not reported.	TBARS decreased significantly in the DSO group (1.91 ± 0.00 nmol/mL → 1.08 ± 0.05 nmol/mL) and increased in the DCON group (1.82 ± 0.01 nmol/mL → 2.26 ± 0.07 nmol/mL). SOD (2.79 ± 0.02 U/mL → 4.91 ± 0.10 U/mL), CAT (5.18 ±0.04 U/mL → 6.41 ± 0.06 U/mL), and GPx (141.55 ± 0.12 U/mL → 147.14 ± 0.17 U/mL) increased in the DSO group, indicating improved antioxidant activity, while these markers decreased in the DCON group (SOD: 2.81 ± 0.02 U/mL → 2.34 ± 0.30 U/mL, CAT: 5.14 ± 0.04 U/mL → 3.42 ± 0.05 U/mL, GPx: 139.06 ± 1.24 U/mL → 101.97 ± 1.80 U/mL).

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TABLE 1 (Continued)

Study	Population	Intervention	Comparison	Outcome measures	Glycemic control	Lipid profiles	Inflammatory markers	Oxidative stress markers
Khajebishak et al. (42)	60 obese type 2 diabetic patients (both genders), aged 30–50 years, BMI >30 and <40 kg/m^2 from Iran	PSO capsules (n = 26) (1 g/day) for 8 weeks; Randomized controlled clinical trial	Placebo capsules (n = 26) (paraffin) for 8 weeks	**Primary:** FBG, HbA1c, Insulin, HOMA-IR, QUICKI, GLUT-4 Gene Expression	GLUT-4 gene expression increased significantly in the PSO group compared to the placebo group. FBG decreased substantially in the PSO (161.46 ± 34.44 mg/dL → 143.50 ± 24.2 mg/dL) group, with no significant change in the placebo group (156.54 ± 31.90 mg/dL → 154.65 ± 31.48 mg/dL); HbA1c showed a slight decrease in the PSO group (7.53% ± 0.92% → 7.25% ± 0.80%) with no significant change in the placebo group (7.65% ± 1.07% → 7.52% ± 1.04%). QUICKI improved significantly in the PSO group (0.30 ± 0.02 → 0.31 ± 0.03); there was no change in the placebo group.	Not reported.	Not reported.	Not reported.
Akrami et al. (45)	60 adults (30–60 years) with MetSyn in Iran, with 81.17 kg ± 11.23 kg for FO and 84.50 ± 14.89 kg for SO	25 mL/day FO for 7 weeks (n = 30); Randomized controlled trial	25 mL/day SO for 7 weeks (n = 30)	**Primary:** BP, serum lipids, FBG, MDA	There is no significant between-group difference in FBG. However, significant within-group reductions in FBG were observed in FO (95.31 mg/dL ± 20.40 mg/dL → 90.46 mg/dL ± 21.90 mg/dL) and SO (93.96 mg/dL ± 23.18 mg/dL → 93.85 mg/dL ± 42.54 mg/dL) groups.	There were significant reductions in TC (FO, 215.15 mg/dL ± 42.74 mg/dL → 196.92 mg/dL ± 36.91 mg/dL; SO, 215.54 mg/dL ± 30.18 mg/dL → 190.35 mg/dL ± 33.34 mg/dL), LDL-C (FO, 128.23 mg/dL ± 29.80 mg/dL → 121.04 mg/dL ± 26.88 mg/dL; SO, 132.08 mg/dL ± 25.01 mg/dL → 117.73 mg/dL ± 27.63 mg/dL), and TG (FO, 209.27 mg/dL ± 106.34 mg/dL → 156.81 mg/dL ± 65.69 mg/dL; SO, 196.00 mg/dL ± 76.18 mg/dL → 142.54 mg/dL ± 66.07 mg/dL) within both groups.	Not reported.	There was a marginally significant reduction in MDA in the FO group (7.01 nmol/dL ± 1.70 nmol/dL → 5.59 nmol/dL ± 0.59 nmol/dL) vs. a non-significant reduction in the SO group (6.64 nmol/dL ± 0.94 nmol/dL → 6.12 nmol/dL ± 1.03 nmol/dL).

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TABLE 1 (Continued)

Study	Population	Intervention	Comparison	Outcome measures	Glycemic control	Lipid profiles	Inflammatory markers	Oxidative stress markers
Jenkins et al. (37)	141 adults with T2DM (HbA1c 6.5%−8.5%) treated with oral antihyperglycemic agents in Canada, with 31 ± 6 kg/m^2 for control and 30 ± 5 kg/m^2 for test diets	Low-GL diet with 31 g canola oil/day ($n = 70$) provided as a supplement (4.5 slices of canola oil–enriched bread daily) for 3 months; Randomized, parallel design clinical trial	A whole-grain diet with 7.5 slices/day ($n = 71$) of whole-wheat bread	**Primary:** HbA1c; **Secondary:** Framingham CVD risk score, RHI	HbA1c significantly decreased in the test diet (−5.15 mmol/mol [−5.92 mmol/mol, −4.38 mmol/mol]). There are more significant benefits in those with higher systolic BP.	Test diet led to significant reductions in TC (−0.30 mmol/L [−0.38 mmol/L, −0.22 mmol/L]), LDL-C (−0.20 mmol/L [−0.27 mmol/L, −0.13 mmol/L]), TG (−0.15 mmol/L [−0.24 mmol/L, −0.07 mmol/L]), and HDL-C (−0.03 mmol/L [−0.05 mmol/L, −0.01 mmol/L]) compared to control diet (TC, 0.04 mmol/L [−0.03 mmol/L, 0.12 mmol/L]; LDL-C, 0.04 mmol/L [−0.02 mmol/L, 0.11 mmol/L]; TG, −0.01 mmol/L [−0.09 mmol/L, 0.07 mmol/L]; HDL-C, 0.00 mmol/L [−0.02 mmol/L, 0.02 mmol/L]).	Not reported.	Not reported.
Wei et al. (43)	Men and women aged 18–75 with elevated blood lipids ($n = 36$) from China	Perilla oil capsules (4 capsules, twice daily for 8 weeks) + exercise (30–60 min/day, at least 4 days/week for 8 weeks) ($n = 12$); Prospective, randomized control trial involving men	EG ($n = 12$, 30–60 min per day, at least 4 days per week), MG ($n = 12$, four capsules, taken twice a day)	**Primary:** TC, TG, HDL-C, LDL-C, hs-CRP, PAI-1, TNF-α	Not reported.	Significant reduction in TC (6.2 ± 0.7 mmol/L → 5.3 ± 0.9 mmol/L), TG (3.4 ± 0.8 mmol/L → 2.8 ± 0.7 mmol/L), and LDL-C (4.0 ± 0.8 mmol/L → 3.1 ± 0.7 mmol/L) in EMG compared to EG and MG after 56 days. HDL-C increased significantly in MG (1.4 ± 0.4 mmol/L → 1.6 ± 0.6 mmol/L) and EMG (1.4 ± 0.4 mmol/L → 1.6 ± 0.6 mmol/L).	hs-CRP (3.43 mg/L ± 0.58 mg/L → 2.76 mg/L ± 0.54 mg/L), PAI-1 (37.79 ng/mL ± 5.98 ng/mL → 33.89 ng/mL ± 5.93 ng/mL), and TNF-α (1.23 ng/mL ± 0.19 ng/mL → 0.88 ng/mL ± 0.21 ng/mL) levels significantly reduced in EMG compared to EG (hs-CRP, 3.41 mg/L ± 0.63 mg/L → 2.74 mg/L ± 0.53 mg/L; PAI-1, 38.87 ng/mL ± 6.18 ng/mL → 33.56 ng/mL ± 5.88 ng/mL; TNF-α, 1.21 ng/mL ± 0.19 ng/mL → 0.97 ng/mL ± 0.18 ng/mL) and MG (hs-CRP, 3.38 mg/L ± 0.55 mg/L → 2.77 mg/L ± 0.61 mg/L; PAI-1, 39.24 ng/mL ± 6.23 ng/mL → 34.19 ng/mL ± 6.12 ng/mL; TNF-α, 1.23 ng/mL ± 0.24 ng/mL → 0.94 ng/mL ± 0.22 ng/mL).	Not reported.

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TABLE 1 (Continued)

Study	Population	Intervention	Comparison	Outcome measures	Glycemic control	Lipid profiles	Inflammatory markers	Oxidative stress markers
Alipoor et al. (40)	Hyperlipidemic patients ($n = 38$) from Iran aged 50–70 years with BMI 18.5–30 kg/m^2, total plasma cholesterol >200 mg/dL or total plasma TG >150 mg/dL, and on medical treatment for >3 months	White sesame seeds (40 g/day for 60 days) + 240 kcal removed from diet; 60 days treatment duration ($n = 19$); Randomized control trial	The control group receiving the same drug treatments without sesame seeds ($n = 19$)	**Primary:** Lipid profile (TC, LDL-C, TC/HDL-C ratio); Antioxidant markers (GPx, SOD, TBARS); Anthropometric measurements (Weight, BMI)	Not reported.	Significant decrease in TC (241.2 mg/dL ± 41.2 mg/dL → 221.5 mg/dL ± 45.2 mg/dL), LDL-C (159.7 mg/dL ± 37.8 mg/dL → 144.0 mg/dL ± 43.7 mg/dL), and TC/HDL-C ratio (5.2 ± 1.1 → 4.9 ± 1.2).	Not Reported.	Decreased TBARS (2.9 μmol/L ± 1.0 μmol/L → 1.9 μmol/L ± 1.0 μmol/L), increased GPx (21.4 U/Hb(g) ± 2.2 U/Hb(g) → 22.5 U/Hb(g) ± 2.0 U/Hb(g)) and SOD (1754.9 U/Hb(g) ± 269.9 U/Hb(g) → 1890.9 U/Hb(g) ± 308.4 U/Hb(g)).
Asghari et al. (44)	Males and females ($n = 51$), aged ≥20 years from Iran with BMI ≤35 kg/m^2, serum TC >200 mg/dL, serum TG >150 mg/dl	PSO (800 mg/day, $n = 25$) vs. placebo (800 mg paraffin/day) for 4 weeks; Double-blind, randomized, placebo-controlled clinical trial	Placebo ($n = 26$)	**Primary:** Serum TG, HDL-C; TNF-α; TG/HDL-C ratio	Not reported.	Reduced serum TG (3.45 mmol/L ± 1.56 mmol/L → 2.75 mmol/L ± 1.40 mmol/L) and TG/HDL-C (7.49 ± 4.95 → 5.73 ± 4.55) ratio with PSO; HDL-C (1.25 mmol/L ± 0.39 mmol/L → 1.38 mmol/L ± 0.44 mmol/L) increased with PSO.	No significant changes in TNF-α levels (14.73 pg/mL ± 5.25 pg/mL → 13.28 pg/mL ± 3.79 pg/mL).	Not reported.
Sankar et al. (41)	60 adults with T2DM, mean aged 57–58 years from India	Combination of sesame oil (35 g/day) + glibenclamide (5 mg/day) for 60 days ($n = 20$); Open-label study	Glibenclamide ($n = 22$, 5 mg/day, single dose) for 60 days; Sesame oil ($n = 18$, 35 g/day) for 60 days	**Primary:** Glycemic Control (Blood glucose, HbA1c); Lipid Profiles (TC, LDL-C, TG, HDL-C); Antioxidant activity	Combination therapy significantly reduced glucose (−36%) and HbA1c (−43%).	Substantial decreases in TC, LDL-c, and TG in sesame oil (20%, 33.8%, and 14%, respectively) and combination therapy (22%, 38%, and 15%, respectively); HDL-C increased significantly in sesame oil (+15.7%) and combination therapy (+17%).	Not reported.	Significant improvement in antioxidant activities with sesame oil and combination therapy ($p < 0.001$).

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TABLE 1 (Continued)

Study	Population	Intervention	Comparison	Outcome measures	Glycemic control	Lipid profiles	Inflammatory markers	Oxidative stress markers
Mirmiran et al. (46)	Hyperlipidemic subjects ($n = 51$) from Iran, aged over 20 years, without allergy or liver dysfunction, BMI $\leq$35 kg/m^2, serum TC >5.2 mmol/l, and TG >1.65 mmol/l	PSO (400 mg, twice daily) for 4 weeks ($n = 25$); Double-blind placebo-controlled clinical trial	Placebo ($n = 26$)	**Primary:** Serum TG, HDL-C	There were no significant changes in insulin/glucose concentrations between PSO (0.08 ± 0.04 → 0.09 → 0.04) and control groups (0.08 ± 0.04 → 0.07 ± 0.03).	Reduction in TG (3.45 mmol/L ± 1.56 mmol/L → 2.75 mmol/L ± 1.40 mmol/L) and TG/HDL-C ratio (7.49 ± 4.95 → 5.73 ± 4.55) with PSO; Cholesterol/HDL-C ratio decreased with PSO (5.87 ± 1.67 → 5.45 ± 1.51). HDL-C increased in the PSO group (1.25 mmol/L ± 0.39 mmol/L → 1.38 mmol/L ± 0.44 mmol/L) compared to placebo (1.27 mmol/L ± 0.23 mmol/L → 1.25 mmol/L ± 0.26 mmol/L). There were no significant changes in serum cholesterol and LDL-C concentrations.	Not reported.	Not reported.

apoA1, Apolipoprotein A1; apoB, Apolipoprotein B; BMI, Body Mass Index; BP, Blood Pressure; CAT, Catalase; CVD, Cardiovascular Disease; DCON, Diabetic Control Group; DSO, Diabetic Sesame Oil Group; EG, Exercise Group; EMG, Exercise and Medicine Group; FBG, Fasting Blood Glucose; FO, Flaxseed Oil; GL, Glycemic Load; GLUT-4, Glucose Transporter Type 4; GPx, Glutathione Peroxidase; HbA1c, Glycated Hemoglobin; HDL-C, High-Density Lipoprotein Cholesterol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hs-CRP, High-Sensitivity C-Reactive Protein; LDL-C, Low-Density Lipoprotein Cholesterol; MDA, Malondialdehyde; MetSyn, Metabolic Syndrome; MG, Medicine Group; ORZ, γ-Oryzanol; PAI-1, Plasminogen Activator Inhibitor-1; PSO, Pomegranate Seed Oil; QUICKI, Quantitative Insulin Sensitivity Check Index; RHI, Reactive Hyperemia Index; SOD, Superoxide Dismutase; SO, Sunflower Oil; T2DM, Type 2 Diabetes Mellitus; TAG, Triglycerides; TBARS, Thiobarbituric Acid Reactive Substances; TC, Total Cholesterol; TG, Triglycerides; TNF-α, Tumor Necrosis Factor-α; WC, Waist Circumference.

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TABLE 2 Reporting of bias assessment based on selection, performance, detection, attrition, and reporting bias following the COCHRANE handbook for interventions assessment.

Study	D1	D2	D3	D4	D5	D6	D7	Overall
Nikooyeh et al. (36)								
Haldar et al. (39)								
Aslam et al. (38)								
Khajebishak et al. (42)								
Akrami et al. (45)								
Jenkins et al. (37)								
Wei et al. (43)								
Alipoor et al. (40)								
Asghari et al. (44)								
Sankar et al. (41)								
Mirmiran et al. (46)								

D1, bias due to confounding; D2, bias due to selection of participants; D3, bias in classification of interventions; D4, bias due to deviations from intended interventions; D5, bias due to missing data; D6, bias in measurement of outcomes; D7, bias in selection of the reported result. , critical; , serious; , moderate; , low.

significant effects were observed on HDL-C or Apolipoprotein A1 (apoA1) concentrations. The strengths of this study include its parallel design, which allows for a direct comparison between oil blends and a control oil. The reductions in key cardiovascular risk factors and blood pressure underscore the potential benefits of the oil blends. Nonetheless, the study's limitations include a modest increase in body weight, which could affect the overall interpretation of the results. Additionally, the lack of significant changes in HDL-C highlights the need for further research to determine the full impact of these oils on cardiovascular health. A significant limitation of Haldar et al.'s study is that they treated patients with fiber, seed oils, and olive oil. Therefore, it remains unclear whether the observed effects were mainly attributed to the impact of oil or fiber.

Aslam et al. (38) explored the effects of white sesame seed oil compared to soybean oil on glycaemic control and inflammatory and oxidative markers in adults with type 2 diabetes. The study found that sesame seed oil significantly improved glycaemic control, with reductions in blood glucose levels and HbA1c, alongside increases in insulin levels. This suggests that sesame seed oil could benefit diabetes management strategies, potentially offering a natural alternative to conventional therapies. The study also demonstrated that sesame seed oil substantially improved oxidative stress markers. Specifically, Thiobarbituric Acid Reactive Substances (TBARS) levels, a marker of lipid peroxidation, decreased significantly in the sesame oil group, indicating reduced oxidative damage. Additionally, antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) were elevated in the sesame oil group, reflecting enhanced antioxidant activity. These findings support the potential of sesame seed oil in mitigating oxidative stress, which is a critical factor in the progression of diabetes-related complications. However, there are limitations to consider. The study's open-label design could introduce bias, as participants and researchers were aware of the treatment allocations. This may have influenced the participants' behavior or the researchers' observations.

Khajebishak et al. (42) investigated the impact of PSO supplementation on glycaemic control and gene expression related to glucose metabolism in obese type 2 diabetic patients. The study was a randomized, double-blind clinical trial involving 60 participants randomly assigned to receive either 1 g/day of PSO or placebo capsules for 8 weeks. The results indicated that PSO supplementation led to a significant improvement in fasting blood glucose levels, with a decrease from 161.46 to 143.50 mg/dL and an increase in GLUT-4 gene expression, which is crucial for glucose uptake in cells. The study also observed a slight but non-significant reduction in HbA1c in the PSO group, suggesting potential benefits in long-term glycaemic control. In addition, the PSO group showed a significant improvement in the Quantitative Insulin Sensitivity Check Index (QUICKI), an index of insulin sensitivity, which implies enhanced insulin action. However, there were no significant changes in insulin levels, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), or HOMA-β between the PSO and placebo groups, indicating that while PSO might improve some aspects of glycaemic control and insulin sensitivity, it does not impact all metabolic parameters uniformly. One of the key strengths of this study is its double-blind design, which minimizes bias and increases the reliability of the results. The significant increase in GLUT-4 gene expression and improvement in QUICKI highlights the potential of PSO as an adjunctive therapy for managing type 2 diabetes, particularly in improving glucose uptake and insulin sensitivity. The study also found no significant changes in insulin levels or HOMA-IR, which suggests that while PSO has some positive effects, its impact on overall insulin resistance and secretion might be limited.

Akrami et al. (45) compared the effects of FO and SO on MetSyn symptoms in individuals with MetSyn. The study found that both oils reduced total cholesterol, LDL-C, and

triglycerides. Notably, FO was associated with a significant decrease in Malondialdehyde (MDA), an oxidative stress marker, whereas SO showed no substantial changes in MDA. Both oils effectively improved lipid profiles, but there were no significant differences in fasting blood sugar levels. The findings highlight the potential of both FO and SO in managing lipid levels and reducing oxidative stress. The study's findings underscore the value of dietary oils in managing lipid levels and hypertension. However, the two groups' lack of significant differences in fasting blood sugar levels limits the conclusions about their impact on glycaemic control. Furthermore, the study did not assess the impact on inflammatory and oxidative markers, which could have provided additional insights into the broader health implications of the oils.

Jenkins et al. (37) investigated the impact of a low-GL diet supplemented with canola oil on glycaemic control and lipid profiles in adults with type 2 diabetes. Their study found that the low-GL diet led to significant reductions in HbA1c and improved lipid profiles compared to a whole-grain diet, suggesting that dietary modifications focused on glycaemic load can effectively manage blood glucose levels. Including canola oil in the low-GL diet also positively influences lipid profiles, potentially lowering cardiovascular risk. One of the study's strengths is its clear focus on dietary modification strategies and their impact on multiple health parameters. Additionally, the study did not explore the effects of the diet on inflammatory and oxidative markers, which could provide a more comprehensive view of its health impacts.

Wei et al. (43) conducted a prospective, randomized control trial to examine the combined effects of perilla oil supplementation and exercise on lipid profiles and inflammatory markers in adults with elevated blood lipids. The study involved 36 participants who were divided into three groups: Exercise only (EG), perilla oil only (MG), and a combination of both (EMG). The results showed significant reductions in total cholesterol, triglycerides, and LDL-C in the EMG group compared to the EG and MG groups. Additionally, HDL-C levels increased significantly in both the MG and EMG groups. Notably, the EMG group also experienced significant reductions in inflammatory markers such as high-sensitivity C-Reactive Protein (hs-CRP), Plasminogen Activator Inhibitor-1 (PAI-1), and Tumor Necrosis Factor-alpha (TNF-α) compared to the other groups. The combination of perilla oil and exercise offers enhanced benefits over either intervention alone, particularly in improving lipid profiles and reducing inflammation. The strength of this study lies in its comprehensive approach, combining both dietary and physical activity interventions, which are crucial for managing hyperlipidemia. The study did not assess potential interactions between the exercise regimen and perilla oil supplementation, which could provide further insights into their combined effects.

Alipoor et al. (40) investigated the impact of white sesame seed consumption on lipid profiles and antioxidant markers in hyperlipidemic patients. The study involved 38 randomized participants who received either white sesame seeds (40 g/day) or a control diet for 60 days. The results demonstrated a significant decrease in total cholesterol, LDL-C, and the total cholesterol to HDL-C ratio in the sesame seed group. Additionally, there were improvements in antioxidant markers, with decreased levels of TBARS and increased GPx and SOD activities. The findings suggest that sesame seeds have a beneficial effect on both lipid profiles and oxidative stress. The study's strengths include its clear focus on dietary intervention and its use of both lipid and antioxidant markers. Additionally, the study did not explore potential changes in inflammatory markers, which could offer a more comprehensive view of the health benefits of sesame seeds.

Asghari et al. (44) examined the effects of PSO supplementation on serum triglycerides, HDL-C, and inflammatory markers in adults with elevated cholesterol. The study found that PSO supplementation (800 mg/day for 4 weeks) reduced serum triglycerides and the triglyceride to HDL-C ratio, increasing HDL-C. Despite these positive changes, there were no significant alterations in TNF-α levels, an inflammatory marker. The study's strength lies in its double-blind, placebo-controlled design, which enhances the reliability of the findings. The significant improvements in triglyceride levels and HDL-C suggest that PSO may be effective in managing dyslipidemia. However, the lack of impact on TNF-α levels suggests that while PSO can improve lipid profiles, its effects on inflammation may be limited.

Sankar et al. (41) evaluated the effectiveness of sesame oil alone, glibenclamide alone, and their combination in patients with type 2 diabetes. The combination therapy showed superior improvements in glycaemic control and lipid profiles compared to either treatment alone, indicating that combining therapies can enhance treatment outcomes. The study also observed improvements in antioxidant activity with sesame oil and its combination with glibenclamide, suggesting added benefits in managing oxidative stress. While the study highlights the potential synergistic effects of combining sesame oil with glibenclamide, the open-label design introduces potential bias, as both participants and researchers were aware of the treatment assignments. Despite these limitations, the study provides valuable insights into the potential for combination therapies to improve glycaemic control and antioxidant status, which are crucial for managing diabetes effectively. The focus on oxidative stress adds another layer of understanding to the benefits of sesame oil. However, the study did not address inflammatory markers, which could further elucidate the full spectrum of health benefits.

Mirmiran et al. (46) explored the impact of PSO on lipid profiles and body composition in hyperlipidemic individuals. The study found that PSO supplementation (400 mg twice daily for 4 weeks) reduced triglycerides and improved HDL-C. However, there were no significant changes in serum cholesterol, LDL-C, glucose concentrations, or body composition. The study highlights the potential of PSO to improve triglyceride levels and HDL-C, but its effects on other lipid parameters and metabolic health were not significant. The double-blind design and focus on specific lipid markers add robustness to the findings.

Figure 2 provides a visual summary of the potential effects of seed oils on key health parameters. The figure illustrates how seed oils may influence diabetes mellitus, dyslipidemia, chronic inflammation, and oxidative stress. Each circle highlights specific actions and outcomes associated with seed oils in these

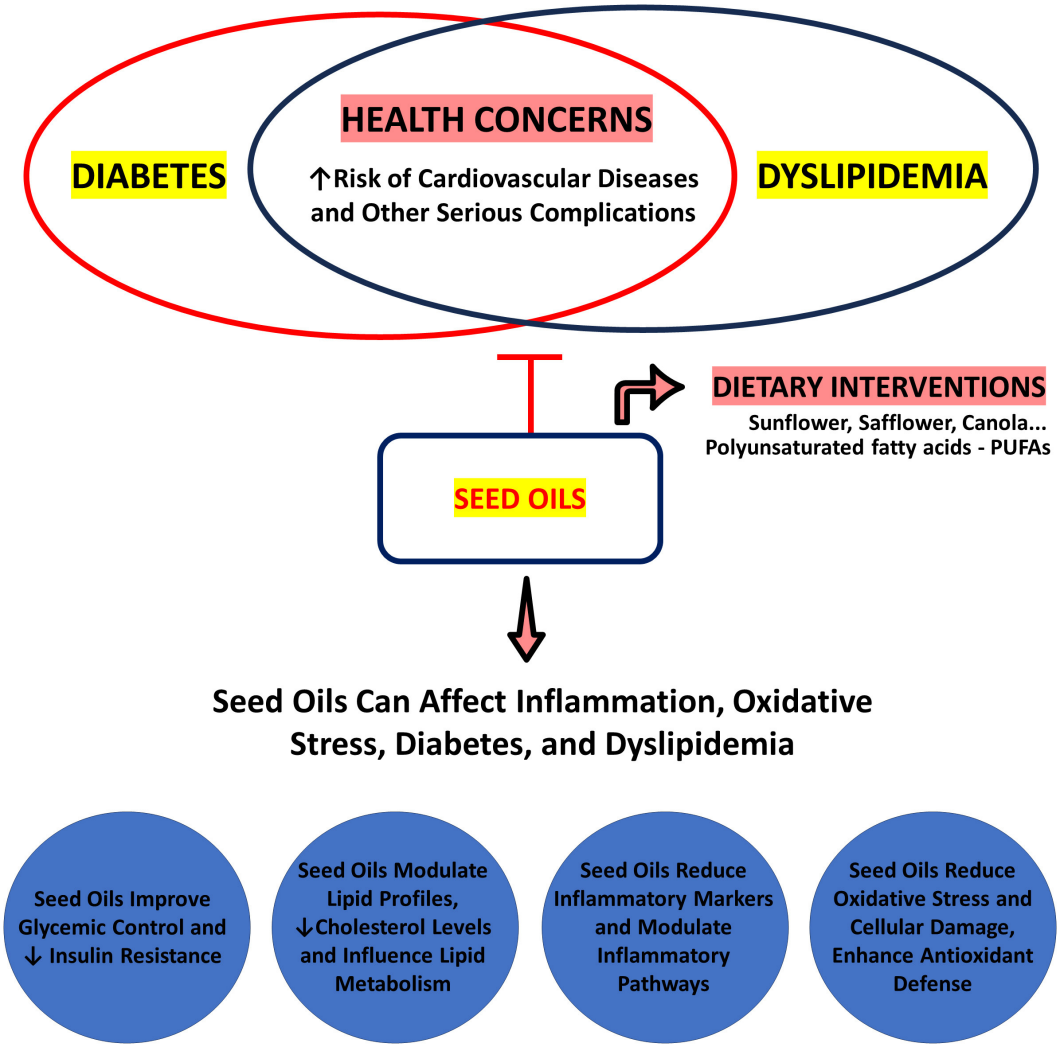

FIGURE 2
Impact of seed oils on diabetes, dyslipidemia, inflammation, and oxidative stress. Reproduced from Servier Medical Art, licensed under CC BY 4.0.

contexts, offering a comprehensive overview of their impact on metabolic and inflammatory pathways. This visual representation aims to clarify the relationships between seed oils and various health markers, setting the stage for a detailed analysis in the subsequent sections.

5 Conclusions, limitations, and future research endeavors

This systematic review consolidates evidence from various studies examining the impact of seed oils on glycemic control, lipid profiles, and markers of inflammation and oxidative stress. The findings from these studies highlight the potential therapeutic benefits of different seed oils in managing metabolic disorders, particularly type 2 diabetes and related conditions. PSO has demonstrated potential in improving glycemic control, significantly reducing fasting blood glucose levels, and enhancing GLUT-4 gene expression. However, its impact on other metabolic parameters, such as insulin levels and HOMA-IR, was limited, indicating that while PSO may offer some benefits, it may not uniformly affect all aspects of metabolic health. Sesame seed oil has shown promise in improving glycemic control and reducing oxidative stress, as evidenced by significant decreases in oxidative stress markers and increases in antioxidant enzyme activity. This suggests that sesame seed oil could be valuable to diabetes management strategies. Additionally, studies on flaxseed and sunflower seed oils reveal their positive effects on lipid profiles, with FO particularly notable for reducing oxidative stress markers. The review also underscores the benefits of combining dietary interventions with physical activity, as shown by the study on perilla oil. This integrated approach resulted in improved lipid profiles and reduced inflammatory markers, highlighting the importance of comprehensive lifestyle modifications in managing metabolic conditions.

Nutrigenomics emerges from studying food and dietary components' impacts on gene expression concerning genetic variants and other nutritional factors. It focuses on the interaction between nutrients and functional foods with the genome at the molecular level, allowing insights into the role of specific food compounds or dietary constituents that may influence human health (47). In this scenario, future research into seed oils and their effects on metabolic health should explore genetic studies, as they could reveal how individual genetic variations affect responses to seed oil supplementation since genetic variances may influence the individual response to dietary intakes and supplements (48). By identifying genetic markers associated with varied responses, researchers can develop personalized treatment strategies that optimize the benefits of seed oils based on individual genetic profiles, thereby permitting the practical and objective translation from conventional dietary guidelines to genome-guided nutrition, as evidenced by Lagoumintzis and Patrinos (49). This approach could lead to more targeted and effective interventions, improving outcomes for people with different genetic backgrounds, which is a crucial step in developing alternative and more cost-effective treatment strategies to diabetes and dyslipidemia, as evidenced by recent vital publications (50, 51). Immunological assays represent another essential research direction. These studies can provide detailed insights into how seed oils influence immune system dynamics by assessing changes in immune cell profiles, cytokine levels, and other markers of immune function. As evidenced by Yamasaki et al. (52), dietary interventions with oils derived from pomegranate seeds modulate the immune system and affect the lipid profiles of the treated mice by modulating inflammation derived from immune interactions and adipose tissue dysfunction. Understanding how seed oils modulate inflammation and oxidative stress at the cellular level could uncover new mechanisms of action, potentially identifying novel therapeutic targets and enhancing strategies for managing chronic inflammatory conditions. Diabetes is an inflammatory disease (53), and dyslipidemia strictly correlates with dietary inflammatory indexes (54). Ribonucleic Acid (RNA)-based therapies also offer significant potential for advancing seed oil research. Exploring small interfering RNAs (siRNAs) or messenger RNA (mRNA) therapies to target specific metabolic pathways influenced by seed oils could enhance their therapeutic efficacy since seed oils have already been identified as epigenetic modulators in many diseases (55), including diabetes (56). Such approaches allow for precise modulation of gene expression related to metabolic health, potentially amplifying the beneficial effects of seed oils and mitigating any adverse outcomes. This innovative line of research could lead to more effective treatments that leverage the molecular impacts of seed oils.

Many of the included studies addressed oils against diabetes and dyslipidemia ranging from milligrams to grams treatment options, which limits the understanding of the findings and the generability to the broader population. Because of this, long-term and dose-response studies are essential to fully understanding the sustainability and optimal use of seed oils. Research beyond typical short-term intervention periods is needed to assess seed oil supplementation's long-term benefits and potential risks. Additionally, investigating different dosages will help determine the most effective and safe levels of seed oil intake for various health outcomes. This comprehensive approach will provide valuable insights for clinical practice and public health recommendations. Expanding research to include more diverse and broader populations is also critical. Future studies should involve individuals with various health conditions beyond type 2 diabetes, such as cardiovascular diseases, MetSyn, and obesity. This will improve the generalizability of findings and help identify which groups might benefit most from seed oil interventions. Furthermore, examining the effects of seed oils across different demographic groups—including varying ages, ethnic backgrounds, and lifestyle factors—will ensure that recommendations are relevant and applicable to a broader audience. In summary, pursuing these research directions will deepen our understanding of seed oils' therapeutic potential, enable more personalized treatment strategies, and enhance the applicability of findings across diverse populations and health conditions.

Several limitations were identified in the reviewed studies. Many had relatively short intervention periods, which may not capture the full range of benefits or potential adverse effects of seed oil supplementation. Larger sample sizes were often lacking, which could affect the statistical power and generalizability of the results. Some studies had open-label designs or lacked control for potential biases, which could impact the reliability of the outcomes. Additionally, not all studies assessed a comprehensive range of biomarkers, and many focused on specific populations, limiting the applicability of the findings to the general population. Finally, the included studies varied in dosage and intervention periods, which could impact the comparability and generalizability of the results, making it difficult to draw definitive conclusions. Most included studies did not evaluate the utilized interventions' nutritional composition and chemical profiles. If they had done so, it would have undoubtedly enhanced the strength of the data since most of the included oils might possess similar bioactive compounds and nutritional values. Addressing these limitations through longer, more rigorously designed studies with diverse populations and comprehensive biomarker assessments will be crucial for further elucidating seed oil supplementation's health benefits and potential drawbacks.

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

LuF: Writing – original draft, Writing – review & editing. LíF: Writing – original draft, Writing – review & editing. VD: Writing – original draft, Writing – review & editing. JS: Writing – original draft, Writing – review & editing. BL: Writing – original draft, Writing – review & editing. AC: Writing – original draft, Writing – review & editing. EL: Writing – original draft, Writing – review & editing. CR: Writing – original draft, Writing – review & editing.

VM: Writing – original draft, Writing – review & editing. EF: Writing – original draft, Writing – review & editing. VC: Writing – original draft, Writing – review & editing. RD: Writing – original draft, Writing – review & editing. SB: Writing – original draft, Writing – review & editing.

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References

1. Kane JP, Pullinger CR, Goldfine ID, Malloy MJ. Dyslipidemia and diabetes mellitus: role of lipoprotein species and interrelated pathways of lipid metabolism in diabetes mellitus. *Curr Opin Pharmacol.* (2021) 61:21–7. doi: 10.1016/j.coph.2021.08.013

2. Kalra S, Raizada N. Dyslipidemia in diabetes. *Indian Heart J.* (2024) 76:S80–S2. doi: 10.1016/j.ihj.2023.11.002

3. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J.* (2020) 41:111–88. doi: 10.1093/eurheartj/ehz455

4. Marx N, Federici M, Schütt K, Müller-Wieland D, Ajjan RA, Antunes MJ, et al. 2023 ESC guidelines for the management of cardiovascular disease in patients with diabetes. *Eur Heart J.* (2023) 44:4043–140. doi: 10.1093/eurheartj/ehad192

5. Hu Y, Chen X, Hu M, Zhang D, Yuan S, Li P, et al. Medicinal and edible plants in the treatment of dyslipidemia: advances and prospects. *Chin Med.* (2022) 17:113. doi: 10.1186/s13020-022-00666-9

6. Liu H, Wang F, Xia H, Pan D, Yang L, Wang S, et al. Comparison of the effects of 3 kinds of oils rich in omega-3 polyunsaturated fatty acids on glycolipid metabolism and lipoprotein subfractions. *Food Sci Hum Wellness.* (2023) 12:2221–31. doi: 10.1016/j.fshw.2023.03.042

7. Gandhi GR, Hillary VE, Antony PJ, Zhong LLD, Yogesh D, Krishnakumar NM, et al. A systematic review on anti-diabetic plant essential oil compounds: dietary sources, effects, molecular mechanisms, and safety. *Crit Rev Food Sci Nutr.* (2024) 64:6526–45. doi: 10.1080/10408398.2023.2170320

8. Li H, Kang S, Sun L. A study on the evaluation of polyenoic vegetable oils and their female health benefits based on time series analysis model: the case of peony seed oil. *J Healthc Eng.* (2022) 2022:3127698. doi: 10.1155/2022/3127698

9. Parikh M, Maddaford TG, Austria JA, Aliani M, Netticadan T, Pierce GN. Dietary flaxseed as a strategy for improving human health. *Nutrients.* (2019) 11:1171. doi: 10.3390/nu11051171

10. Šamec D, Loizzo MR, Gortzi O, Çankaya I T, Tundis R, Suntar I, et al. The potential of pumpkin seed oil as a functional food-A comprehensive review of chemical composition, health benefits, and safety. *Compr Rev Food Sci Food Saf.* (2022) 21:4422–46. doi: 10.1111/1541-4337.13013

11. Çakir M, Özer CO, Var GB. Utilization of sunflower oil-based oleogel fordeep-fried coated chicken products. *J Oleo Sci.* (2023) 72:399–407. doi: 10.5650/jos.ess22365

12. Fadiloglu EE, Yildiz Turp G, Celebioglu C, Sel G. Influence of different cooking methods on quality characteristics and nutritional value of gluten-free beef burger patties formulated with walnut oil, safflower oil and buckwheat. *Meat Sci.* (2023) 204:109251. doi: 10.1016/j.meatsci.2023.109251

13. Saleem M, Ali H, Bilal M, Atta BM, Ahmad N. Quality analysis of canola and mustard oil using fluorescence spectroscopy. *J Fluoresc.* (2023) 33:1695–704. doi: 10.1007/s10895-023-03185-4

14. Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *Int J Mol Sci.* (2015) 16:12871–90. doi: 10.3390/ijms160612871

15. Rodríguez-Blázquez S, Gómez-Mejía E, Rosales-Conrado N, León-González ME, García-Sánchez B, Miranda R. Valorization of prunus seed oils: fatty acids composition and oxidative stability. *Molecules.* (2023) 28:207045. doi: 10.3390/molecules28207045

16. Šmíd V, Dvorák K, Šedivý P, Kosek V, Leníček M, Dezortová M, et al. Effect of omega-3 polyunsaturated fatty acids on lipid metabolism in patients with metabolic syndrome and NAFLD. *Hepatol Commun.* (2022) 6:1336–49. doi: 10.1002/hep4.1906

17. Dash UC, Bhol NK, Swain SK, Samal RR, Nayak PK, Raina V, et al. Oxidative stress and inflammation in the pathogenesis of neurological disorders: mechanisms and implications. *Acta Pharmaceutica Sinica B.* (2024). doi: 10.1016/j.apsb.2024.10.004

18. Gambini J, Stromsnes K. Oxidative stress and inflammation: from mechanisms to therapeutic approaches. *Biomedicines.* (2022) 10:40753. doi: 10.3390/biomedicines10040753

19. de Luca C, Olefsky JM. Inflammation and insulin resistance. *FEBS Lett.* (2008) 582:97–105. doi: 10.1016/j.febslet.2007.11.057

20. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. *Immunity.* (2022) 55:31–55. doi: 10.1016/j.immuni.2021.12.013

21. Henein MY, Vancheri S, Longo G, Vancheri F. The role of inflammation in cardiovascular disease. *Int J Mol Sci.* (2022) 23:2906. doi: 10.3390/ijms232112906

22. Sorriento D, Iaccarino G. Inflammation and cardiovascular diseases: the most recent findings. *Int J Mol Sci.* (2019) 20:63879. doi: 10.3390/ijms20163879

23. Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxid Med Cell Longev.* (2016) 2016:5698931. doi: 10.1155/2016/5698931

24. Hussain T, Tan B, Yin Y, Blachier F, Tossou MC, Rahu N. Oxidative stress and inflammation: what polyphenols can do for us? *Oxid Med Cell Longev.* (2016) 2016:7432797. doi: 10.1155/2016/7432797

25. Manzoor MF, Arif Z, Kabir A, Mehmood I, Munir D, Razzaq A, et al. Oxidative stress and metabolic diseases: relevance and therapeutic strategies. *Front Nutr.* (2022) 9:994309. doi: 10.3389/fnut.2022.994309

26. Boarescu PM, Boarescu I, Pop RM, Roşian S H, Bocşan IC, Rus V, et al. Evaluation of oxidative stress biomarkers, pro-inflammatory cytokines, and histological changes in experimental hypertension, dyslipidemia, and type 1 diabetes mellitus. *Int J Mol Sci.* (2022) 23:1438. doi: 10.3390/ijms23031438

27. Lu X, Xie Q, Pan X, Zhang R, Zhang X, Peng G, et al. Type 2 diabetes mellitus in adults: pathogenesis, prevention and therapy. *Signal Transduct Target Ther.* (2024) 9:262. doi: 10.1038/s41392-024-01951-9

28. Ojo OA, Ibrahim HS, Rotimi DE, Ogunlakin AD, Ojo AB. Diabetes mellitus: from molecular mechanism to pathophysiology and pharmacology. *Med Novel Technol Dev.* (2023) 19:100247. doi: 10.1016/j.medntd.2023.100247

29. Papantoniou E, Arvanitakis K, Markakis K, Papadakos SP, Tsachouridou O, Popovic DS, et al. Pathophysiology and clinical management of dyslipidemia in people living with HIV: sailing through rough seas. *Life.* (2024) 14:449. doi: 10.3390/life14040449

30. Alam S, Sarker MMR, Sultana TN, Chowdhury MNR, Rashid MA, Chaity NI, et al. Antidiabetic phytochemicals from medicinal plants: prospective candidates for new drug discovery and development. *Front Endocrinol.* (2022) 13:800714. doi: 10.3389/fendo.2022.800714

31. Langhi C, Vallier M, Bron A, Otero YF, Maura M, Le Joubioux F, et al. A polyphenol-rich plant extract prevents hypercholesterolemia and modulates gut microbiota in western diet-fed mice. *Front Cardiovasc Med.* (2024) 11:1342388. doi: 10.3389/fcvm.2024.1342388

32. Tian M, Bai Y, Tian H, Zhao X. The chemical composition and health-promoting benefits of vegetable oils-a review. *Molecules.* (2023) 28:6393. doi: 10.3390/molecules28176393

33. Schwingshackl L, Bogensberger B, Benčič A, Knüppel S, Boeing H, Hoffmann G. Effects of oils and solid fats on blood lipids: a systematic review and network meta-analysis. *J Lipid Res.* (2018) 59:1771–82. doi: 10.1194/jlr.P085522

34. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* (2021) 372:n71. doi: 10.1136/bmj.n71

35. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The cochrane collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* (2011) 343:d5928. doi: 10.1136/bmj.d5928

36. Nikooyeh B, Zargaraan A, Ebrahimof S, Kalayi A, Zahedirad M, Yazdani H, et al. Daily consumption of γ-oryzanol-fortified canola oil, compared with unfortified canola and sunflower oils, resulted in a better improvement of certain cardiometabolic biomarkers of adult subjects with type 2 diabetes: a randomized controlled clinical trial. *Eur J Med Res.* (2023) 28:416. doi: 10.1186/s40001-023-01409-8

37. Jenkins DJ, Kendall CW, Vuksan V, Faulkner D, Augustin LS, Mitchell S, et al. Effect of lowering the glycemic load with canola oil on glycemic control and cardiovascular risk factors: a randomized controlled trial. *Diabetes Care.* (2014) 37:1806–14. doi: 10.2337/dc13-2990

38. Aslam F, Iqbal S, Nasir M, Anjum AA. White sesame seed oil mitigates blood glucose level, reduces oxidative stress, and improves biomarkers of hepatic and renal function in participants with type 2 diabetes mellitus. *J Am Coll Nutr.* (2019) 38:235–46. doi: 10.1080/07315724.2018.1500183

39. Haldar S, Wong LH, Tay SL, Jacoby JJ, He P, Osman F, et al. Two blends of refined rice bran, flaxseed, and sesame seed oils affect the blood lipid profile of Chinese adults with borderline hypercholesterolemia to a similar extent as refined olive oil. *J Nutr.* (2020) 150:3141–51. doi: 10.1093/jn/nxaa274

40. Alipoor B, Haghighian MK, Sadat BE, Asghari M. Effect of sesame seed on lipid profile and redox status in hyperlipidemic patients. *Int J Food Sci Nutr.* (2012) 63:674–8. doi: 10.3109/09637486.2011.652077

41. Sankar D, Ali A, Sambandam G, Rao R. Sesame oil exhibits synergistic effect with anti-diabetic medication in patients with type 2 diabetes mellitus. *Clin Nutr.* (2011) 30:351–8. doi: 10.1016/j.clnu.2010.11.005

42. Khajebishak Y, Payahoo L, Alivand M, Hamishehkar H, Mobasseri M, Ebrahimzadeh V, et al. Effect of pomegranate seed oil supplementation on the GLUT-4 gene expression and glycemic control in obese people with type 2 diabetes: a randomized controlled clinical trial. *J Cell Physiol.* (2019) 234:19621–8. doi: 10.1002/jcp.28561

43. Wei M, Xiong P, Zhang L, Fei M, Chen A, Li F. Perilla oil and exercise decrease expressions of tumor necrosis factor-alpha, plasminogen activator inhibitor-1 and highly sensitive C-reactive protein in patients with hyperlipidemia. *J Tradit Chin Med.* (2013) 33:170–5. doi: 10.1016/S0254-6272(13)60120-X

44. Asghari G, Sheikholeslami S, Mirmiran P, Chary A, Hedayati M, Shafiee A, et al. Effect of pomegranate seed oil on serum TNF-α level in dyslipidemic patients. *Int J Food Sci Nutr.* (2012) 63:368–71. doi: 10.3109/09637486.2011.631521

45. Akrami A, Nikaein F, Babajafari S, Faghih S, Yarmohammadi H. Comparison of the effects of flaxseed oil and sunflower seed oil consumption on serum glucose, lipid profile, blood pressure, and lipid peroxidation in patients with metabolic syndrome. *J Clin Lipidol.* (2018) 12:70–7. doi: 10.1016/j.jacl.2017.11.004

46. Mirmiran P, Fazeli MR, Asghari G, Shafiee A, Azizi F. Effect of pomegranate seed oil on hyperlipidaemic subjects: a double-blind placebo-controlled clinical trial. *Br J Nutr.* (2010) 104:402–6. doi: 10.1017/S0007114510000504

47. Selvi MK, Sowmya B, Kannan T, Latha M, Jena I, Arun Kumar V, et al. Chapter 2 - Advances in personalized food and nutrition. In: *Research and Technological Advances in Food Science*. Eds Prakash B. Academic Press. (2022). p. 31-60. doi: 10.1016/B978-0-12-824369-5.00004-X

48. Niforou A, Konstantinidou V, Naska A. Genetic variants shaping inter-individual differences in response to dietary intakes-A narrative review of the case of vitamins. *Front Nutr.* (2020) 7:558598. doi: 10.3389/fnut.2020.558598

49. Lagoumintzis G, Patrinos GP. Triangulating nutrigenomics, metabolomics and microbiomics toward personalized nutrition and healthy living. *Hum Genomics.* (2023) 17:109. doi: 10.1186/s40246-023-00561-w

50. Chung WK, Erion K, Florez JC, Hattersley AT, Hivert M-F, Lee CG, et al. Precision medicine in diabetes: a consensus report from the American diabetes association (ADA) and the European association for the study of diabetes (EASD). *Diabetes Care.* (2020) 43:1617–35. doi: 10.2337/dci20-0022

51. Tomlinson B, Lin CH, Chan P, Lam CW. Personalized medicine in lipid-modifying therapy. *Per Med.* (2021) 18:185–203. doi: 10.2217/pme-2020-0110

52. Yamasaki M, Kitagawa T, Koyanagi N, Chujo H, Maeda H, Kohno-Murase J, et al. Dietary effect of pomegranate seed oil on immune function and lipid metabolism in mice. *Nutrition.* (2006) 22:54–9. doi: 10.1016/j.nut.2005.03.009

53. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol.* (2011) 11:98–107. doi: 10.1038/nri2925

54. Chen X, Hou C, Yao L, Li J, Gui M, Wang M, et al. Dietary inflammation index is associated with dyslipidemia: evidence from national health and nutrition examination survey, 1999–2019. *Lipids Health Dis.* (2023) 22:149. doi: 10.1186/s12944-023-01914-z

55. Alsanosi S, Sheikh RA, Sonbul S, Altayb HN, Batubara AS, Hosawi S, et al. The potential role of nigella sativa seed oil as epigenetic therapy of cancer. *Molecules.* (2022) 27:92779. doi: 10.3390/molecules27092779

56. Pinto LC, Cerqueira-Lima AT. Suzarth SdS, de Souza R, Tosta BR, da Silva HB, et al. *Anonna muricata L* (soursop) seed oil improves type 1 diabetes parameters *in vivo* and *in vitro. PharmaNutrition.* (2018) 6:1–8. doi: 10.1016/j.phanu.2017.11.00

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Association of dietary inflammatory index on all-cause and cardiovascular mortality in U.S. adults with metabolic dysfunction associated steatotic liver disease

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Backgrounds: An inflammatory diet is pivotal in metabolic dysfunction-associated steatotic liver disease (MASLD) development. However, it remains unclear whether Dietary Inflammatory Index (DII), which serves as a reliable indicator to assess pro-inflammatory diet, have associative effects on mortality outcomes of MASLD.

Methods: Participants in the National Health and Nutrition Examination Survey (NHANES) database from 1999 to 2018 years were included. Kaplan–Meier (KM) curves were used to estimate survival probabilities, while Cox regression analysis and restricted cubic splines (RCS) were employed to assess the association between DII and mortality outcomes. The concordance index (C-index) evaluated the accuracy of multivariate-adjusted DII for mortality among MASLD participants.

Results: The cohort consisted of 4,510 men and 4,323 women with a median age of 52 years. Multivariate-adjusted Cox regression analysis revealed that high levels of DII were significantly associated with the all-cause mortality of participants with MASLD (multivariable-adjusted hazard ratio (aHR) = 1.28, 95% confidence interval (CI) 1.10–1.49, $p = 0.002$, DII aHR for cardiovascular mortality = 1.28, 95% CI 1.07–1.53, $p = 0.006$). The C-index for the multivariate model, integrating DII and other clinical variables, was 0.837 for all-cause mortality and 0.860 for cardiovascular mortality. RCS analysis showed a positive linear relationship between DII and all-cause mortality rate (p for nonlinearity = 0.057), with no significant nonlinearity for cardiovascular mortality ($p = 0.953$). Subgroup analyses indicated stronger associations in participants <65 years, married, with a college education, non-smokers, non-drinkers, and those without hypertension.

Conclusion: Elevated DII levels are linked to higher mortality in adults with MASLD, underscoring the index's utility in predicting mortality risks. These findings shows that dietary interventions targeted inflammation may be helpful in this population.

KEYWORDS

MASLD, dietary inflammatory index, cardiovascular, mortality, NHANES

1 Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously termed non-alcoholic fatty liver disease (NAFLD), was officially renamed in June 2023 (1). The prevalence of MASLD is rising rapidly, affecting 32.4% of the population in 2022, making it one of the most common chronic liver diseases (2, 3). MASLD is characterized by the accumulation of fat in hepatocytes, excluding the impact of viruses, alcohol, and autoimmune factors (4). It affects over one-third of the global population and is associated with significant morbidity and mortality due to its progression to metabolic dysfunction-associated steatohepatitis (MASH), cirrhosis, and hepatocellular carcinoma. Beyond liver-specific outcomes, MASLD contributes to systemic metabolic dysfunction, increasing the risk of cardiovascular disease and all-cause mortality (5).

Cardiovascular complications and systemic metabolic dysfunction are the primary causes of mortality in individuals with MASLD (6). Given the strong links between diet, inflammation, and metabolic dysfunction—key drivers of MASLD progression—dietary management is a cornerstone of nonpharmacological strategies for MASLD. Dietary patterns with low inflammatory potential may help mitigate disease progression and improve health outcomes. Additionally, bioactive compounds, such as polyphenols and omega-3 fatty acids, have demonstrated anti-inflammatory properties that may support liver health in MASLD populations (7, 8). However, despite the potential benefits of specific dietary components, the overall inflammatory potential of the diet and its impact on MASLD-related health outcomes remain insufficiently explored.

To address this, the Dietary Inflammatory Index (DII) has been widely applied as a tool to quantify dietary inflammatory potential, providing a standardized assessment of the inflammatory impact of an individual's diet. Developed by Shivappa et al. in 2014 (9), the DII quantifies the effects of dietary components on inflammatory markers such as IL-1β, IL-6, TNF-α, and C-reactive protein, assigning each food parameter a score based on its pro-inflammatory or anti-inflammatory properties. Higher DII scores denote a diet with greater inflammatory potential, whereas lower scores reflect anti-inflammatory effects.

Studies in populations with obesity and aging have linked higher DII scores to increased mortality, highlighting the impact of dietary inflammation in metabolically compromised populations. For example, in a prospective cohort study involving 3,521 adults within the normal-weight BMI range, a pro-inflammatory diet, as indicated by high DII scores, was associated with an increased risk of cardiovascular disease (CVD) mortality among those with central obesity (10). Another study utilizing data from the National Health and Nutrition Examination Survey (NHANES) found that lower DII scores were linked to a decreased risk of all-cause mortality in aging populations (11). Moreover, MASLD is a systemic metabolic disorder that significantly increases the risk of cardiovascular disease-related mortality and various malignancies (12, 13). Unlike general obesity or aging populations, individuals with MASLD exhibit distinct inflammatory profiles driven by hepatic steatosis, insulin resistance, and metabolic dysregulation. Given the chronic inflammatory state of MASLD, the DII not only assesses dietary inflammation but also reflects how pro-inflammatory diets may exacerbate existing inflammation and accelerate disease progression. A high DII diet can increase pro-inflammatory cytokines (IL-6, TNF-α), oxidative stress, and gut dysbiosis, further aggravating hepatic inflammation and metabolic dysfunction. Thus, DII serves as a key marker for both dietary inflammation and its role in MASLD progression.

Given the significant burden of MASLD and its progression to more severe liver diseases and associated comorbidities, it is critical to investigate how dietary inflammatory potential, as measured by DII, impacts all-cause and cardiovascular mortality in this population. The present study employed data from the NHANES from 1998 to 2018. The primary objective was to examine the association between DII and all-cause and CVD mortality in United States adults diagnosed with MASLD. By analyzing this relationship, we hope to provide insights that could inform dietary recommendations and interventions aimed at reducing inflammation and improving long-term health outcomes in this population.

2 Materials and methods

2.1 Data source

Data for this study were derived from the NHANES database between 1999 and 2018. NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the United States, conducted by the National Center for Health Statistics (NCHS) as part of the Centers for Disease Control and Prevention (CDC). NHANES employs a stratified, multistage probability sampling design to produce a sample that is representative of the noninstitutionalized U.S. population (14). To ensure high data quality, NHANES uses rigorous standardized protocols in data collection, including thorough training for interviewers and examiners, regular calibration of measurement equipment, and standardized data collection environments. Extensive quality control procedures, such as double-checking entries and cross-referencing data, are applied throughout the process to maintain accuracy and validity. Furthermore, NHANES data is continually reviewed and updated to reflect changes in population demographics and health trends, making it a widely trusted resource for epidemiological research. Specific description of the NHANES database can be found on the website.[1]

2.2 Participants selection

To evaluate the association between the DII and all-cause and cardiovascular mortality of adults with MASLD, only participants with the diagnosis of MASLD were included. Therefore, during the 10 cycles of interviews (1999 to 2018), 101,316 participants were reviewed. After excluding the participants aged below 18 years old, there were 59,204 participants left. Additionally, participants with missing data on DII, body measurements, and metabolic dysfunction-associated factors were excluded.Then, participants presenting clinical features of FLI < 60, with other causes of SLD, a

1 https://www.cdc.gov/nchs/nhanes/

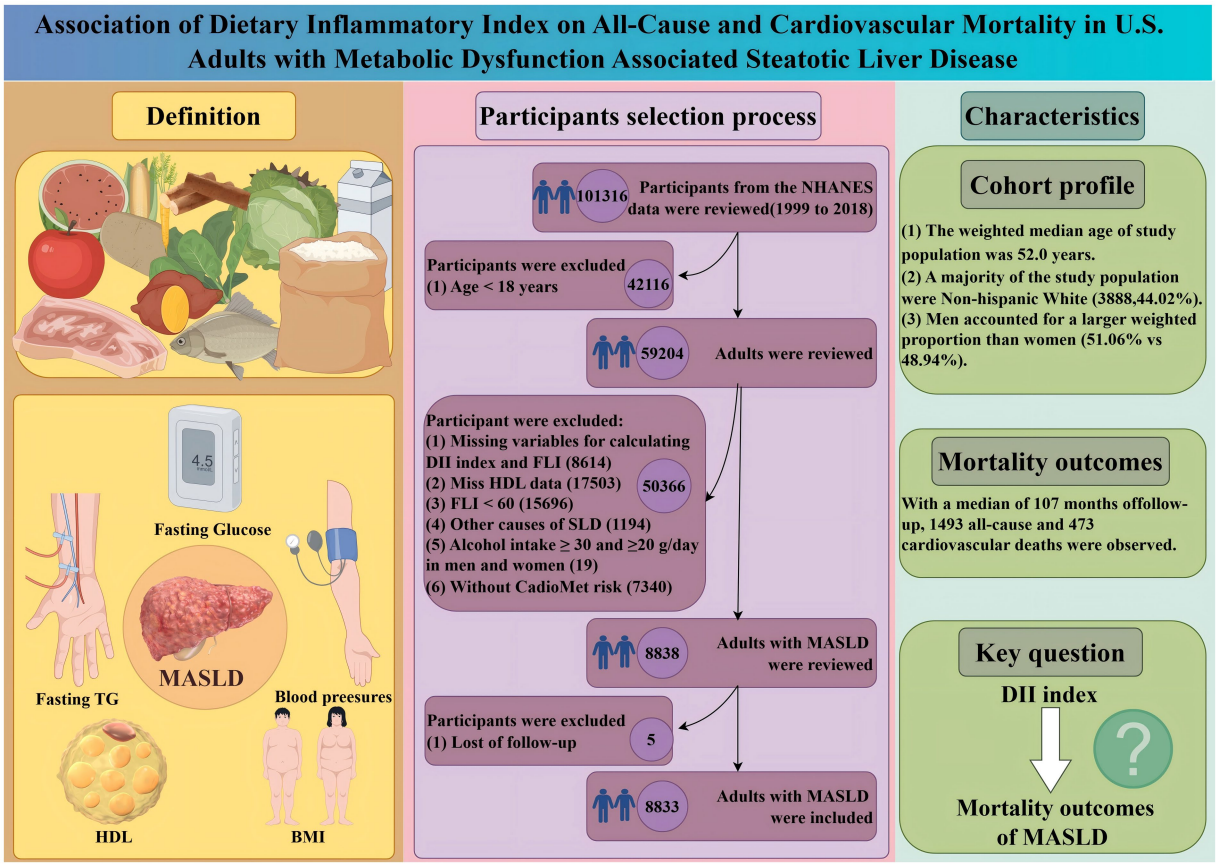

FIGURE 1
Scheme of the aim of the study and participants selection process. We aim to evaluate the association between varied DII with the mortality outcomes of adults with MASLD. MASLD, metabolic dysfunction-associated steatotic liver disease; DII, diet infammatory index; BMI, body mass index; HDL, high-density lipoprotein; TG, triglyceride. Other protential causes of SLD: viral hepatitis, autoimmune liver disease, genetic liver diseases, drug- or medication-induced liver disease, and alcohol-related liver disease.

history of moderate to heavy alcohol intake, lacking cardiometabolic risk factors, or lost to follow-up were further excluded. Finally, there were 8,833 participants with MASLD were included in this study (Figure 1).

Participants with missing data for critical variables, such as FLI, DII, and key metabolic dysfunction-associated factors, were excluded. However, minor missingness in covariates used in the regression models was addressed using multiple imputation, as described in Section 2.5.

2.3 Assessment of MASLD

Direct ultrasonographic assessments of hepatic steatosis were missing in most of the interview cycles. Thus, hepatic steatosis was determined by using the fatty liver index (FLI), which was a reliable tool to evaluate steatotic liver disease (SLD), with high sensitivity and specificity (15, 16). The equation is listed below (17):

TG refers to triglyceride, GGT refers to gamma-glutamyl transferse, BMI refers to body mass index, and WC refers to waist circumference (15, 17). Based on prior research, participants with an FLI below 60 were deemed unlikely to have hepatic steatosis, whereas those with an FLI of 60 or higher were considered likely to have the condition. Therefore, participants with FLI ≥ 60 were diagnosed with SLD (18).

To meet the diagnostic criteria for MASLD, participants with viral hepatitis, autoimmune liver disease, genetic liver disorders, drug- or medication-induced liver disease, alcohol-related liver disease, as well as individuals whose daily alcohol intake met or exceeded 30 g/day for men and 20 g/day for women, were excluded. Consequently, MASLD was defined as SLD with a combination of the presence of at least one cardiometabolic risk factor (19):

(1) BMI ≥ 25 kg/m^2 or WC ≥ 94 cm for males and ≥ 80 cm for females;

$$FLI = \left(e^{0.953\times\ln(TG)+0.139\times BMI+0.718\times\ln(GGT)+0.053\times WC-15.745}\right)/\left(1+e^{0.953\times\ln(TG)+0.139\times BMI+0.718\times\ln(GGT)+0.053\times WC-15.745}\right)\times 100$$

(2) FBG ≥ 100 mg/dL or 2-h post-load glucose levels ≥140 mg/dL or hemoglobin A1c ≥ 5.7% or diabetes mellitus (DM) or undergoing hypoglycemic therapy for DM;
(3) Blood pressure ≥ 130/85 mmHg or undergoing antihypertensive drug treatment;
(4) Fasting plasma triglycerides ≥150 mg/dL or undergoing lipid-lowering treatment;
(5) Plasma HDL-cholesterol <40 mg/dL for males and < 50 mg/dL for females or undergoing lipid-lowering treatment.

2.4 DII measurements

The DII is a scoring index developed to quantify the overall inflammatory potential of an individual's diet. This index was created by Shivappa et al., who conducted an extensive review of the literature to determine the impact of various foods and dietary constituents on six inflammatory markers: IL-1β, IL-4, IL-6, IL-10, TNF-α, and CRP. The DII assigns pro-inflammatory and anti-inflammatory scores to each food parameter based on the findings of these studies. These scores are then weighted to obtain an overall inflammatory effect score for each specific food parameter.

A lower DII score indicates a diet with a greater capacity to reduce inflammation, whereas a higher DII score suggests a diet with a greater capacity to promote inflammation. In this study, the DII was computed using data from the NHANES database, specifically from 24-h dietary recall (24HDR) data, which were collected during the health examination phase.

A total of 27 nutrients were utilized in the computation of the DII score, including alcohol, vitamins B_{12}/B_6, β-carotene, caffeine, carbohydrates, cholesterol, total fat, fiber, folate, iron, magnesium, zinc, selenium, monounsaturated fat acid (MUFA), niacin, n-3 fatty acids, n-6 fatty acids, protein, polyunsaturated fatty acid (PUFA), riboflavin, saturated fat, thiamin, and vitamins A/C/D/E.

Although the DII can evaluate the inflammatory effects of up to 45 food components, prior studies have demonstrated that using fewer than 30 food components does not significantly affect the DII's evaluative ability (20). In this study, the NHANES data allowed for the use of 27 food parameters to calculate the DII, ensuring a comprehensive assessment of the dietary inflammatory potential.

2.5 Clinical characteristics and covariates

We collected the demographic characteristics of participants with MASLD from the NHANES database.

Sociodemographic characteristics, including gender (male or female), age, race/ethnicity (Hispanic, non-Hispanic White, non-Hispanic Black, or other races), marital status (not married, married), education level (≤ high school, college, or > college), and poverty income ratio (PIR: < 1.3, 1.3–3.5, > 3.5), were obtained during the household interview phase. PIR was defined as the ratio of family income to the federal poverty threshold, adjusted for family size.

Physical and laboratory data collected during the health examination phase included measurements of waist circumference (WC), height, BMI, triglycerides (TG), total cholesterol (TC), glutamic-pyruvic transaminase (ALT), and high-density lipoprotein (HDL).

Hypertension status was defined using a combination of self-reported physician diagnoses (household interview phase), antihypertensive medication use, and blood pressure measurements taken during the health examination phase (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg).

2.6 Outcome measurements

The main outcome of this study was the all-cause mortality of participants with MASLD. The secondary outcome was the cardiovascular mortality of the participants with MASLD. The ICD-10 (International Statistical Classification of Diseases, 10th revision) was used to check the causes of mortality. The mortality of the follow-up population were obtained from the NHANES public-use linked mortality file as of December 31, 2019, which was correlated with the National Death Index (NDI) through a probability matching algorithm. The period of follow-up was calculated from the date when the interview was initially taken to either the date of the patient's death or December 31, 2019 (21).

2.7 Statistical analysis

According to the analytic guideline of the NHANES database[2] (accessed on 17 June 2024), NHANES' person-level sample weights were applied to adjust for the multistage sampling design, which includes stratification and clustering. These adjustments ensure that the results are representative of the U.S. adult population with MASLD and provide accurate variance estimates.

To address missingness in secondary covariates, multiple imputation by chained equations (MICE) was employed. Five imputed datasets were generated, incorporating all variables used in the regression models. Missingness for covariates, such as PIR, blood pressure, and laboratory measures (e.g., HDL and ALT), was typically below 10%. The pooled results from imputed datasets were combined using Rubin's rules to ensure robust inference and minimize bias.

In this study, continuous variables were summarized as medians with interquartile ranges (IQRs) to represent their central tendency and variability. Categorical variables were presented as frequencies with weighted percentages. Comparisons of continuous variables between survivors and non-survivors were conducted using the Kruskal–Wallis test. Categorical variables were compared using the Chi-Squared test to assess proportional differences.

Cox proportional hazard models were used to estimate the association of DII with all-cause and cardiovascular mortality of the participants with MASLD. The selections of controlled covariates in the present study were based on previous literature evaluating the survival of MASLD (22–24). Specifically, model 1 served as the unadjusted analysis. Besides, brief adjustments for age, gender, and race were made in Model 2. In the fully adjusted model, we accounted for age, gender, race, marital status, educational level, poverty income ratio (PIR), plasma glucose concentration, alcohol

2 https://wwwn.cdc.gov/nchs/nhanes/tutorials/weighting.aspx

use, BMI, serum levels of TC, ALT, HDL and TR. The concordance index (C-index) (25) was employed to assess the model accuracy of the multivariate-adjusted DII indices for mortality outcomes among participants with MASLD. Kaplan–Meier (KM) curves with NHANES sample weights were used to illustrate survival patterns across quartiles of the DII among participants with MASLD. For cardiovascular mortality, the cumulative incidence function (CIF) was used to account for competing risks, calculated with the cmprsk package in R.

To evaluate potential non-linear associations between the DII and mortality outcomes, restricted cubic splines (RCS) with three knots were employed. The selection of knots for the RCS curves was guided by the minimization of Akaike's Information Criterion (AIC). A likelihood ratio test was used to compare the spline model to a linear model. *p*-values for non-linearity were reported, with insufficient evidence for non-linearity ($p > 0.05$) being interpreted as lack of support for significant deviation from linearity.

To check the robust associations between DII with all-cause and cardiovascular mortality of the participants with MASLD, three sets of sensitive analyses were conducted to validate the main findings. First, we excluded participants who died within 2 years after the interview, which could reduce the potential reverse causality between exposure and outcome. Second, we tested the association between DII with mortality outcomes of adults who were interviewed between 1999 and 2006 years, which could check the impact of the different cycles we have chosen on the association. Third, we also checked the mortality prediction value of DII in a more generalizable population by applying FLI ≥ 30 as the diagnostic criteria for SLD.

All statistical analyses of the present study were conducted by using the R software.[3]

3 Results

3.1 The baseline characteristics of adults with MASLD

Over the period from 1999 to 2018, the basic characteristics of the 8,833 participants enrolled in the study are summarized in Table 1. The median age of the study population was 52.00 years, and the median BMI was 32.98 kg/m2. Male participants accounted for 51.06% (4,510 cases) of the total population, while females made up 48.94% (4,323 cases). The majority of adults with MASLD were non-Hispanic White (3,888 cases, 44.02%), and non-Hispanic Black individuals constituted 20.23% of the population (1,787 cases). Over half of the participants (54.75%) had an educational level of high school or below, and 28.53% had a college education. Additionally, 61.49% of the participants were current smokers, and 7.85% were current drinkers. Regarding comorbidities, 21.83% of the participants had a history of DM, and 10.30% had hypertension. The median DII was 1.60. During the median follow-up period, there were 1,493 all-cause deaths and 473 cardiovascular-related deaths. Non-survivors were more likely to be male, older, non-Hispanic White, have lower educational levels, be unmarried, have comorbidities, tend to smoke, have a lower BMI, and lower socioeconomic status, and have a higher DII compared to survivors (all $p < 0.05$).

3.2 Association between DII with mortality outcomes of adults with MASLD

All-cause mortality was significantly associated with higher quartile levels of the DII among MASLD participants compared to those with lower quartile levels ($p < 0.0001$ by log-rank test, Figure 2). Consistently, participants with high levels of DII presented lower cardiovascular-specific survival probabilities ($p = 0.012$ by log-rank test) compared to other subgroups (Figure 3). The multivariate-adjusted Cox regression analysis revealed that the 4th quartile level of the DII was significantly associated with all-cause mortality [adjusted hazard ratio (aHR) = 1.28, 95% confidence interval (CI) = 1.10–1.49, $p = 0.002$]. However, the association between the high quartile levels of the DII and cardiovascular mortality was not statistically significant [aHR = 1.23, 95% CI = 0.92–1.63, $p = 0.156$] among adults with MASLD. When incorporated into a multivariate model including other clinically relevant variables, the DII yielded a C-index of 0.837 for all-cause mortality and 0.860 for cardiovascular mortality among participants with MASLD (Figures 4, 5). Finally, the multivariate-adjusted RCS analysis indicated a positive association between DII levels and mortality risk from all-cause and cardiovascular causes among MASLD adults. The *p*-values for non-linearity were 0.057 for all-cause mortality and 0.953 for cardiovascular mortality, suggesting insufficient evidence to support a non-linear relationship. (Figures 6, 7).

3.3 Sensitivity analyses

Subgroup analyses were conducted to determine whether demographic characteristics and clinical factors influenced the relationship between the Dietary Inflammatory Index (DII) and mortality outcomes in individuals with MASLD. The results showed consistent associations between DII and all-cause mortality across gender, age, BMI, marital status, and ALT levels (*P* for interaction >0.05). However, a significant interaction was observed with education level (*P* for interaction = 0.048), indicating that educational attainment may modify the association between DII and all-cause mortality (Supplementary Figure S1). For cardiovascular mortality, consistent results were observed across subgroups stratified by gender, race, marital status, BMI, smoking status, and ALT levels (*P* for interaction >0.05). A significant interaction effect was found for age (*P* for interaction = 0.036), suggesting a stronger association between DII and cardiovascular mortality in individuals under 65 years (Supplementary Figure S2).

Meanwhile, we conducted a series of sensitive analyses to check the robustness of the primary findings (Supplementary Tables S1–S3). First, consistent associations between the DII with all-causes as well as cardiovascular mortality were observed after excluding the participants who died within 2 years. Second, among adults interviewed in earlier cycles, the DII maintained significant associations with mortality outcomes in those with MASLD. Last, the DII demonstrated similar associations with mortality outcomes in

3 version 4.2.3, https://www.r-project.org/

10.3389/fnut.2025.1478165

TABLE 1 The demographic characteristics of adult with MASLD in the present study.

Variables	Total (n = 8,833)	Survivors (n = 7,340, 83.10%)	Non-survivors (n = 1,493, 16.90%)	H/χ^2	p
DII, M(Q_1, Q_3)	1.60 (0.06, 2.83)	1.56 (−0.01, 2.80)	1.74 (0.35, 2.96)	**14.10**	**<0.001**
Age, M(Q_1, Q_3)	52.00 (37.00, 65.00)	48.00 (34.00, 61.00)	70.00 (61.00, 77,00)	**1562.92**	**<0.001**
BMI, M(Q_1, Q_3)	32.98 (29.92, 37.20)	33.29 (30.20, 37.56)	31.51 (28.73, 35.42)	**137.02**	**<0.001**
WAIST, M(Q_1, Q_3)	109.90 (103.20, 118.80)	109.70 (103.10, 118.70)	110.50 (104.00, 119.00)	3.06	0.080
TC, M(Q_1, Q_3)	196.00 (170.00, 255.00)	197.00 (170.00, 225.00)	194.00 (167.00, 226.00)	1.64	0.200
TR, M(Q_1, Q_3)	145.00 (103.00, 207.00)	143.00 (101.00, 204.25)	154.00 (112.00, 222.00)	**38.62**	**<0.001**
ALT, M(Q_1, Q_3)	26.00 (18.00, 40.00)	26.00 (18.00, 29.00)	28.00 (19.00, 46.00)	**37.24**	**<0.001**
GLU, M(Q_1, Q_3)	104.00 (96.00, 118.40)	103.00 (95.00, 106.00)	111.00 (99.70, 135.70)	**206.19**	**<0.001**
FLI, M(Q_1, Q_3)	85.27 (73.73, 94.38)	85.36 (73.67, 94.52)	84.88 (74.05, 93.54)	0.75	0.388
HDL, M(Q_1, Q_3)	46.00 (39.00, 54.00)	46.00 (39.00, 54.00)	46.00 (39.00,54.00)	0.90	0.342
Gender, n (%)				**53.84**	**<0.001**
Male	4,510 (51.06)	3,618 (49.29)	892 (59.75)		
Female	4,323 (48.94)	3,722 (50.71)	601 (40.25)		
Race, n (%)				**175.66**	**<0.001**
Mexican American	1944 (22.00)	1,697 (23.12)	247 (16.54)		
Hispanic	760 (8.61)	692 (9.43)	68 (4.55)		
Non-Hispanic White	3,888 (44.02)	3,018 (41.12)	870 (58.27)		
Non-Hispanic Black	1787 (20.23)	1,511 (20.59)	276 (18.49)		
Others	454 (5.14)	422 (5.75)	32 (2.14)		
Education level, n (%)				**101.56**	**<0.001**
≤ High school	4,836 (54.75)	3,841 (52.33)	995 (66.64)		
College	2,520 (28.53)	2,200 (29.97)	320 (21.44)		
> College	1,477 (16.72)	1,299 (17.70)	178 (11.92)		
Marital status, n (%)				**11.96**	**<0.001**
Not married	3,291 (37.26)	2,675 (36.44)	616 (41.26)		
Married	5,542 (62.74)	4,665 (63.56)	877 (58.74)		
PIR, n (%)				**49.60**	**<0.001**
< 1.3	2,909 (32.93)	2,388 (32.53)	537 (35.96)		
1.3–3.5	3,470 (39.29)	2,814 (38.34)	650 (43.54)		
> 3.5	2,454 (27.78)	2,138 (29.13)	306 (20.50)		
Smoking, n (%)				**198.61**	**<0.001**
No	3,401 (38.51)	2,114 (28.80)	406 (27.20)		
Yes	5,432 (61.49)	5,226 (71.20)	1,087 (72.80)		
Alcohol use, n (%)				**13.37**	**<0.001**
No	8,140 (92.15)	6,729 (76.18)	1,411 (15.97)		
Yes	693 (7.85)	611 (15.97)	82 (0.93)		
Hypertension, n (%)				**92.87**	**<0.001**
No	7,923 (89.70)	6,687 (91.10)	1,236 (82.79)		
Yes	910 (10.30)	653 (8.90)	257 (17.21)		
DM, n (%)				**23.07**	**<0.001**
No	6,905 (78.17)	5,668 (77.22)	1,237 (82.85)		
Yes	1928 (21.83)	1,672 (22.78)	256 (17.15)		

M, median; Q, quartile; DII, Dietary Inflammatory Index; TC, total cholesterol; TR, triglyceride; HDL, high-density lipoprotein; GLU, glucose; ALT, glutamic-pyruvic transaminase; FLI, fatty liver index; BMI, body mass index; PIR, poverty income ratio; DM, diabetes mellitus.

Non-normally distributed variables are displayed as median with 1st and 3rd quartile (M, Q1, and Q3). Categorical variables are displayed as numbers with weighted percentages (n, %). Bold values indicate statistical significance ($p < 0.05$).

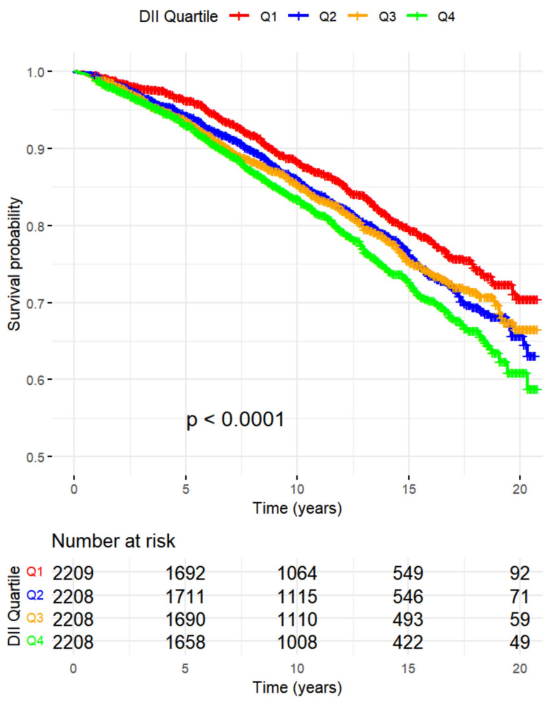

FIGURE 2
Kaplan–Meier Survival Curves for All-Cause Mortality by DII Quadrtiles with NHANES Sample Weight.

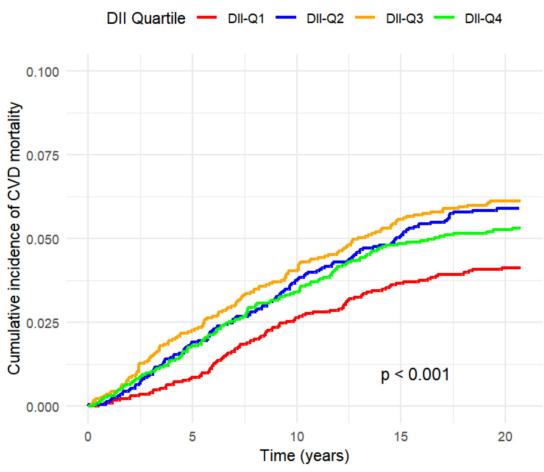

FIGURE 3
Cumulative Incidence Function for CVD Mortality by DII Quartiles Considering Competing Risks.

adults subjected to more lenient inclusion criteria for the diagnosis of SLD (FLI ≥ 30).

4 Discussion

In the present investigation, we ascertained that a heightened DII is markedly linked to increased risks of all-cause mortality in adults diagnosed with MASLD. More precisely, MASLD subjects within the highest quartile of the DII were associated with an aHR of 1.28 for all-cause mortality (95% CI: 1.10–1.49, $p = 0.002$), denoting a substantial elevation in mortality risk compared to those with lower DII scores. Although a trend toward increased cardiovascular mortality was noted (aHR = 1.23, 95% CI: 0.92–1.63, $p = 0.156$), statistical significance was not achieved. When the DII was evaluated alongside additional clinical variables, the model demonstrated a C-index of 0.837 for all-cause mortality and 0.860 for cardiovascular mortality, underscoring the potential utility of integrating dietary and clinical factors in understanding mortality risk in MASLD populations. Further analysis using multivariate-adjusted RCS revealed a positive linear relationship between DII scores and mortality risks, suggesting a direct association. While the test for nonlinearity was not significant for cardiovascular mortality (P for nonlinearity = 0.953), it was marginally significant for all-cause mortality (P for nonlinearity = 0.057). These results emphasize the importance of considering dietary inflammation in the context of MASLD, paving the way for personalized nutritional interventions aimed at reducing disease burden and improving patient outcomes.

Prior research has demonstrated that a substantial association exists between higher DII levels and elevated inflammatory factors, particularly CRP, IL-6, and so on (26, 27). Therefore, the DII calculated in this study likely reflects the impact of the participants' diets on their inflammatory status, making it a valuable metric for further analysis. Recent studies have highlighted that elevated DII scores are significantly correlated with a heightened risk of cardiovascular disease, diabetes, various common cancers, and an increase in all-cause mortality (16, 28). A study with 15,291 diabetic individuals in the U.S. found that after 45 months, those on a pro-inflammatory diet (DII > 0) had a 71% higher risk of all-cause mortality compared to those on an anti-inflammatory diet (DII < 0) (HR, 1.71; 95% CI, 1.13–2.58; $p = 0.011$) (29). A similar pattern was observed in hyperlipidemic patients, reinforcing the connection between dietary inflammation and increased mortality risk (30). A meta-analysis encompassing 15 studies across four continents revealed a consistent linear positive dose–response relationship between DII scores and all-cause mortality (31). The findings from the aforementioned studies on the DII in various populations mirror the results of this study. Another consideration is the impact of dietary fluctuations on metabolic health. Studies suggest that dietary inconsistency, especially alternating between inflammatory and anti-inflammatory diets, may exacerbate metabolic dysfunction, oxidative stress, and inflammation, potentially posing greater risks than a consistently high DII diet (32). Sudden dietary shifts have been linked to impaired insulin sensitivity and increased cytokine activity, both of which are central to MASLD pathogenesis (33, 34). Future research should investigate the long-term effects of dietary fluctuations.

There is still uncertainty as to why MASLD patients' DII scores are associated with death risk. Potential mechanisms include the following: High DII diets exacerbate liver inflammation, speeding up the progression to fibrosis and cirrhosis by increasing systemic inflammation and insulin resistance common in MASLD. Additionally, such diets contribute to oxidative stress by increasing reactive oxygen species that damage hepatocytes (35, 36). The immune response is also implicated, pro-inflammatory diets activate the immune system, increasing cytokine production such as TNF-α and IL-6, which further aggravate liver damage and fibrosi. Moreover, the gut-liver axis

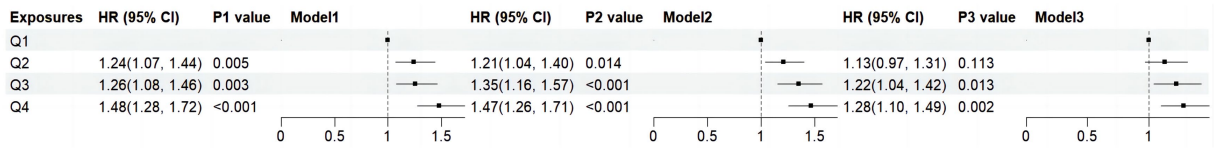

FIGURE 4
Forest plots show the association between DII with the all-cause mortality among adults with MASLD. DII, Dietary Inflammatory Index; HR: hazard ratio; CI: confidence interval; MASLD, metabolic dysfunction-associated steatotic liver disease; Q, quartile. Model 1: unadjusted; Model 2: adjusted for age, gender, race; Model 3: adjusted for age, gender, race, marital status, educational level, poverty income ratio, plasma glucose concentration, smoking status, alcohol use, hypertension, BMI, HDL, ALT, TR and TC.

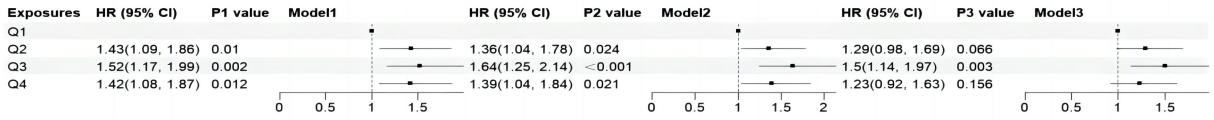

FIGURE 5
Forest plots show the association between DII with the cardiovascular mortality among adults with MASLD. DII, Dietary Inflammatory Index; HR, hazard ratio; CI, confidence interval; MASLD, metabolic dysfunction-associated steatotic liver disease; Q, quartile. Model 1: unadjusted; Model 2: adjusted for age, gender, race; Model 3: adjusted for age, gender, race, marital status, educational level, poverty income ratio, plasma glucose concentration, smoking status, alcohol use, hypertension, BMI, HDL, ALT, TR and TC.

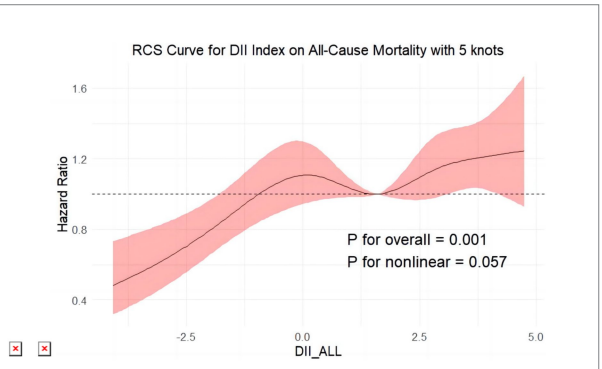

FIGURE 6
Restricted cubic splines reflect the dose-effect relationships between DII the all-cause mortality among adults with MASLD. DII, Dietary Inflammatory Index. The y-axis represents hazard ratio (HR), with the shaded area indicating the 95% confidence interval.

plays a crucial role in MASLD progression, as dietary components shape gut microbiota composition. Pro-inflammatory diets (high in saturated fats, refined carbs, low fiber) disrupt microbiota balance, increasing intestinal permeability and lipopolysaccharide (LPS) translocation, which triggers hepatic inflammation via Kupffer cell activation (37). Conversely, fiber, polyphenols, and probiotics support gut homeostasis, increasing short-chain fatty acids (SCFAs) with anti-inflammatory and hepatoprotective effects. Anti-inflammatory and antioxidant-rich diets improve gut microbiota composition, reducing inflammation and oxidative stress (38). Targeting gut dysbiosis through dietary modifications may help reduce MASLD burden and improve outcomes. These interconnected mechanisms underscore the significant impact of dietary choices on the health outcomes of individuals with MASLD.

In the subgroup analysis, a stronger association was observed between DII and all-cause mortality among individuals with higher educational attainment. While higher education is generally linked to greater health literacy and healthier dietary habits, research suggests that factors such as social stress, work burden, and mental health challenges among highly educated individuals may contribute to chronic inflammation, amplifying the impact of a pro-inflammatory diet on mortality risk (39). This finding may reflect broader socioeconomic influences on dietary inflammation rather than being solely attributable to nutritional literacy. Similarly, the stronger association of DII scores with cardiovascular mortality in younger adults may be attributed to their higher consumption of processed, pro-inflammatory foods, which are rich in refined sugars, saturated fats, and additives. Studies have shown that such diets elevate inflammatory markers like C-reactive protein (CRP) and interleukin-6 (IL-6), contributing to vascular damage, arterial stiffness, and worsening liver inflammation and fibrosis, thereby accelerating MASLD progression (40). Prolonged exposure to these foods also induces oxidative stress, further damaging endothelial cells and promoting plaque formation. In contrast, older adults are more likely to adopt dietary adjustments as part of chronic disease management, including anti-inflammatory eating patterns like the Mediterranean or DASH diets, which reduce inflammation and oxidative stress (41).

The current study has several strengths. First, it is the first to investigate the prognostic implications of the DII on mortality outcomes among U.S. adults with MASLD. Second, the analysis is based on a robust dataset from NHANES, which provides a large, nationally representative sample and extensive follow-up data over two decades. Last, the study's methodological rigor is evident through the comprehensive use of sensitivity analyses, which verify the stability and robustness of the results across various conditions and adjustments.

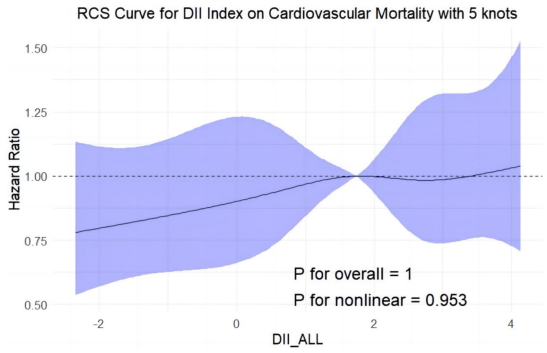

FIGURE 7
Restricted cubic splines reflect the dose-effect relationships between DII the cardiovascular mortality among adults with MASLD. DII, Dietary Inflammatory Index. The y-axis represents hazard ratio (HR), with the shaded area indicating the 95% confidence interval.

Some limitations need to be addressed in future studies. Potential selection bias in this study arises from the exclusion of participants with missing data on MASLD status and DII. However, the use of multiple imputation for covariates with minor missingness helps mitigate the impact of missing data. Additionally, Potential unmeasured confounders for this study include medication use and pre-existing CVD or other comorbidities. However, they may be correlated with observed confounders, which could reduce unmeasured confounding. Survival bias is also possible, as participants with higher DII who survived until the NHANES survey may represent a healthier subset, potentially underestimating the true association with mortality. Measurement errors are possible but likely minimal due to NHANES' robust design and methods. Future studies incorporating clinically verified data, additional confounders, left truncation adjustments, or longitudinal follow-up from MASLD diagnosis could enhance the validity of these associations.

In conclusion, our findings suggest that elevated levels of DII are associated with increased risks of all-cause and cardiovascular mortality in U.S. adults with MASLD. Furthermore, DII demonstrates an enhanced associative effect on mortality outcomes in adults with MASLD, making it a simple and easily calculable clinical biomaker for managing MASLD.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The studies involving humans were approved by the ethics protocol was approved by the Research Ethics Review Board of National Center for Health Statistics [https://www.cdc.gov/nchs/nhanes/irba98.htm (accessed on 17 June 2024)], and informed consent was signed by all recruited participants. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LT: Conceptualization, Validation, Writing – review & editing. TW: Conceptualization, Data curation, Writing – original draft. XD: Data curation, Writing – review & editing. QL: Validation, Visualization, Writing – original draft. YH: Validation, Visualization, Writing – review & editing. TZ: Supervision, Writing – review & editing. YY: Data curation, Methodology, Writing – review & editing.

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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.

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Supplementary material

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References

1. Song R, Li Z, Zhang Y, Tan J, Chen Z. Comparison of NAFLD, MAFLD, MASLD and pure-MASLD characteristics and mortality outcomes in United States adults. *Liver Int*. (2023) 42:1144–57. doi: 10.1111/liv.15240

2. Younossi Z, Koenig A, Abdelatif D, Fazel Y, Henry L, Wymer M. The global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. (2016) 64:73–84. doi: 10.1002/hep.28431

3. Tesfai K, Pace J, El-Newihi N, Martinez ME, Tincopa M, Loomba R. Disparities for Hispanic adults with metabolic dysfunction-associated Steatotic liver disease in the US: a systematic review and Meta-analysis. *Clin Gastroenterol Hepatol*. (2024) (in press)) 23:236–49. doi: 10.1016/j.cgh.2024.06.038

4. Powell EE, Wong VW-S, Rinella M. Non-alcoholic fatty liver disease. *Lancet*. (2021) 397:2212–24. doi: 10.1016/S0140-6736(20)32511-3

5. Chan KE, Ong EYH, Chung CH, Ong CEY, Koh B, Tan DJH, et al. Longitudinal outcomes associated with metabolic dysfunction-associated Steatotic liver disease: a meta-analysis of 129 studies. *Clin Gastroenterol Hepatol*. (2024) 22:488–498.e14. doi: 10.1016/j.cgh.2023.09.018

6. Targher G, Byrne CD, Tilg H. MASLD: a systemic metabolic disorder with cardiovascular and malignant complications. *Gut*. (2024) 73:691–702. doi: 10.1136/gutjnl-2023-330595

7. European Association for the Study of the Liver. EASL-EASD-EASO clinical practice guidelines on the Management of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). *J Hepatol*. (2024) 81:492–542. doi: 10.1016/j.jhep.2024.04.031

8. Petermann-Rocha F, Wirth MD, Boonpor J, Parra-Soto S, Zhou Z, Mathers JC, et al. Associations between an inflammatory diet index and severe non-alcoholic fatty liver disease: a prospective study of 171,544 UK biobank participants. *BMC Med*. (2023) 21:123. doi: 10.1186/s12916-023-02793-y

9. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr*. (2014) 17:1689–96. doi: 10.1017/S1368980013002115

10. Sun S-N, Ni S-H, Li Y, Liu X, Deng J-P, Ouyang X-L, et al. Association between dietary inflammatory index with all-cause and cardiovascular disease mortality among older US adults: a longitudinal cohort study among a nationally representative sample. *Arch Gerontol Geriatr*. (2024) 118:105279. doi: 10.1016/j.archger.2023.105279

11. Choi MK, Park Y-MM, Shivappa N, Hong O-K, Han K, Steck SE, et al. Inflammatory potential of diet and risk of mortality in Normal-weight adults with central obesity. *Clin Nutr*. (2023) 42:208–15. doi: 10.1016/j.clnu.2022.11.019

12. Lee H-H, Lee HA, Kim E-J, Kim HY, Kim HC, Ahn SH, et al. Metabolic dysfunction-associated Steatotic liver disease and risk of cardiovascular disease. *Gut*. (2024) 73:533–40. doi: 10.1136/gutjnl-2022-325805

13. Younossi ZM, Paik JM, Stepanova M, Ong J, Alqahtani S, Henry L. Clinical profiles and mortality rates are similar for metabolic dysfunction-associated Steatotic liver disease and non-alcoholic fatty liver disease. *J Hepatol*. (2024) 80:694–701. doi: 10.1016/j.jhep.2024.01.014

14. Johnson CL, Dohrmann SM, Burt VL, Mohadjer LK. National Health and nutrition examination survey: sample design, 2011-2014. *Vital Health Stat*. (2014) 2162:1–33.

15. Hébert JR, Shivappa N, Wirth MD, Hussey JR, Hurley TG. Perspective: the dietary inflammatory index (DII)—lessons learned, improvements made, and future directions. *Adv Nutr*. (2019) 10:185–95. doi: 10.1093/advances/nmy071

16. Hariharan R, Odjidja EN, Scott D, Shivappa N, Hébert JR, Hodge A, et al. The dietary inflammatory index, obesity, type 2 diabetes, and cardiovascular risk factors and diseases. *Obes Rev*. (2022) 23:e13349. doi: 10.1111/obr.13349

17. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The fatty liver index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol*. (2006) 6:33. doi: 10.1186/1471-230X-6-33

18. Younossi ZM, Stepanova M, Younossi Y, Golabi P, Mishra A, Rafiq N, et al. Epidemiology of chronic liver diseases in the USA in the past three decades. *Gut*. (2020) 69:564–8. doi: 10.1136/gutjnl-2019-318813

19. Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, et al. The US Department of Agriculture Automated Multiple-Pass Method Reduces Bias in the collection of energy intakes. *Am J Clin Nutr*. (2008) 88:324–32. doi: 10.1093/ajcn/88.2.324

20. Zhang J, Jia J, Lai R, Wang X, Chen X, Tian W, et al. Association between dietary inflammatory index and atherosclerosis cardiovascular disease in U.S. adults. *Front Nutr*. (2023) 9:1044329. doi: 10.3389/fnut.2022.1044329

21. Cao C, Cade WT, Li S, McMillan J, Friedenreich C, Yang L. Association of Balance Function with all-Cause and Cause-Specific Mortality among US adults. *JAMA Otolaryngol Head Neck Surg*. (2021) 147:460–8. doi: 10.1001/jamaoto.2021.0057

22. Ramanathan R, Patwa SA, Ali AH, Ibdah JA. Thyroid hormone and mitochondrial dysfunction: therapeutic implications for metabolic dysfunction-associated Steatotic liver disease (MASLD). *Cells*. (2023) 12:2806. doi: 10.3390/cells12242806

23. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet*. (2005) 365:1415–28. doi: 10.1016/S0140-6736(05)66378-7

24. Saklayen MG. The global epidemic of the metabolic syndrome. *Curr Hypertens Rep*. (2018) 20:12. doi: 10.1007/s11906-018-0812-z

25. Harrell FE, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. *JAMA*. (1982) 247:2543–6.

26. Shivappa N, Hébert JR, Marcos A, Diaz L-E, Gomez S, Nova E, et al. Association between dietary inflammatory index and inflammatory markers in the HELENA study. *Mol Nutr Food Res*. (2017) 61:1600707. doi: 10.1002/mnfr.201600707

27. Tabung FK, Smith-Warner SA, Chavarro JE, Wu K, Fuchs CS, Hu FB, et al. Development and validation of an empirical dietary inflammatory index. *J Nutr*. (2016) 146:1560–70. doi: 10.3945/jn.115.228718

28. Shivappa N, Godos J, Hébert JR, Wirth MD, Piuri G, Speciani AF, et al. Dietary inflammatory index and cardiovascular risk and mortality—a Meta-analysis. *Nutrients*. (2018) 10:200. doi: 10.3390/nu10020200

29. Tan J, Liu N, Sun P, Tang Y, Qin W. A Proinflammatory diet may increase mortality risk in patients with diabetes mellitus. *Nutrients*. (2022) 14:2011. doi: 10.3390/nu14102011

30. Huang J, Zhang Y, Li J, Li H, Wei Y, Sun M. Association of Dietary Inflammatory Index with all-cause and cardiovascular disease mortality in hyperuricemia population: a cohort study from NHANES 2001 to 2010. *Medicine (Baltimore)*. (2023) 102:e36300. doi: 10.1097/MD.0000000000036300

31. Zhang J, Feng Y, Yang X, Li Y, Wu Y, Yuan L, et al. Dose-response Association of Dietary Inflammatory Potential with all-cause and cause-specific mortality. *Adv Nutr*. (2022) 13:1866–81. doi: 10.1093/advances/nmac054

32. Sepehrinia M, Khanmohammadi S, Rezaei N, Kuchay MS. Dietary inflammatory potential and metabolic (dysfunction)-associated steatotic liver disease and its complications: a comprehensive review. *Clin Nutr ESPEN*. (2025) 65:162–71. doi: 10.1016/j.clnesp.2024.11.032

33. Beyoğlu D, Popov YV, Idle JR. Metabolomic hallmarks of obesity and metabolic dysfunction-associated Steatotic liver disease. *Int J Mol Sci*. (2024) 25:12809. doi: 10.3390/ijms252312809

34. Papadopoulos G, Giannousi E, Velliou R-I, Nikolakopoulou P, Avdi AP, Chatzigeorgiou A. T cell-mediated adaptive immunity in the transition from metabolic dysfunction-associated steatohepatitis to hepatocellular carcinoma. *Front Cell Dev Biol*. (2024) 12:1343806. doi: 10.3389/fcell.2024.1343806

35. Montserrat-Mesquida M, Quetglas-Llabrés M, Abbate M, Montemayor S, Mascaró CM, Casares M. Oxidative stress and pro-inflammatory status in patients with non-alcoholic fatty liver disease. *Antioxidants (Basel)*. (2020) 9:759. doi: 10.3390/antiox9080759

36. Adiels M, Taskinen M-R, Borén J. Fatty liver, insulin resistance, and dyslipidemia. *Curr Diab Rep*. (2008) 8:60–4. doi: 10.1007/s11892-008-0011-4

37. Scarpellini E, Scarcella M, Tack JF, Scarlata GGM, Zanetti M, Abenavoli L. Gut microbiota and metabolic dysfunction-associated Steatotic liver disease. *Antioxidants*. (2024) 13:1386. doi: 10.3390/antiox13111386

38. Hamamah S, Iatcu OC, Covasa M. Dietary influences on gut microbiota and their role in metabolic dysfunction-associated Steatotic liver disease (MASLD). *Nutrients*. (2024) 17:143. doi: 10.3390/nu17010143

39. Martinez-Lacoba R, Pardo-Garcia I, Amo-Saus E, Escribano-Sotos F. Socioeconomic, demographic and lifestyle-related factors associated with unhealthy diet: a cross-sectional study of university students. *BMC Public Health*. (2018) 18:1241. doi: 10.1186/s12889-018-6149-3

40. Boccatonda A, D'Ardes D, Moronti V, Santilli J, Cipollone A, Lessiani G, et al. From MASLD to PAD: looking for cardiovascular disease starting from metabolic status. *Medicina*. (2024) 60:1781. doi: 10.3390/medicina60111781

41. Sullivan VK, Appel LJ, Seegmiller JC, McClure ST, Rebholz CM. A low-sodium DASH dietary pattern affects serum markers of inflammation and mineral metabolism in adults with elevated blood pressure. *J Nutr*. (2021) 151:3067–74. doi: 10.1093/jn/nxab236

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Thiamine, gastrointestinal beriberi and acetylcholine signaling

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Research has highlighted numerous detrimental consequences of thiamine deficiency on digestive function. These range from impaired gastric and intestinal motility to aberrant changes in pancreatic exocrine function, gastric acidity and disturbances in gut barrier integrity and inflammation. Thiamine and its pharmacological forms, as a primary or adjunctive therapy, have been shown to improve symptoms such as nausea, constipation, dysphagia and intestinal dysmotility, in both humans and animals. This review aims to explore molecular mechanisms underlying the therapeutic action of thiamine in gastrointestinal dysfunction. Our analysis demonstrates that thiamine insufficiency restricted to the gastrointestinal system, i.e., lacking well-known symptoms of dry and wet beriberi, may arise through (i) a disbalance between the nutrient influx and efflux in the gastrointestinal system due to increased demands of thiamine by the organism; (ii) direct exposure of the gastrointestinal system to oral drugs and gut microbiome, targeting thiamine-dependent metabolism in the gastrointestinal system in the first line; (iii) the involvement of thiamine in acetylcholine (ACh) signaling and cholinergic activity in the enteric nervous system and non-neuronal cells of the gut and pancreas, employing both the coenzyme and non-coenzyme actions of thiamine. The coenzyme action relies on the requirement of the thiamine coenzyme form – thiamine diphosphate – for the production of energy and acetylcholine (ACh). The non-coenzyme action involves participation of thiamine and/or derivatives, including thiamine triphosphate, in the regulation of ACh synaptic function, consistent with the early data on thiamine as a co-mediator of ACh in neuromuscular synapses, and in allosteric action on metabolic enzymes. By examining the available evidence with a focus on the gastrointestinal system, we deepen the understanding of thiamine's contribution to overall gastrointestinal health, highlighting important implications of thiamine-dependent mechanisms in functional gastrointestinal disorders.

KEYWORDS

thiamine, gastrointestinal beriberi, acetylcholine, intestinal ThDP-dependent enzymes, intestinal transport of thiamine, intestinal metabolism of thiamine, functional gastrointestinal disorder

1 Introduction

Gastrointestinal diseases are highly prevalent worldwide and account for a significant portion of global disease burden (1) and healthcare costs (2). Globally, a substantial proportion of these diseases are linked to infectious and transmissible causes, whereas in developed countries the vast majority are non-communicable and are rising in prevalence (3). The most common diagnoses in gastroenterology are a group of disorders known as Functional Gastrointestinal Disorders (FGIDs). With growing recognition in recent years of the nervous system's involvement in such disorders, they are now broadly defined as disorders of gut-brain

interaction (4). According to the Rome IV criteria, such disorders can involve any combination of motility disturbance, altered mucosal and immune function, dysbiosis of gut microbiota, visceral hypersensitivity, and altered central nervous system processing (5). The estimated prevalence is 10–40% (6), although the actual prevalence is unknown (7). A recent global study shows that 49% of females and 37% of males meet the diagnostic criteria for at least one FGID (7). The five most prevalent FGIDs are irritable bowel syndrome, functional dyspepsia, functional constipation, functional diarrhea, and functional bloating/abdominal distention. However, the FGID classification also includes a diverse range of other disorders, many of which exhibit overlapping clinical features. These include epigastric pain syndrome, functional dysphagia, heartburn, reflux hypersensitivity, belching disorder, bloating/distention, centrally mediated abdominal pain, fecal incontinence, and others.

The molecular and cellular mechanisms underpinning the pathophysiology of FGIDs are complex and have been reviewed elsewhere (5). Prominent features include epithelial barrier dysfunction, delayed or accelerated gastrointestinal transit due to abnormal function of smooth muscles, dysfunctional enteric nervous system and/or immunity, gut microbial dysbiosis, bile acid malabsorption, and alterations in the gut-brain axis (8–12). Therapeutic interventions include pharmacologic agents, dietary and lifestyle changes, probiotics, antibiotics, fecal microbial transplant, and stress management. Pharmacological treatments range from prokinetics and antispasmodics to centrally acting neuromodulators, albeit with varied success (13). As research continues to unearth a multitude of pathophysiological mechanisms involved in FGIDs, it is important to reveal specific primary causes. Similar symptoms may originate from different impairments, but therapies mitigating the primary impairment, rather than the convergent symptoms, are the most efficient ones (14).

Publications on FGIDs, which are ameliorated by administration of thiamine (vitamin B1) (15–23), suggest that thiamine insufficiency in the gastrointestinal system and/or its innervation may contribute causally to the development of these disorders, also in the absence of systemic thiamine deficiency. Our review aims at understanding the molecular mechanisms underlying this potential of thiamine to counteract many pathological alterations commonly observed in FGIDs. In addition to thiamine's role as a precursor of an essential coenzyme in energy production from glucose oxidation (24), contribution to gastrointestinal function of the non-coenzyme action of thiamine (25, 26), presumably regulating not only the coenzyme-dependent pathway of acetylcholine (ACh) biosynthesis (27), but also ACh signaling at neuromuscular junctions (28) and in non-neuronal cells (29), is considered. Our focus on an intestine-expressed set of proteins which are well-known or suggested to be involved in transport, transformations and action of thiamine and its derivatives, underscores the significance of polar distribution of different thiamine transporters to organize the nutrient flux in enterocytes. We draw attention to various factors that may perturb enterocytic balance between the thiamine influx from the lumen and systemic delivery to other tissues, providing insights into the molecular mechanisms of local thiamine insufficiency in gastrointestinal system. Although this form of thiamine deficiency, which we call gastrointestinal beriberi, is initially limited to the gastrointestinal system, it mostly progresses to systemic thiamine deficiency, often triggered by stresses of different etiologies. That is why the distinction between specific gastrointestinal beriberi and the gastrointestinal manifestations of systemic beriberi resulting from gastrointestinal dysfunction remain unclear and are not well defined. However, in line with the existing classification of beriberi affecting nerves (dry beriberi) and cardiovascular system (wet beriberi), it is plausible to use the term gastrointestinal beriberi in an analogous, tissue-directed, way.

Our analysis of the gastrointestinal beriberi mechanisms employs, in particular, case studies of patients after bariatric surgery – a well-defined group, often developing systemic thiamine deficiency. Using available data on the time-dependent transcriptomic changes in enterocytes of these patients, we demonstrate long-term perturbations in their thiamine metabolism, which may be of diagnostic significance. Existence of genetic variants of thiamine-dependent proteins, insufficient molecular identification of these proteins, as well as the age- and sex-dependent differences in thiamine metabolism (30), may contribute to the observed variety of clinical manifestations of thiamine deficiency (31, 32). Clearly, this may also affect the outcomes of systemic and meta-analyses of thiamine's effects in different populations and patient groups (32). As a result, our review calls upon more attention to personalized approaches in characterization of individual thiamine homeostasis, based on the knowledge of the thiamine-dependent proteins and their tissue-specific features and functions.

2 Thiamine (vitamin B1) and its metabolic significance

Thiamine is an essential water-soluble vitamin, naturally occurring in a wide variety of foods. Rich dietary sources include different types of meat, legumes and whole grains. In the human body, the major intracellular derivative of thiamine is its diphosphorylated form, thiamine diphosphate (ThDP). This essential coenzyme of ThDP-dependent enzymes is absolutely necessary not only for oxidative glucose metabolism, but also for coupling metabolic pathways of carbohydrates and amino acids, as well as for controlling *de novo* synthesis of major neurotransmitters, ACh and glutamate, from glucose. The control is determined by ThDP-dependent biosynthesis and/or distribution of the neurotransmitter precursors acetyl-CoA and 2-oxoglutarate, formed upon the oxidation of the glycolytic product pyruvate in the TCA cycle (Figure 1).

The mammalian ThDP-dependent enzymes (24) include cytosolic transketolase (TKT) participating in the pentose phosphate pathway; the mitochondrial multienzyme complexes of 2-oxo acids, i.e., of pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase (OGDH), 2-oxoadipate dehydrogenase (OADH) and branched chain 2-oxo acid dehydrogenase (BCODH), linked to amino acids through their transamination to the 2-oxo acids; and peroxisomal 2-hydroxy acyl-CoA lyase (HACL) participating in the α-oxidation of long and very long chain 3-methyl or 2-hydroxy even-chain fatty acids (33).

TKT, as part of the cytosolic pentose phosphate pathway, along with the mitochondrial multienzyme complexes of pyruvate (PDHC) and 2-oxoglutarate dehydrogenases (OGDHC), are essential for energy production from glucose oxidation. The oxidation-generated reducing equivalents accumulate as cytosolic NADPH produced by the pentose phosphate pathway, and mitochondrial NADH, produced by 2-oxo acid dehydrogenases. In addition, the function of TKT in the pentose phosphate pathway supports the generation of phosphoribose

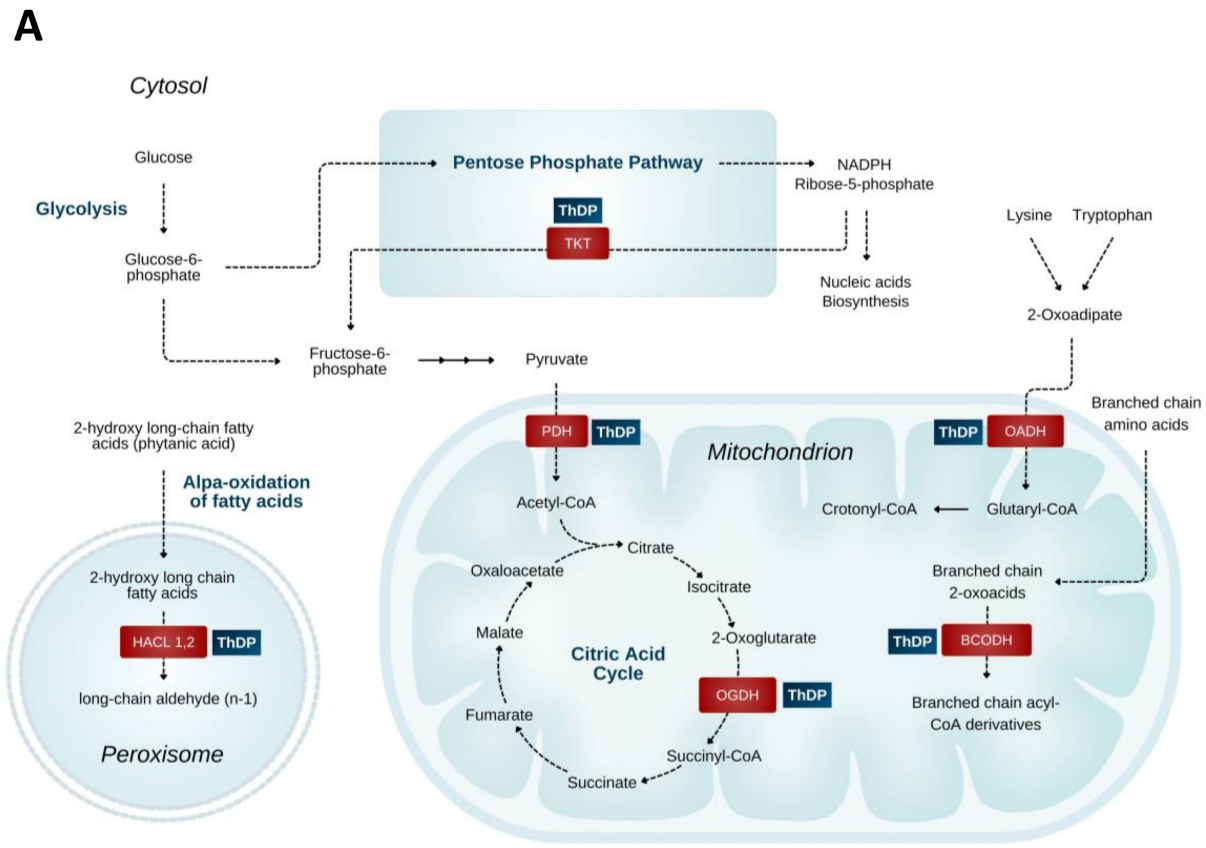

FIGURE 1
Coenzyme role of ThDP in metabolism of the gut cells. **(A)** Metabolic pathways involving enzymes that use the coenzyme derivative of thiamine, thiamine diphosphate (ThDP). **(B)** The relative abundance of mRNAs for the ThDP-dependent enzymes in different cells of intestine (colonocytes, enterocytes of ileum, enterocytes of jejunum) and in enterocyte organoids. The transcript signals for the genes of interest are normalized to the sum of the average mRNA signals of GAPDH, ACTB and TUBA1A as described earlier (149). The signals of these mRNAs for the three transcripts used for the normalization, are comparable, producing similar normalization ratios of the transcripts of interest across the different GEO datasets used. Transcriptomics data is taken from the GEO database. Identificators of the assessed experiments are: colonocytes experiments GSE13367 [Platform GPL570, (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array, 10 datasets: GSM337520, GSM337526, GSM337529, GSM337530, GSM337532, GSM337533, GSM337537, GSM337539, GSM337540, GSM337544] and GSE30292 [Platform GPL570, (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array, 3 datasets: GSM750882, GSM750883, GSM750884]; jejunum enterocytes experiments GSE214758 [Platform GPL20795, HiSeq X Ten (*Homo sapiens*), 9 datasets: GSM6615629, GSM6615631, GSM6615633, GSM6615637, GSM6615641, GSM6615643, GSM6615645,

(Continued)

FIGURE 1 (Continued)

GSM6615647, GSM6615649], GSE113819 {Platform GPL17586 (HTA-2_0) Affymetrix Human Transcriptome Array 2.0 [transcript (gene) version], 5 datasets: GSM3120595, GSM3120597, GSM3120599, GSM3120601, GSM3120603} and GSE30292 [Platform GPL570, (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array, 2 datasets: GSM750891, GSM750892]; ileum enterocytes experiment GSE30292 [Platform GPL570, (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array, 3 datasets: SM750888, GSM750889, GSM750890]; enterocyte organoid experiment GSE242765 [Platform GPL18573, Illumina NextSeq 500 (*Homo sapiens*), 1 dataset GSM7770156]. Normalized mRNA levels from all the datasets for the same cell type are averaged, and the data are shown as mean ± SEM.

for nucleic acid synthesis. PDHC links cytosolic glycolysis to the mitochondrial TCA cycle by oxidizing pyruvate, the end product of glycolysis. The PDHC-catalyzed reaction generates not only NADH, which is oxidized in the mitochondrial respiratory chain, but also acetyl-CoA, which feeds into the TCA cycle and is a precursor of acetylcholine (ACh) in cholinergic cells. OGDHC catalyzes the rate-limiting step of the TCA cycle. The substrate of the OGDHC reaction, 2-oxoglutarate, is a precursor for glutamate biosynthesis from glucose. The OGDHC reaction product succinyl-CoA provides for the only mitochondrial phosphorylation reaction of ADP to ATP, at the substrate-level, i.e., phosphorylation beyond mitochondrial respiratory chain.

In addition to the ubiquitous ThDP-dependent enzymes, enterocytes express a number of the ThDP-dependent isoenzymes, i.e., the enzymes with similar catalytic functions, encoded by separate genes. These include transketolase-like 1 and 2 proteins, TKTL1 and TKTL2, as well as the ubiquitous (PDHA1) and testis-specific (PDHA2) isoenzymes of the α-subunit of PDH (PDHA) (Figure 1B). Deciphering physiological roles of isoenzymes is often challenging. Inactivation of TKTL1 aggravates colitis in a murine knockout model (34, 35). Isoenzymes of HACL1 and HACL2 are shown to provide α-oxidation of 3-methyl and 2-hydroxy long chain fatty acids, respectively, with HACL2 playing an important role in ceramide formation in the stomach (33). Isoenzymes of OGDH are the brain-specific OGDH-like protein (OGDHL) and OADH, encoded by OGDHL and DHTKD1 genes, correspondingly (Figure 1). OGDH and OGDHL differ in their regulatory properties, including 2-oxoglutarate saturation (36). OGDH(L) and 2-oxoadipate dehydrogenase (OADH) have different substrate specificity, preferring 2-oxoglutarate and 2-oxoadipate, correspondingly (37, 38). The OGDH isoenzymes encoded by OGDHL and DHTKD1 genes, link eosinophilic esophagitis to mitochondrial dysfunction (39).

Functional significance of the ThDP-dependent enzyme isoforms, which are the enzyme variants arising from alternative splicing of transcripts from a single gene, or through post-translational modifications, is studied even less than that of isoenzymes. An exception is the established regulatory significance of the OGDH splice variants lacking Ca^{2+}-dependent regulation. Unlike skeletal muscle and heart, which predominantly express Ca^{2+}-sensitive isoforms, other tissues, in particular pancreatic islets, have significant expression of Ca^{2+}-independent OGDH isoforms, which are thought to be involved in the distribution of 2-oxoglutarate flux to oxidation in the TCA cycle and glutamate biosynthesis (40).

As mentioned above, the coenzyme role of thiamine is important for metabolism of α-amino acids, as they are transaminated to 2-oxo acids. In addition to PDHC and OGDH(L)C, this action of thiamine is mediated by two other ThDP-dependent multienzyme complexes, i.e., those of branched chain 2-oxo acid dehydrogenase (BCODH) and OADH, functioning in the pathways of degradation of branched-chain α-amino acids, and lysine and tryptophan, respectively (Figure 1). The corresponding acyl-CoAs generated by these complexes undergo additional transformations before entering the TCA cycle in the form of acetyl- or succinyl-CoA. As a result, catalytic function of the multienzyme complexes of 2-oxo acid dehydrogenases may provide energy from oxidation of both carbohydrates and amino acids. Thiamine-dependent metabolic regulation is important for optimizing the energy source: by increasing efficiency of glucose oxidation, thiamine prevents excessive degradation of amino acids (41).

In recent years, the role of different acyl-CoAs in the posttranslational acylation of protein lysine residues has acquired increasing attention (42). Histone acylations are considered to be especially important for intestinal epithelium adaptations to environmental signals (43). Remarkably, nuclear localization of the 2-oxo acid dehydrogenase complexes has recently been discovered, in addition to their traditional mitochondrial localization (44–47). This finding is in good accordance with independent research that acylations of both metabolic proteins and histones depend on the function of 2-oxo acid dehydrogenase complexes (48, 49). The changes in histone acylation, mediated by ThDP-dependent 2-oxo acid dehydrogenase complexes, provide a mechanism for thiamine-dependent transcriptional regulation.

Positioning of ThDP-dependent 2-oxo acid dehydrogenase complexes at the intersections of major pathways of central metabolism and transcriptional regulation endows these systems with a functional role of "signaling hubs," regulating multiple cellular processes, such as intracellular redox status, growth, protein signaling, and calcium homeostasis (50–52).

Expression of the considered ThDP-dependent enzymes and isoenzymes in enterocytes (Figure 1B) underscores both the common and isoenzyme-specific dependence of gastrointestinal metabolism on thiamine.

3 Gastrointestinal beriberi

As animals do not synthesize thiamine, their thiamine need is satisfied by dietary intake and biosynthesis by gut microbiota. Thus, gastrointestinal dysfunction impairing the absorption of nutrients inevitably results in insufficient organismal thiamine levels, i.e., thiamine deficiency (TD). Indeed, the most well-known TD state is Wernicke-Korsakoff syndrome, first described by Wernicke in a female whose digestive tract was severely damaged by sulfuric acid (53). On the other hand, gastrointestinal disturbances are critical early indicators of severe thiamine deficiency causing pediatric Wernicke Encephalopathy (54).

An estimated 2–3% of the body's thiamine may originate from microbial synthesis within the colon, although its precise nutritional significance remains to be fully understood. Studies on the gut microbiome have identified distinct genera of bacteria capable of synthesizing thiamine, including *Lactobacillus*, *Bifidobacterium*, *Escherichia coli*, *Enterococcus*, and *Clostridium* (55, 56). Furthermore, high expression of genes involved in thiamine biosynthesis and transport are prevalent in *Prevotella*, *Desulfovibrio* (57), and *Bacteroides* (58). On the other hand, the microbiome houses many genera of bacteria that fail to grow in the absence of thiamine (59), and depend on its external sources (60). The abundance of one such family, *Ruminococcaceae*, is positively correlated with thiamine intake in humans. Dietary thiamine restriction in rodents also decreases relative abundance of this family, which is accompanied by reduced fecal butyrate concentrations (61). ThDP is used as a cofactor for the microbial enzyme pyruvate-ferredoxin oxidoreductase, which catalyzes the conversion of pyruvate to acetyl-CoA in the pathway for butyrate synthesis (56). Hence, bacteria require thiamine for production of short-chain fatty acids, which play important anti-inflammatory and signaling roles in the gut. It is therefore not surprising that thiamine from dietary sources may alter the composition of microbes in the gut, and a deficit of thiamine may result in bacterial dysbiosis (58, 59).

Remarkably, in a trial using high-dose thiamine for fatigue related to inflammatory bowel disease (IBD), the relative abundances of *Faecalibacterium prausnitzii*, a member of the *Ruminococcacea* family, and *Roseburia hominis*, a member of the *Lachnospiraceae* family, inversely correlate with fatigue severity both pre- and post-treatment with thiamine (62).

When TD becomes severe and chronic, it is known as beriberi. Dry beriberi affects the central nervous system (CNS) and peripheral nerves, whereas wet beriberi involves the cardiovascular system. In 2004, Dr. Michael Donnino introduced the term "gastrointestinal beriberi" (63) to describe a distinct clinical entity caused by TD in the gastrointestinal system. It is broadly defined as a combination of several possible symptoms: anorexia (lack of appetite), nausea, unexplained vomiting, abdominal distention, constipation, reflux and epigastric pain, and occasionally intestinal paralysis. Since its original definition in the medical literature, multiple case reports have been published by independent researchers (Table 1). Although manifestations of neurological dysfunction often point to TD and may sometimes accompany gastrointestinal beriberi (17, 64), it has been also noted that chronic mild deficiency may present with gastrointestinal symptoms under entirely normal neurological exams in well-nourished, non-alcoholic patients (65–68). In one of the early reports cited by Donnino (69), severe manifestations of gastrointestinal beriberi are also preceded in time by much milder symptoms. These include abdominal distension, belching, and alternating constipation and diarrhea. Furthermore, early into the study, all participants exhibited achlorhydria or hypochlorhydria, delayed gastric emptying, and reduced intestinal motility. Similarly, early gastrointestinal symptoms - such as a full sensation in the epigastrium, gastric reflux, hypochlorhydria, impaired gastric and intestinal motility, and constipation - are reported by Shimanoza and Katsura in 30–50% of patients with TD (70). In another study, thiamine therapy leads to the disappearance of both gastrointestinal (dysphagia) and CNS/cardiovascular (dyspnea and blurred vision) symptoms. However, after thiamine is discontinued, dysphagia re-appears at the time when other symptoms are not observed (71). One case report describes the onset of dysphagia and gastroparesis in a patient with Crohn's disease 2 months before developing Wernicke encephalopathy, which resolved with high dose thiamine (72). A recent analysis (19) has found gastrointestinal symptoms in 46 out of 52 patients with diagnosed TD. In some cases, symptoms precede the classical neurological signs by up to several months, suggesting that digestive dysfunction may, in fact, be an early indicator of TD before progressing to other bodily

TABLE 1 Therapeutic action of thiamine administration in human and animal studies of FGIDs.

GI symptoms resolved after thiamine administration	Intervention	Sample size
Humans		
Nausea, vomiting, abdominal pain, anorexia in patients with Wernicke encephalopathy (19)	I.v. infusions of 300-600 mg of thiamine for 5–10 days, followed by oral thiamine at 100-300 mg	$n = 42$
Nausea, vomiting, anorexia preceding Wernicke encephalopathy (17)	Single i.v. injection of 1,000 mg of thiamine	Case report ($n = 1$)
Constipation in patients with thiamine deficiency after Roux-en-Y Gastric Bypass. Decreased blood levels of thiamine with increased folate levels are shown (18)	I.m. injections of 100–200 mg of thiamine monthly, some patients taking 200 mg oral thiamine daily	$n = 11$
Perturbed intestinal motility/gas release in post-hysterectomy patients (16)	I.m. injection of 100 mg thiamine for 2 days	$n = 80$
Dysphagia and gastroparesis preceding Wernicke encephalopathy in Crohn's disease (72)	I.v. injections of 200 mg thiamine three times, followed by I.m. injections of 100 mg/day for several months	Case report ($n = 1$)
Intestinal paralysis in post-hysterectomy patients (20)	I.m. injection of 100 mg thiamine for 3 days	$n = 60$
Animals		
Ruminal epithelial barrier dysfunction, oxidative stress and apoptosis, induced by high-concentrate diet in goats (22)	Thiamine 200 mg/kg dry feed for 12 weeks	$n = 8$
Perturbed colonic integrity and mucosal inflammation, induced by high-concentrate diet in goats (21)	Thiamine 200 mg/kg dry feed for 12 weeks	$n = 8$
Experimental constipation induced by atropine and papaverine in rats (23)	S.c. injection of 100 mg/kg TTFD	$n = 4$
Ulcerative colitis rat model (15)	I.p. injection of 20 mg/kg thiamine per day for 5 days	$n = 6$

systems. Dysphagia, or esophageal dysmotility, is common after bariatric surgery, known to be associated with TD, and is restored following thiamine administration (73, 74). A high prevalence of constipation and gastrointestinal paralysis is also associated with TD (70). Numerous reports demonstrate significant impairments in intestinal motility (small and large) in TD subjects, which normalizes with thiamine repletion (18, 75–78). Dietary thiamine intake is inversely associated with constipation (79). Abnormal gastric acidity is reported to be one of the most common and early signs of TD (70). In this study, four patterns of acid secretion are identified in response to thiamine administration: (i) Hypoacidity which gradually improves with thiamine repletion; (ii) Hypoacidity which shifts to high acidity in the recovery phase after thiamine administration, followed by eventual normalization; (iii) Hyperacidity in the early stage of TD, followed by normalization with thiamine therapy; (iv) Hyperacidity, followed by low acidity during the recovery phase after thiamine administration, which normalizes later. More recently, aberrant changes in gastric output have been demonstrated in TD. Achlorhydria is found in both human (80–83) and animal (84) studies. TD is also known to induce gastric ulceration (85). Epigastric pain, early satiety, and gastroesophageal reflux are common initial symptoms of TD and gastrointestinal beriberi (19, 70). Based on modern diagnostic criteria, this combination of symptoms could now be broadly classified as functional dyspepsia, which is often associated with gastric hypoacidity (86). Thiamine in conjunction with other therapies has been used to successfully treat functional dyspepsia (87).

Thus, a wide range of gastrointestinal disorders respond positively to thiamine administration. The therapeutic action of thiamine administration in human and animal studies are summarized in Table 1.

An important factor for digestion and overall gastrointestinal health is pancreatic function (88). Of note, the pancreas has a high thiamine content (89) which it maintains through constant uptake from circulation (90). TD is associated with a dramatic reduction of pancreatic thiamine content by up to 75% (89). Depletion of pancreatic thiamine can result in oxidative stress (91), which is considered to be a driving factor in development of pancreatitis. Furthermore, some researchers speculate that thiamine depletion in the pancreas may be a necessary antecedent for pancreatic inflammation (91). Indeed, chronic alcoholism and nicotine consumption are independent risk factors for pancreatitis, and both inhibit pancreatic thiamine uptake via downregulation of thiamine transporters (90). A Spanish review published in 1944 reports that hyposecretion of pancreatic enzymes is a common feature of TD (92), and administration of thiamine in children could increase the release of the pancreatic enzymes trypsin, amylase and lipase (93). Animal research also highlights substantial abnormalities in pancreatic function during TD, evidenced by a reduction in stored pancreatic protein and digestive enzyme content, contrasted by an abnormally large enzyme secretion (94). Acute pancreatitis and encephalopathy are recently reported as a consequence of TD and successfully treated with thiamine administration (95).

As a result, thiamine is known to positively influence perturbed gastrointestinal function. In many cases, this positive influence occurs when CNS symptoms of TD are absent, pointing to the gastrointestinal system as the primary site of TD. The findings suggest that TD restricted to the gastrointestinal tract may occur, while other TD-sensitive systems, such as the neurological and cardiovascular systems, do not display their specific symptoms. That said, chronic impairment of thiamine availability in the gastrointestinal system should also reduce resilience of other body systems. Obvious neurological and/or cardiovascular symptoms may represent a culmination of gastrointestinal beriberi, probably triggered by challenges that increase metabolic demands or decrease intracellular transport of thiamin, such as stress, infection, trauma, drug administration. For instance, a case study describes persistent gastrointestinal beriberi followed by the onset of neurological symptoms in a male patient after one session of heavy drinking (17). Metformin, a widely used antidiabetics entering cells through the thiamine transporters, is known to reduce intestinal thiamine content in mice (96). Thus, different comorbidities may transition a vulnerable state of gastrointestinal beriberi—where TD is confined to the gastrointestinal tract, but is not obviously affecting other body systems—into dry or wet beriberi, characterized by specific symptomatic manifestations where potentially life-threatening situations promote the diagnosis of TD.

In conclusion, TD limited to the gastrointestinal system may be an overlooked and underdiagnosed cause of the increasingly common gastrointestinal disorders encountered in modern medical settings. Left unattended, it may progress to wet or dry beriberi, most often observed as Wernicke encephalopathy. However, how is it possible that the gastrointestinal system—which, unlike other systems, is directly exposed to nutrients—suffers from this nutrient deficiency more than the rest of the body? The following sections examine the available evidence on molecular mechanisms of thiamine transport, metabolism, and function in the gastrointestinal system, offering insights for the interpretation of medical studies on thiamine-responsive gastrointestinal disorders that highlight mechanistic connections between common disorders of gastrointestinal tract and TD.

4 Thiamine metabolism in intestinal cells

4.1 Thiamine transport in brush border and basolateral membranes of enterocytes

Due to its hydrophilic nature, thiamine is not membrane-permeable, with its entry into the cell depending on membrane proteins dedicated to thiamine transport. Enterocytic absorption of dietary thiamine from the lumen and further transport of thiamine from the intestine to the blood occur via active transport through a number of transporters (Figure 2A). Thiamine transport capacity in human intestinal biopsy samples is highest in the duodenum, followed by the colon and stomach (97). Although THTR1 and THTR2 transporters, encoded by the SLC19A2 and SLC19A3 genes, respectively, are the most widely known and well-characterized, a number of newly characterized transporters of thiamine and its phosphates have been added to the list in recent decades. Figure 2 shows those expressed in intestine (A) along with their relative expression in enterocytes, their organoids and colonocytes (B).

Thiamine transporter SLC35F3 has been identified in studies investigating its variant, which is associated with human hypertension and lower levels of erythrocytic thiamine; expression of the protein in *Escherichia coli* increases thiamine transport (98). Thiamine is also

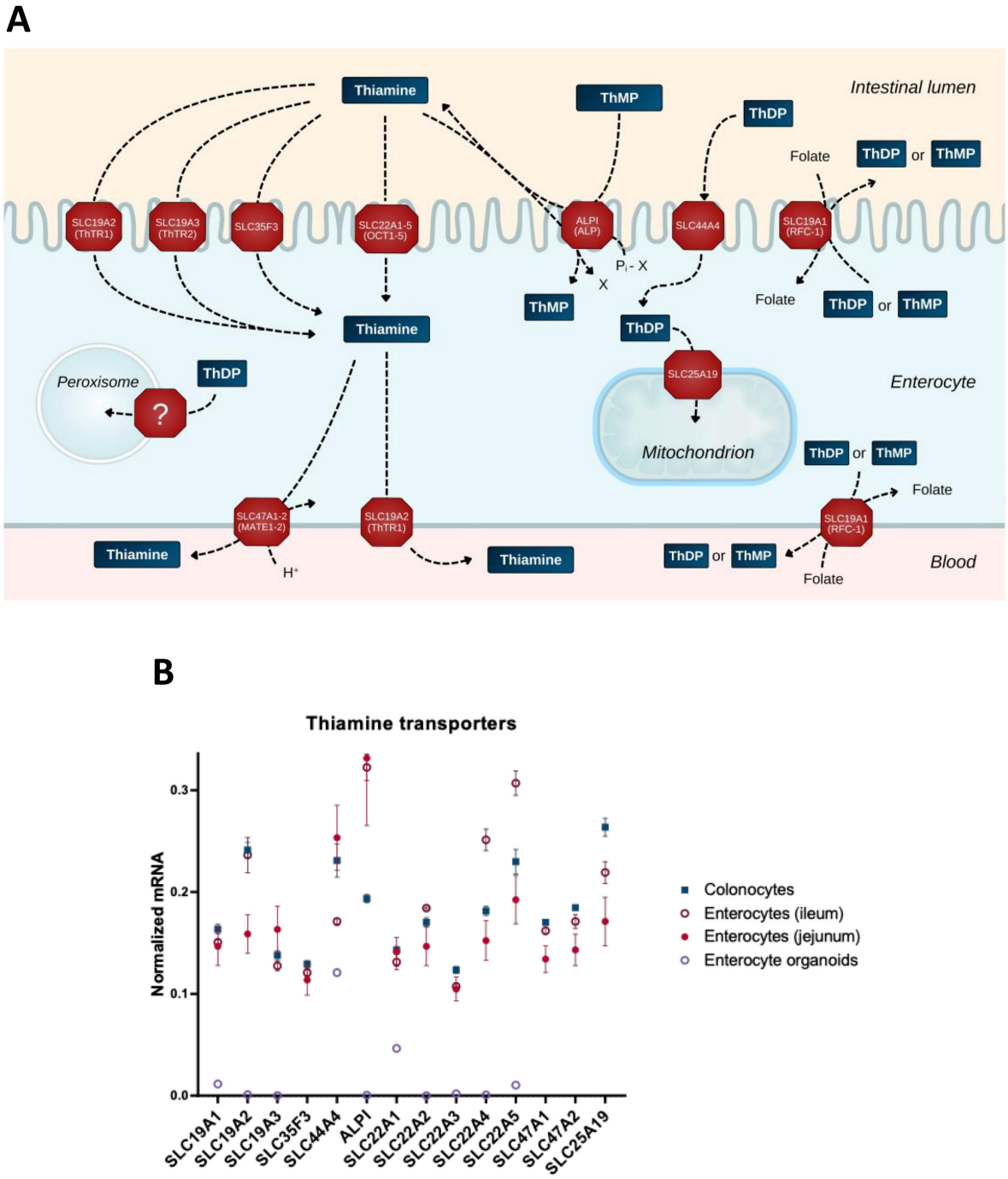

FIGURE 2
Transporters of thiamine and its derivatives in the gut cells. **(A)** Schematic presentation of the transport processes through transporters expressed in the gut cells. **(B)** The relative abundance of mRNAs for the transporters of thiamine and its derivatives in different intestinal cells (colonocytes, enterocytes of ileum, enterocytes of jejunum) and in enterocyte organoids. The normalized mRNA signals are calculated as described in the legend to Figure 1 using the same datasets. The data are shown as mean ± SEM.

transported through OCT1 (SLC22A1) (96) and OCT2 (SLC22A2) (99). Although the substrate specificity of other OCT family members is insufficiently characterized, their principal ability to transport organic cations justifies inclusion of SLC22A3–5 in Figure 2.

Intestinal alkaline phosphatase (ALPI, Figure 2) plays an essential role in the absorption of dietary B vitamins including thiamine (100). This enzyme not only exhibits phosphatase activity, dephosphorylating thiamine phosphates in the lumen, but may also transphosphorylate extracellular thiamine to intracellular thiamine monophosphate (ThMP), at the expense of intracellular phosphate donors such as beta-glycerophosphate or creatine phosphate (101). Transporters of MATE (multidrug and toxin extrusion) family (SLC47A) extrude thiamine in exchange for a proton; SLC47A1 and SLC47A2-K are characterized by a Km for thiamine in micromolar range (102), supporting the

physiological relevance of thiamine extrusion by these transporters (Figure 2A).

Differences in the net intestinal transport of thiamine have been studied by Rindi and co-workers using basolateral and brush border membrane vesicles (100, 103, 104). However, apart from the SLC19A transporter family, little is known about the polar distribution of transporters in enterocytes and colonocytes. The gut cells have an apical, or brush border, membrane facing the lumen, and a basolateral membrane facing the circulatory system. This polarity is in accord with the dual function of intestinal epithelium: the same cells absorb nutrients from the lumen through the brush border membrane and deliver them to the blood through the basolateral membrane (Figure 2A). Available data on the polarity are taken into account in Figure 2A. These refer to the excretory function of MATE transporters (102, 105, 106) and the identification of SLC19A2 on both membrane types in epithelial cells (107). In contrast, the SLC19A3 protein is shown to localize specifically to the apical (brush border) membrane (108). This transporter is well-characterized in terms of its structure and function, with a number of drugs decreasing thiamine transport through SLC19A3 (109). With the brush border membrane directly exposed to drugs inhibiting thiamine influx through SLC19A3, and nutrient efflux through the basolateral membrane to satisfy permanent systemic demands for thiamine, the steady-state concentration of thiamine in the gut epithelium may be decreased. The disbalance between the inhibited enterocytic influx and unchanged or increased systemic demand may cause specific vulnerability of the gut epithelium to toxic effects of the thiamine-competitive drugs targeting SLC19A3. As a result, gastrointestinal TD may develop and exist as a steady-state, providing other tissues with thiamine levels sufficient for normal conditions, but inadequate for metabolic challenges.

As luminal ThDP from the diet is supposed to be hydrolyzed by ALPI, while ThDP is known to be produced inside the cell, the role of SLC44A4 in transporting extracellular ThDP has been enigmatic. Single nucleotide polymorphisms in SLC44A4 are key risk factors for ulcerative colitis in a collection of different studies (110–113). In view of the high expression of this transporter in colon, it has been proposed to participate in absorption of the microbiota-generated ThDP (25). However, this view leaves unanswered the question why microbes would extrude ThDP to the lumen. Besides, the transporter is also expressed in other tissues, and independent data (114–116) show a less drastic difference between human colonocytes and enterocytes in its expression (Figure 2B), compared to the initial finding of a 10-fold higher expression in the colon relative to other regions of gastrointestinal tract in humans (117). On the other hand, according to recent structure–function characterization of reduced folate transport through SLC19A1, ThDP is the best substrate to be exchanged for folate (118). The participation of ThDP in cellular folate absorption (Figure 2A) endows SLC44A4 with a physiologically relevant function to return ThDP that is extruded in exchange for folate, back to the cell (Figure 2A). Employment of the ThDP-folate exchange by microbes may explain the high SLC44A4 expression in the colon. Yet, the expression of this transporter in different parts of the gut (Figure 2B) does not exclude its possible participation in the gut's absorption of ThDP from the diet, especially given the presence of other folate transporters on the brush border membrane of enterocytes, i.e., those encoded by SLC46A1, FOLR1, and FOLR2 (119).

Interestingly, males display lower plasma folate levels than females (120–122), and only males demonstrate a positive correlation between the folate deficiency risk scores calculated from the polymorphisms in the folate pathway genes SLC19A1 and MTHFR (123). At the same time, it is known that thiamine intake is higher in males vs. females (124). The opposite sex-dependent differences in the levels of folate and thiamine highlight the physiological significance of the folate/ThDP exchange through SLC19A1. This is further supported by a study of the SLC19A1 variant rs1051266:G (125). In model experiments employing HEK293 cells in a thiamine-deficient medium, the SLC19A1 variant rs1051266:G exhibits a strong decrease in ThDP efflux compared to the canonic SLC19A1 sequence rs1051266:A. Association of the SLC19A1 variant rs1051266:G with Wernicke-Korsakoff encephalopathy (125), a condition known to develop due to the thiamine deficiency in the brain, underscores the role of SLC19A1 in delivering not only folate but also thiamine to CNS (28). Both SLC19A1 and SLC19A2 deliver vitamins to systemic tissues (119), correspondent to their locations in the basolateral membrane (Figure 2). ThDP transport through the folate transporter encoded by SLC19A1 is further supported by cases of thiamine-responsive dysphagia that have been observed at normal laboratory values of blood thiamine, but increased serum folate levels (71). The blood transports thiamine or its monophosphate (ThMP), both of which also penetrate the blood–brain barrier (26, 126) through saturable transporters (127).

4.2 Interconversions of thiamine and its natural derivatives in intestinal cells

To perform its coenzyme function (Figure 1A), thiamine or ThMP entering the cells (Figure 2A) must be transformed into the coenzyme form, ThDP. Animals synthesize ThDP by diphosphorylation of thiamine in the thiamine diphosphokinase (or thiamine pyrophosphokinase, TPK)-catalyzed reaction (Figure 3A). Another well-characterized protein of thiamine metabolism is thiamine triphosphatase, encoded by ThTPA gene, which hydrolyzes thiamine triphosphate (ThTP) to ThDP (128, 129). The enzymes catalyzing other transformations of thiamine derivatives are not unambiguously identified. Given the poorly characterized specificity of enzymes involved in thiamine transformation and the often unknown roles of genomics-identified isoenzymes, all human isoenzymes are included in Figure 3 when there is evidence that at least one isoenzyme can catalyze the reactions with thiamine or its derivatives. In doing this, we would like to draw attention to possible roles of the isoenzymes, as shown in Figure 3, in thiamine metabolism. This may further help decipher their physiological significance, e.g., when the data on phenotypes of the mutated isoenzymes in humans may appear.

ThMP kinase, which produces ThDP through ThMP phosphorylation and is known in thiamine-synthesizing organisms, has not been identified in animals. Accordingly, to be transformed into ThDP by TPK, intracellular ThMP should first be dephosphorylated. ThMP hydrolysis is known to be catalyzed by prostatic acid phosphatase ACP3, which is localized to plasmatic membrane and linked to the antinociceptive action of thiamine and its derivatives (130–132). Cells of the gut express all isoenzymes of acid phosphatase (ACP), i.e., ACP 1–7 (Figure 3B). According to the UniProt database, these isoenzymes have diverse locations and

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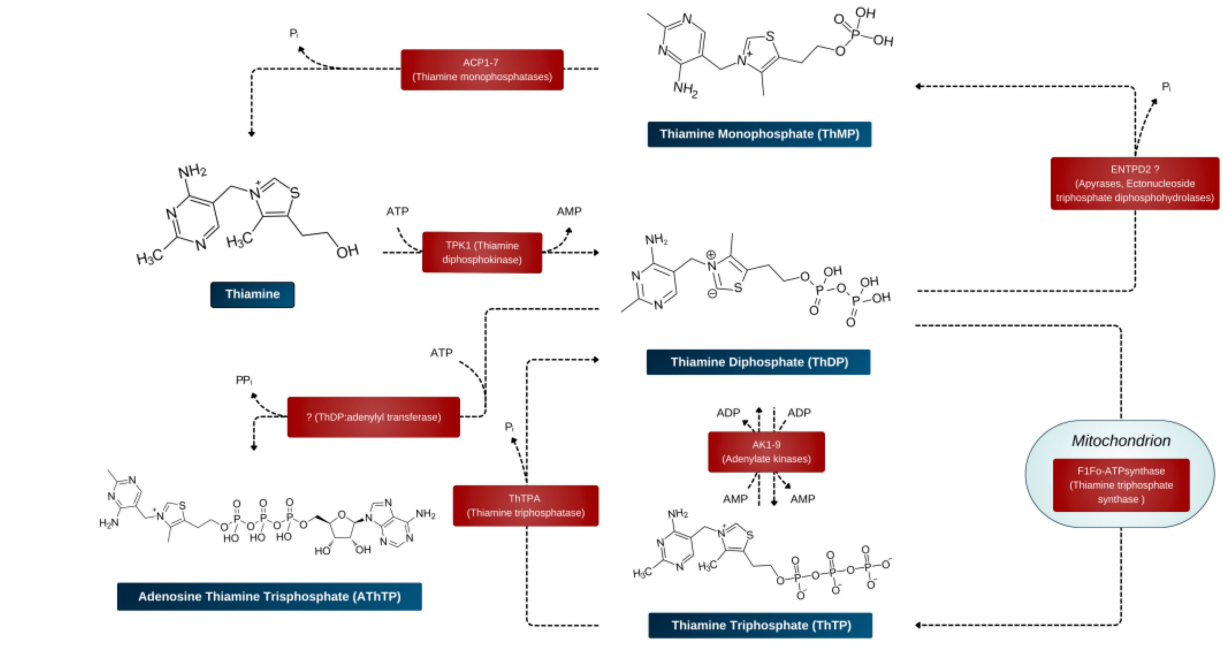

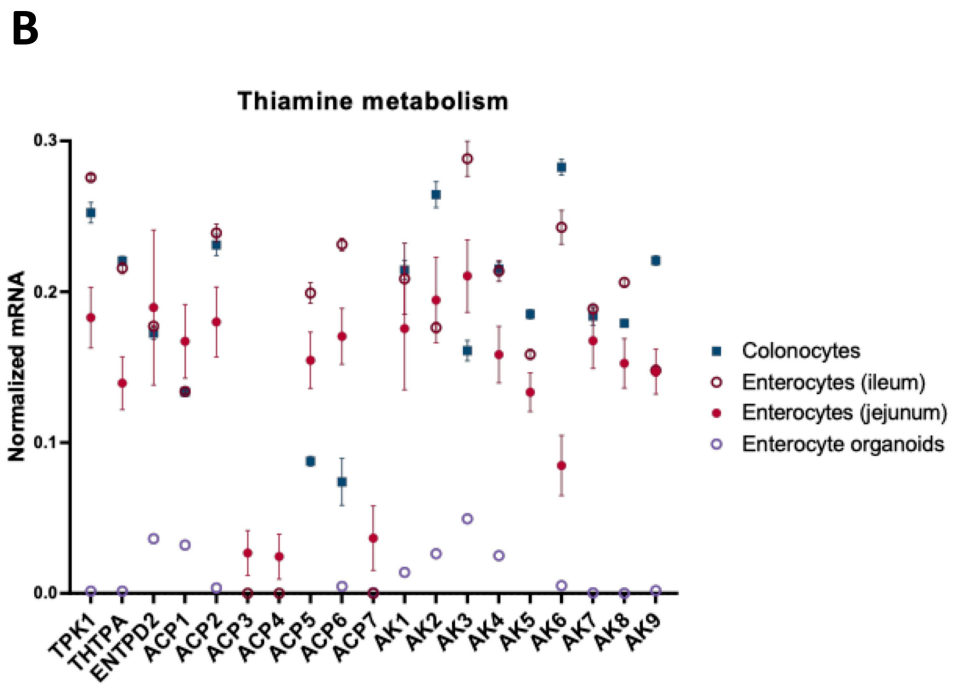

FIGURE 3
Enzymatic transformations of thiamine and its derivatives in the gut cells. **(A)** Schematic presentation of the reactions and available information on their catalysts. **(B)** The relative abundance of mRNAs for the enzymes of thiamine metabolism in different intestinal cells (colonocytes, enterocytes of ileum, enterocytes of jejunum) and in enterocyte organoids. The normalized mRNA signals are calculated as described in the legend to Figure 1 using the same datasets. The data are shown as mean ± SEM.

substrate specificities, acting on protein phosphotyrosine residues and a number of alkyl, aryl and acyl orthophosphates of low molecular mass. ACP1 has cytosolic location, whereas ACP2 and ACP5 are lysosomal enzymes. ACP4 is a transmembrane protein with 50% homology to the prostatic and lysosomal acid phosphatases, highly expressed in the testis and involved in mineralization of tooth enamel (133, 134). ACP6 is mitochondrial, supposed to participate in lipid metabolism through its characterized activity of hydrolyzing lysophosphatidic acid (135). Based on similarity to ACP5, ACP7 is predicted to be a putative tartrate-resistant phosphatase, a member of purple acid phosphatase family of metallophosphoesterase superfamily. The most probable locations of ACP7 are supposed to be extracellular space and cytosol. Studies on substrate specificities of ACP isoenzymes 1–7 are scarce and fragmentary, not enabling a conclusion on their specific catalysis of ThMP hydrolysis. Additionally, the substrate specificity may differ in the isoforms of each gene product, arising due to posttranscriptional (alternative splicing) or posttranslational modifications. Hence, Figure 3 shows all of the ACP isoenzymes expressed in the gut as potential catalysts of ThMP hydrolysis.

A deficiency of acid phosphatase activity in the lysosomal fraction, presumably due to impairment of the ACP2 protein, manifests as intermittent vomiting, hypotonia, lethargy, opisthotonos, terminal bleeding and death in early infancy (136). Many of these symptoms are also present in the thiamine deficiency states (137), supporting involvement of ACP2 in thiamine metabolism. Pathogenic mutation of the tartrate-resistant acid phosphatase ACP5 leads to a strong predisposition to autoimmune diseases, associated with the accumulation of phosphorylated osteopontin, involved in immune regulation and in bone resorption (138). As shown in the next sections, thiamine is tightly linked to immunity. Furthermore, both ACP5 (30) and a ThMP analog benfotiamine (139) are involved with Akt signaling. These lines of evidence favor catalytic activity of ACP5 in ThMP hydrolysis.

Thiamine-specific phosphatases of the bovine brain synaptosomes have been studied by binding them to a thiamine-modified affine sorbent, followed by MS identification of the eluted proteins. Only bacterial paralogues of mammalian proteins with phosphatase activities are identified by the procedure, probably due to poor coverage of the bovine genome. According to the sequence and structural alignment of these identified phosphatases, an apyrase encoded by the ENTPD2 gene, that is expressed in different tissues, including colon and small intestine, may possess the ThDP phosphatase activity in mammals (140). Apyrases, or ectonucleoside diphosphohydrolases, may catalyze hydrolysis of triphosphonucleotides to their monophosphates. Furthermore, these membrane-bound and soluble hydrolases may also act as monophosphatases of the diphosphonucleotides, catalyzing the hydrolysis of ThDP to ThMP (Figure 3).

Thiamine triphosphate is a non-coenzyme derivative of thiamine, probably involved in acetylcholine neurotransmission (141–143). Unlike the substrate-specific enzyme that hydrolyzes ThTP, synthesis of ThTP is currently known to be catalyzed only by the enzymes producing ATP through ADP phosphorylation. That is, ThTP is synthesized by adenylate kinase 1 (AK1) in cytoplasm and by F_1F_o-ATP synthase in the mitochondria (140). Mitochondrial ThTP synthesis is tissue-specific and involves an unidentified regulator of F_1F_o-ATP synthase linked to pyruvate oxidation (140). Other isoenzymes of AK, shown in Figure 3, are expressed in the gut, having different cellular locations. Their ability to synthesize ThTP and the substrate specificity has not been tested.

The adenylated form of ThTP (AThTP) is found in mammalian tissues, but the enzyme(s) of its synthesis lose their activity during purification, and this interferes with their identification (140). Levels of ThTP and AThTP are higher in fast growing, non-differentiated cells, highlighting the significance of these thiamine derivatives in cellular differentiation (144).

Metabolic transformations and known roles of thiamine and its different natural derivatives are summarized in Table 2.

TABLE 2 Metabolism and roles of thiamine and its derivatives in gastrointestinal system.

Thiamine compound	Role in gastrointestinal system	Chemical transformations in gastrointestinal system
Thiamine	Is transported to enterocytes, directly through the thiamine and OCT transporters, or coupled to ThMP production in transphosphorylation by intestinal alkaline phosphatase (ALPI). May be exchanged for proton through MATE. Important for systemic supply.	Precursor for ThDP biosynthesis by thiamine diphosphokinase (TPK). May be degraded or modified to thiamine antagonists by thiaminases I and II, identified in fish and microbes. Thiamine degradation in mammalian tissues suggests existence of mammalian thiaminases.
ThMP	May be exchanged for folate through SLC19A1. Important for systemic supply.	Hydrolysed in lumen by intestinal alkaline phosphatase (ALPI). Product of thiamine transphosphorylation by intestinal alkaline phosphatase (ALPI).
ThDP	Essential coenzyme of central metabolism. May be exchanged on external folate through SLC19A1 and returned to enterocyte through SLC44A4.	Product of the reactions catalyzed by thiamine diphosphokinase (TPK) or thiamine triphosphatase (ThTPase). May be hydrolysed to ThMP by apyrase(s) (ENTPD). In the reaction with ATP, catalyzed by unidentified enzyme(s), produces regulatory derivative, adenylated ThTP.
ThTP and adenylated ThTP	Specific action in gastrointestinal system is not characterized. In general, these derivatives regulate metabolic enzymes involved in ACh production in mammals through different mechanisms. ThTP regulates rapsyn - scaffolding protein of ACh synapses. ThTP is presumed to be involved into thiamine co-release with ACh at the synapses.	ThTP is synthesized from ThDP by adenylate kinase(s) (KAD) and hydrolysed to ThDP by thiamine triphosphatase (ThTPase). ThTP may be hydrolysed to ThMP by apyrase(s) (ENTPD). In the brain, but not liver, mitochondria ThTP may be formed by ATP synthase in the proton-gradient-dependent manner.

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5 Transcriptomics of the enterocytes before and after gastric bypass implies increased thiamine demand in humans long term after the bypass

Thiamine deficiency often develops after bariatric surgery, including gastric bypass (145–147). Independent published data provide transcriptomics analysis of the enterocytes at one (15–45 days) (148) and 6–9 (115) months after gastric bypass. We have used these data to answer the question of what happens to thiamine-dependent metabolism after gastric bypass. To compare the levels of mRNAs for proteins involved in thiamine-associated metabolism across different experiments, they are normalized to the summarized transcripts of GAPDH+ACTB+TUBA1A, as described previously (149). The normalized levels are shown as averages between the datasets before and after gastric bypass (Figure 4), and in each of the analyzed datasets (Figure 5). As seen from Figures 4, 5, no gross changes are observed in human jejunum enterocytes

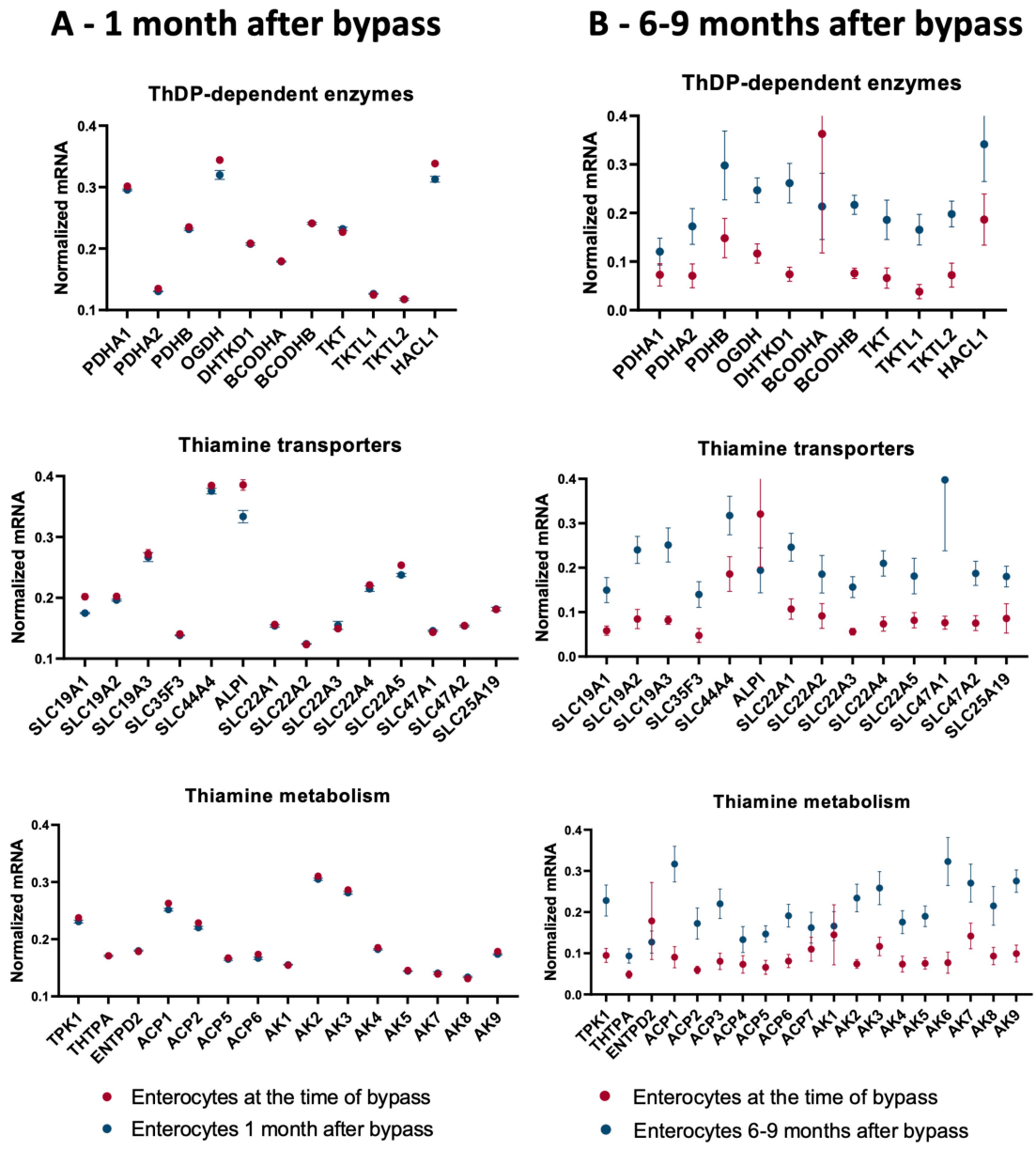

FIGURE 4
Changes in the averaged levels of mRNA for proteins of thiamine-dependent metabolism after gastric bypass. The relative abundance of mRNAs for the ThDP-dependent enzymes, thiamine transporters and enzymes of thiamine metabolism in jejunum enterocytes before and after gastric bypass is shown. The normalized mRNA signals are calculated as described in the legend to Figure 1. The data are shown as mean ± SEM. **(A)** Experiment GSE113819 {Platform GPL17586 (HTA-2_0) Affymetrix Human Transcriptome Array 2.0 [transcript (gene) version], 5 datasets for enterocytes before bypass: GSM3120595, GSM3120597, GSM3120599, GSM3120601, GSM3120603 - and 5 datasets for enterocytes 1 month after bypass: GSM3120594, GSM3120596, GSM3120598, GSM3120600, GSM3120602}. **(B)** Experiment GSE214758 [Platform GPL20795, HiSeq X Ten (*Homo sapiens*), 9 datasets for enterocytes before bypass: GSM6615629, GSM6615631, GSM6615633, GSM6615637, GSM6615641, GSM6615643, GSM6615645, GSM6615647, GSM6615649 - and 9 datasets for enterocytes 6–9 months after bypass: GSM6615630, GSM6615632, GSM6615638, GSM6615640, GSM6615642, GSM6615644, GSM6615646, GSM6615648, GSM6615650].

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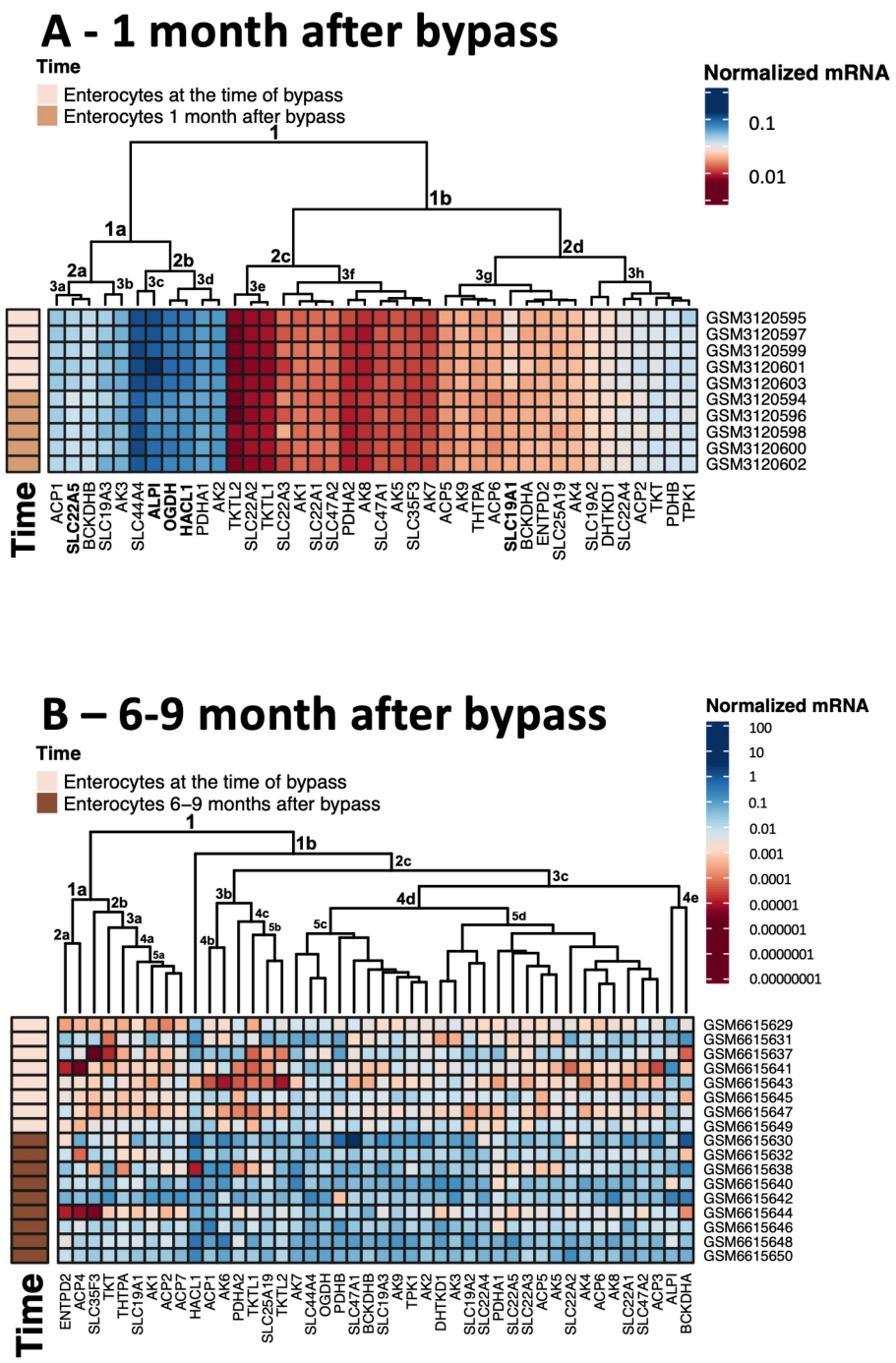

FIGURE 5
Heatmaps showing transcriptomics changes in the thiamine-dependent metabolism after gastric bypass. The transcriptomics experiments are identified in Figure 4. The normalized mRNA signals for the enzymes of thiamine-dependent metabolism in jejunum enterocytes before and after gastric bypass are shown in decimal logarithm scale according to the color code legend accompanying each heatmap. The heatmaps are produced using R program. **(A)** The normalized mRNA signals before and 1 month after gastric bypass. **(B)** The normalized mRNA signals before and 6–9 months after gastric bypass. The dataset GSM6615633 is excluded, as many transcripts are not identified in this experiment.

1 month after gastric bypass, similar to findings in the mouse model of the bypass, where an initial metabolic perturbation in response to the surgery, manifesting on the 9th day, is reversed to the control state 2 months post-bypass (148). However, a comparison of either the average transcript levels (Figure 4A), or separate datasets of human enterocytes (Figure 5A) reveals minor, but consistent decreases in transcripts for ALPI > SLC19A1 ≈ OGDH ≈ HACL1 > SLC22A5 1 month after the bypass, suggesting a decrease in thiamine-dependent metabolism.

Clusterization of transcript levels groups the transcripts according to their expression level and coincidence of the variations in different datasets (Figure 5). For instance, cluster 1a in Figure 5A

comprises proteins whose transcript levels are higher than those of proteins in cluster 1b. Interestingly, each of the clusters comprises one of the well-characterized thiamine transporters: SLC19A3 shares the cluster 1a with thiamine/ThDP-dependent proteins of higher expression, while SLC19A2 shares the cluster 1b with thiamine/ThDP-dependent proteins of lower expression. Overall, the three well-defined clusters 1a, 2c, 2d comprise transcripts of the high, low and intermediary levels of expression, correspondingly. Transcripts of the thiamine/ThDP-dependent proteins which are most obviously decreasing 1 month after the bypass (Figure 4A, gene names in bold in Figure 5A) belong to the proteins with the high (ALPI, OGDH, HACL1, SLC22A5 in cluster 1a) or intermediary (SLC9A1in cluster 2d) expression (Figure 5A). Increasing levels of clusterization (2, 3 etc.) group proteins of increasing transcriptional coincidence, i.e., proteins whose transcript levels show coupled variations across the datasets. For instance, despite the overall similarity between the transcription profiles before and 1 month after the bypass, the transcripts show featured variations in each of the cluster 2a or 2b. In particular, variations in the transcripts for the ThDP-dependent enzymes with relatively high enterocytic expression, i.e., OGDH, HACL1 and PDHA1, are coupled with those in the transcripts for ALPI and ThDP transporter SLC44A4, all belonging to the cluster 2b including the three proteins with decreased transcripts 1 month after the bypass (Figure 5A in bold). The cluster 2a includes proteins with a lower, compared to the cluster 2b, expression, with the decreasing transcriptional level of SLC22A5 1 month after the bypass coupled to the variations of BCKDHB and SLC19A3 transcripts (Figure 5A). Reduced folate/ThDP exchanger SLC19A1, demonstrating decreased transcript levels 1 month after the bypass, shares the cluster (3 g) with mitochondrial ThDP transporter SLC25A19, ThTPase and BCKDHA (Figure 5A).

In contrast to the transcriptomic pattern 1 month after the bypass, human jejunum enterocytes 6–9 months after the bypass exhibit significant perturbations in their transcriptomics profiles (115). In particular, significant changes in the levels of transcripts of genes encoding TCA cycle enzymes and associated proteins are observed (115). In good agreement with this finding, our analysis of the selected transcripts characterizing thiamine-dependent metabolism, shows an overall increase in the transcripts for the related proteins (Figures 4, 5). In particular, up-regulation of the transcripts for the rate-limiting enzyme of the TCA cycle, 2-oxoglutarate dehydrogenase (OGDH), and for the TCA-cycle-affiliated proteins, such as subunits of pyruvate dehydrogenase (PDH) and branched chain 2-oxo acids dehydrogenase (BCODH) is observed. The upregulation of ThDP-dependent DHTKD1 protein 6–9 months after the gastric bypass surgery (Figures 4B, 5B) is similar to the long-term increase of DHTKD1 protein observed after another type of surgical intervention (laminectomy) (48). In accord with the upregulated expression of all the ThDP-dependent dehydrogenases, transcripts of the thiamine/ThDP transporters and the major producer of ThDP from thiamine, i.e., thiamine diphosphokinase (TPK), as well as probable ThMP phosphatase ACP1, also undergo a long-term upregulation (Figure 4B).

Heatmap in Figure 5B shows that the first level of clusterization results in separating the ThDP-dependent TKT in cluster 1a from all the other ThDP-dependent enzymes, combined in cluster 1b. That said, the homooligomeric ThDP-dependent enzymes participating in metabolism of glucose and amino acids, i.e., TKT, OGDH, DHTKD1, preserve their cluster partners in both of the studied experiments. TKT has a common cluster (2d in Figures 1A, 5A in Figure 5B) with ThTPA and SLC19A1; OGDH shares its cluster (1a in Figures 5A,C in Figure 5B) with SLC44A4 and SLC19A3; DHTKD1 keeps associated with SLC19A2 and SLC22A4 (clusters 3 h in Figures 5A,D in Figure 5B). For the heterooligomeric ThDP-dependent enzymes, i.e., PDH and BCOADH, the cluster partners of the subunits α and β may be switched. For instance, OGDH shares the cluster 3d with PDHA1 in Figure 5A, while in Figure 5B OGDH occupies the same cluster 5c with PDHB, while PDHA1 is moved to the DHTKD1-comprising cluster 5d. BCKDHB partners with ALPI in the cluster 1a in Figure 5A, but the ALPI partner in cluster 4e of Figure 5B is BCKDHA. As a result, a comparison of the two independent transcriptomics experiments reveals stable associations between specific proteins of thiamine/ThDP metabolism and ThDP-dependent enzymes in enterocytes.

The observed upregulation of not only the ThDP-dependent dehydrogenases limiting and feeding the TCA cycle, but also the thiamine transporters and ThDP-producing enzyme TPK1 (Figures 4B, 5B), may compensate for decreased levels and/or increased demands of thiamine in enterocytes 6–9 months after gastric bypass, facilitated by the higher expression of the proteins binding thiamine or ThDP. Indeed, increased activities of ThDP-dependent dehydrogenases (assayed *in vitro*) are known as a compensatory response to their *in vivo* inhibition (38, 150). Cellular exposure to TD strongly stimulates OGDH activity upon the following incubation with ThDP (29). Hence, the upregulation of the thiamine/ThDP-dependent protein transcripts 6–9 months after gastric bypass (Figures 4B, 5B) maybe a biochemical indicator of thiamine insufficiency in the enterocytes. Remarkably, the upregulation is observed after initial decrease in the five thiamine/ThDP-dependent proteins 1 month after the bypass (Figures 4A, 5A).

According to the overall transcriptomics analysis of the long-term (6–9 months) changes in jejunum enterocytes after the bypass (115), there are interactions between the TCA cycle gene cluster and other significantly affected pathways that include genes linked to the cell cycle G2/M DNA damage checkpoint regulation. The DNA damage checkpoint is also involved in cellular repair and differentiation (151). Strong involvement of thiamine transporters in the differentiation of enterocytes is suggested by approximately 6-fold higher thiamine uptake, corresponding to elevated expression of SLC19A2 and SLC19A3, in the differentiated jejunum epithelial cells of the villi, compared to the non-differentiated jejunum epithelial cells of the crypt (152). Positive action of high doses of thiamine under metabolic perturbations including surgeries (48, 115, 149, 153–155) suggest that cellular protection and/or repair requiring cell differentiation, increase demand of thiamine. After surgical perturbations in enterocytic integrity, elevated cellular growth and differentiation, which occur within days, is followed by temporal organ-specific adaptations to the bypass (148). Both phases are characterized by high metabolic demands. If the increased demand is not met by the increased thiamine supply, a state of TD may easily follow, first in enterocytes, and after some period at the level of organism, most often manifested as Wernicke encephalopathy

(145–147). Thus, bariatric surgery-induced upregulation of thiamine-dependent metabolism in jejunum (Figures 4B, 5B) may manifest metabolic remodeling addressing insufficient levels of thiamine in post-bypass enterocytes.

6 Molecular basis of the thiamine-induced improvements in gastrointestinal disorders

As mentioned above, despite the direct exposure of the gastrointestinal system to nutritional supply, this system often exhibits thiamine-responsive dysfunctions even when no known TD signs are evident in other tissues. The essential role of ThDP in mitochondrial energy production (Figure 1) is universal for all tissues and therefore can hardly explain specific and primary vulnerability of the gastrointestinal system. This energetic role is usually considered in the context of the specific vulnerability to TD of tissues with high energy demand, such as the heart and the brain. These tissues are considered to be the last to decrease their thiamine content during TD and the first to replete it upon thiamine administration, with the liver serving as the thiamine depot for other tissues (156). Nevertheless, if drugs inhibit ThDP-dependent enzymes, as, e.g., omeprazole does (157), direct exposure of the gastrointestinal system to oral drugs may increase its vulnerability compared to other tissues. Based on currently available data, we also suggest several other origins of TD restricted to the gastrointestinal system.

First of all, the dual role of the gastrointestinal system - dedicated not only to nutrient absorption from the lumen, but also to their supply to other tissues via the blood - may present one of the reasons for the intestine-specific susceptibility to TD. That is, the function of the gastrointestinal system requires a balance between thiamine absorption and its supply to other tissues. Mechanisms controlling the balance between the two processes are not well characterized. However, as shown above in the analysis of thiamine/ThDP-dependent metabolism in enterocytes after gastric bypass, increased thiamine demand by other tissues may impair the thiamine status of the gastrointestinal system.

Furthermore, a major difference between the gastrointestinal system and other tissues is the gut microbiome. It is important to note that microbes can synthesize not only thiamine but also thiamine antagonists. This may occur as part of natural metabolism (158) or when microbes are exposed to drugs that undergo aberrant reactions, such as metronidazole (159–161). Microbial enterotoxins alter gene expression in the gut epithelium, leading to enteropathies (162). Proinflammatory cytokines inhibit enterocytic thiamine uptake at the transcriptional level (163). Thiamine transport is also significantly reduced by bacterial lipopolysaccharide and in sepsis (163, 164). Yet another mechanism contributing to the specific vulnerability of the gastrointestinal system to TD may involve the gut-brain axis, which depends on vagal tone and acetylcholine neurotransmission, for which thiamine co-release is known (165–167). In addition to the parasympathetic regulation, acetylcholine is currently suggested to be a paracrine signal of peripheral tissues, particularly in pancreas and epithelial cells (168, 169).

Below, data on molecular mechanisms underlying thiamine-responsive gastrointestinal dysfunctions are considered in more detail.

6.1 Exposure of the intestine to bacterial enzymes degrading thiamine or producing thiamine antagonists

Abnormal proliferation of gut bacteria in the upper small intestine, defined by the term 'small intestinal bacterial overgrowth' (SIBO), is associated with symptoms such as bloating, abdominal pain, excessive foul-smelling flatulence, constipation and/or diarrhea. The etiology of SIBO is varied and complex, with relapse commonly occurring after conventional treatment. Research suggests that SIBO is involved in up to 78% of irritable bowel syndrome (IBS) cases, the most commonly diagnosed disorder of gastrointestinal motility (170). SIBO has been associated with dysfunctional intestinal motility, characterized by inadequate peristaltic action of gastrointestinal smooth muscle (171–174), hypochlorhydria, and pancreatic exocrine insufficiency (175). As mentioned above, such symptoms are often responsive to thiamine administration. A link between SIBO and TD is provided by the notion that SIBO may increase bacterial transformations of thiamine, which are far more variable than those in mammals. In particular, this concerns the action of bacterial thiaminases I and II, which catalyze biosynthesis of thiamine antagonists or thiamine degradation, respectively. Thiaminase I catalyzes the substitution of thiamine heterocycles with catalytically inactive heterocycles from xenobiotics. For instance, in the presence of the antibiotic metronidazole, thiaminase I substitutes the thiazolium ring of thiamine with the imidazolium ring of metronidazole, resulting in a thiamine antagonist which can inhibit TPK (160). Microbes also synthesize naturally occurring thiamine antagonists, such as 2'-methoxyThDP (158). Thiaminase II degrades thiamine, splitting it into two heterocycles. Immobilized thiaminase II has been applied as an anticancer approach to deplete thiamine in cancer cells (176). Thiaminase II in fern extracts exerts an effect on neurotransmission which, similar to that of the thiamine antagonist pyrithiamine, can be counteracted by the addition of thiamine (177). It may thus be suggested that direct exposure of the gut to the microbiome - capable of synthesizing thiamine antagonists or possessing thiamine-degrading thiaminase II - may first of all affect the gut, leading to gastrointestinal beriberi, especially under conditions of SIBO or other disturbances of the gut microbiome. If these conditions are not treated, TD may spread from the gastrointestinal system to other tissues, as observed in metronidazole-induced encephalopathy (159).

6.2 Thiamine supports intestinal barrier integrity

Disturbed intestinal barrier function has been increasingly studied in recent years and is now widely recognized as a prominent feature of many chronic diseases, pertaining not only to the gut (178), but also to neurological, psychiatric, cardiovascular, and autoimmune conditions. Increased intestinal permeability can facilitate the entry of food antigens, bacteria and bacterial components into systemic circulation, which are thought to provoke systemic inflammatory responses. The intestinal epithelial barrier shares several key morphological and functional characteristics with the blood–brain barrier. The tight junctions of both structures are composed of transmembrane proteins such as claudin, occludin, zonula occluden (ZO), and endothelial cell-selective adhesion molecules. Thiamine is necessary for maintaining intestinal epithelial cell bioenergetics, and reduced activity of thiamine-dependent enzymes

may lead to a defective gut barrier (34). TD is known to disrupt the blood brain barrier, featuring loss of occludin, ZO-1 and ZO-2 (179, 180). It is therefore possible that similar mechanisms could be at play in the gastrointestinal tract. Notably, TD leads to disturbed expression of junction protein subtypes in *Ctenopharyngodon idella* (181). Moreover, thiamine administration enhances claudin-1, claudin-4, ZO-1, and occludin in ruminal epithelium (22). Thiamine facilitates the protective action of secretory IgA against immunogenic threats in enterocytes (182). The prevention of intestinal barrier dysfunction by secretory IgA, along with its immunomodulatory properties, may play a role in IBD, including ulcerative colitis and Crohn's disease (183). Poor thiamine status is frequently reported in IBD, mostly assumed to be a consequence of malabsorption (184–187). TD aggravates ulcerative colitis in mice, associated with the promoted infiltration of proinflammatory M1 macrophages into colonic lamina propria (188). The underlying mechanism is TD-induced impairment of PDHC activity, which causes remodeling of glucose metabolism in the macrophages.

Other mechanisms may also contribute to IBD, too. In particular, IBD is associated with hypoxia in enterocytes (189). Hypoxia has recently been shown to downregulate thiamine transporters in a colonic cell line, impairing the thiamine uptake that results in a localized intracellular deficiency (190). Alkaline phosphatase of the brush border of the intestines, which participates in thiamine transport (Figure 2), plays protective roles against pathogenic infection (191) and bacterial toxins (LPS) in the gut, and may counteract inflammation (192).

A combination of exogenous and endogenous factors is known to influence intestinal barrier function, including both acute and/or chronic immune dysregulation (193). TD may induce negative developments in the gastrointestinal system, which can be counteracted by thiamine supplementation. By normalizing or increasing the efficiency of glucose metabolism, thiamine decreases the degradation of amino acids as energy substrates and increases protein synthesis, which is essential for maintaining tight junctions and intestinal barrier integrity (194). Remarkably, TD in rats causes a 42–66% reduction of brush border enzyme activities and a 20% reduction in intestinal weight with significant thinning of the microvillus membrane (195). This may be the result of disturbed function of the ThDP-dependent 2-oxoglutarate dehydrogenase (Figure 1), as a universal effect of its inhibition is perturbation in relative amino acid abundance and a subsequent decrease in protein synthesis (196, 197). In turn, villous atrophy can impair nutrient absorption and is one of the primary mechanisms underpinning extensive nutritional deficiencies found in Celiac and inflammatory bowel diseases (198). Furthermore, atrophy and inflammation of mucosal surfaces in the gut are documented in experimental TD (82), and high doses of thiamine have been trialed in two studies showing that thiamine reduced fatigue associated with IBD (199, 200).

6.3 Thiamine and intestinal inflammation

Thiamine is considered a natural anti-inflammatory compound (201). Studies in both goats (202) and cows (203) indicate that thiamine has anti-inflammatory effects on the ruminal epithelium, ameliorating in particular the intestinal inflammation and barrier permeability caused by high-concentrate diet (27). As considered in Section 3 above, thiamine affects the composition of symbiotic microbiota, and ThDP-dependent bacterial butyrate synthesis in particular. Knockouts of the ThDP transporter *SLC44A4* in mice display an upregulation of genes associated with colonic inflammation, and increased susceptibility to dextran sodium sulfate-induced colitis, accompanied by significant weight loss and shortening of the colon (204).

A number of positive anti-inflammatory actions of thiamine include activation of Nrf2 and decreased ROS levels, enhanced activity of mitochondrial respiratory chain complexes I-IV, downregulation of endoplasmic reticulum stress, and suppression of gene expression associated with mitophagy, oxidative stress, and proinflammatory cytokines (205). Remarkably, the antioxidant effects of thiamine, including Nrf2 activation, are observed even when the thiamine disulfide forms— which should decrease cellular redox potential— are administered (206). These pharmacological forms, such as sulbutiamine or TTFD, may penetrate cell membrane better than thiamine. Inside the cells, they are reduced, particularly by the thioredoxin and glutaredoxin system, simultaneously stimulating antioxidant defense through Nrf2 activation (206). One may suggest that Nrf2 activation is specific to the disulfide forms of thiamine, not inherent in thiamine itself, as only the thiamine disulfides undergo intracellular reduction. The associated shift in the cellular redox state may cause the activation of Nrf2, leading to this additional positive effect on metabolism of high doses of thiamine disulfides, compared to thiamine. The Nrf2 activation along with the thiamine formation may explain why high doses of the thiamine disulfides, expected to decrease cellular redox potential, do not have any negative action even upon the long-term administration (155).

By maintaining integrity of the intestinal barrier via complex mechanisms, the vagus nerve and its major neurotransmitter, acetylcholine (ACh), play a crucial role in coordinating adaptive neural and endocrine responses of the gastrointestinal system, including those against infection and inflammation (207). SIBO and intestinal hypomotility are highly prevalent in patients with anti-ACh receptor antibodies (208, 209).

6.4 Acetylcholine-dependent mechanisms of thiamine action in gastrointestinal disorders

Many independent data associate gastrointestinal disorders with perturbed ACh signaling. ACh exerts tonic effects to maintain constriction of the lower esophageal sphincter, with ACh signaling compromised in a rat model of gastrointestinal reflux disease (210). Drugs which block the action of ACh can cause abnormal relaxation of the sphincter and reflux (211). The anti-cholinergic agents atropine and papaverine induce constipation in animals (23). In contrast, preserving ACh levels through acetylcholinesterase inhibitors improves gastrointestinal reflux disease (212). Pro-cholinergic pharmacological agents also provide symptomatic improvement in FGIDs (213, 214). Pharmacological forms of thiamine exhibit interactions with the ACh-dependent effects. That is, TTFD prevents the gut paralysis induced by atropine or papaverine (23), while sulbutiamine promotes cholinergic neurotransmission, potentiating the action of ACh esterase inhibitors (215). Interestingly, structure of an inhibitor of ACh esterase, acotiamide (brand name acofide), approved in Japan as a prokinetic motility drug against functional dyspepsia (216–218), combines the two heterocycles, one of them

thiazole, that may be considered as structural mimics of the thiazolium and aminopyrimidine heterocycles of thiamine.

An intimate relationship between thiamine and cholinergic neurotransmission relies on both the coenzyme and non-coenzyme actions of thiamine and its derivatives. In cholinergic cells, the ThDP-dependent pyruvate dehydrogenase complex synthesizes the ACh precursor acetyl-CoA (Figure 1) (219, 220). Distribution of this acetyl-CoA between the oxidation in the TCA cycle and participation in ACh synthesis is regulated through limitation of the TCA cycle rate by the ThDP-dependent OGDH, and the non-coenzyme action of thiamine and derivatives on the other enzymes involved (221). TD-perturbed function of ThDP-dependent dehydrogenases of TCA cycle (Figure 1) is a well-known contributor to impaired synthesis of ACh (219). The ensuing mitochondrial dysfunction of cholinergic cells increases their susceptibility to different insults (222–224). In particular, neurons of the gastrointestinal system are highly sensitive to oxidative stress (219, 225, 226), which is a general hallmark of TD (227). Cytosolic oxidative stress can inactivate nicotinic ACh receptors in neurons, decreasing ACh-evoked currents (228). Oxidative stress is considered to contribute to the pathophysiology of IBD (229, 230).

Independent of metabolic action as the coenzyme ThDP, thiamine is essential for axonal membrane excitability, playing a significant role in development of the action potential (231). This non-coenzyme action of thiamine in neuronal signaling is further supported by the identified molecular targets of thiamine and its non-coenzyme derivative ThTP. ThTP-dependent phosphorylation of rapsyn regulates ACh neurotransmission, as rapsyn is a scaffolding protein of the post-synaptic membrane of neuromuscular junctions, specifically associated with nicotinic ACh receptors (232). Hydrolysis of ThTP by synaptic membrane-bound protein(s) different from the well-characterized soluble ThTPase of cytosol, is supposed to be involved with synaptic function (233, 234). Thiamine binds to a bitter taste receptor, which modifies ileum contraction (235) and provokes ACh-induced contraction of jejunum (236). At a high concentration (0.05 mM), thiamine also binds to an isolated nicotinic ACh receptor (237). Probably, the low binding affinity is an artifact of the receptor isolation, as the physiologically relevant affinity of thiamine to the receptor may require protein–protein interaction within the native structure of the synapses. The non-coenzyme action of thiamine in neurotransmission is further supported by the action of the thiamine analog oxythiamine, whose diphosphorylated derivative (oxyThDP), formed by TPK *in vivo*, is an antagonist of the coenzyme action of thiamine. As a result, oxyThDP inhibits ThDP-dependent dehydrogenases, whose function is required for ACh synthesis, particularly pyruvate dehydrogenase complex generating ACh precursor acetyl-CoA (Figure 1). However, in superfused rat brain slices, oxythiamin enhances the release of synaptic acetylcholine, thus mimicking the non-coenzyme action of thiamine in facilitating ACh neurotransmission (238).

ACh is a major neurotransmitter of vagus nerve, coordinating the brain-gut axes (239). Vagus nerve stimulation can lead to gastrointestinal improvements (240), e.g., can increase gastric emptying through acting on the pyloric sphincter (241) and may be a treatment for gastroparesis (242). Potentiation of ACh release through transcutaneous vagal neuromodulation is known to enhance gut motility, reduce inflammation by suppressing TNF-α, and preserve epithelial tight junction integrity through the activation of enteric glial cells (243). The data indicate that TD-perturbed ACh synthesis and neurotransmission in vagus nerve may induce gastrointestinal dysfunction. However, in this case ACh synthesis and signaling are controlled by neurons of central nervous system. As discussed above, sufficient levels of thiamine in the brain are supported at the expense of other tissues, causing TD in these other tissues long before the TD occurs in the brain. Hence, when TD is limited to the gastrointestinal system, the pathology-relevant perturbation of ACh signaling may rather be expected in the enteric nervous system (ENS). ENS may regulate gastrointestinal function both with and without the input of central nervous system (244–246). In particular, the independent function of ENS is manifested in the intestinal peristaltic reflex.

ACh is a primary neurotransmitter of different types of ENS neurons, i.e., intrinsic primary afferent neurons, excitatory motor neurons, and interneurons (245). The thiamine-induced increases in contractions of the intestine smooth muscle and peristalsis, observed in a number of independent *in vitro* experiments on muscle strips of gastrointestinal tract (207, 219–222), support the action of thiamine on ACh signaling in ENS in the absence of central regulation (236). Synaptic co-release of thiamine and ACh, with thiamine facilitating the neurotransmission through its non-coenzyme action, is known from the early works of Minz and von Muralt on neuromuscular junctions (143, 165–167). In accordance with these observations, addition of thiamine and ThMP modulate synaptic transmission, electric and contractile activity of the smooth muscle strips from gastrointestinal tract (247). Thiamine hydrochloride, thiamine nitrate, thiamine propyldisulfide, TTFD, and some other experimental derivatives increase peristalsis of isolated sections of small intestine from rat (248). In isolated sections of murine jejunum and ileum, thiamine regulates parameters of the ACh-induced contraction in the concentration-dependent manner (236). In isolated duodenal and jejunal segments from cats, dogs, rabbits and guinea pigs the thiamine disulfide derivative TTFD exerts an excitatory effect on motility; sensitivity of the effect to an antagonist of muscarinic acetylcholine receptor atropine suggests involvement of the ACh neurotransmission (249). All forms of thiamine including thiamine hydrochloride, S-benzoyl thiamine disulfide, TTFD, increase contractions of isolated segments of small intestine (248).

In human patients with chronic enterocolitis and colitis, thiamine-induced increases in motor activity of stomach, small and large intestine are detected by electrogastrography and balloon-kymography, with no similar effects of vitamins B6, B12 and C (250). In animal models, TTFD can stimulate intestinal peristalsis within a few minutes after administration, also when mesenteric nerves are cut (251, 252). Intravenous administration of TTFD induces a slight increase in tone and a remarkable increase in the amplitude of rhythmic contractions in the jejunal loop of both non-anesthetized and anesthetized dogs for 20 min (253). TTFD applied topically inside the lumen of the intestine can also elicit such excitation effect (249). Nevertheless, in some studies on TTFD, percent weight gain was lower in the TTFD group (254). Two possible explanations for this effect are proposed: (1) reduced food intake due to an irritant effect of the treatment on the gastrointestinal tract (255, 256) or (2) a stimulant effect of TTFD on metabolism via enhanced noradrenaline secretion and thermogenesis (257).

ACh signaling in non-neuronal cells, employing the nicotinic and muscarinic ACh receptors, is comparable to ACh neurotransmission (168, 169). Therefore, ACh signaling in non-neuronal cells is another process potentially affected by TD before the deficiency develops into the well-known dry or wet beriberi. Glial cells of ENS are stimulated by ACh (245). In mammalian intestine, ACh regulates sodium currents in the apical and basolateral membranes (168). In human pancreas, ACh synthesized by alpha-cells is suggested to activate beta-cells (169). Other *in vivo* actions of ACh in pancreas may be linked to its high thiamine content (89). As considered in Section 3, TD in pancreas strongly affects gastrointestinal function. Perturbed ACh signaling by pancreatic cells under TD may contribute to gastrointestinal dysfunction in addition to insufficient pancreatic synthesis of the enzymes needed for digestion.

Thus, cholinergic neurotransmission and ACh-dependent regulation of non-neuronal cells employ ThDP coenzyme function for ACh synthesis and non-coenzyme action of thiamine in ACh signaling. Both may be impaired by TD, contributing to gastrointestinal dysfunction.

7 Conclusion

Various lines of evidence at the molecular level and in model systems provide a mechanistic basis for a number of observations at the physiological level, where thiamine supplementation alleviates gastrointestinal dysfunction, suggesting that insufficient levels of thiamine may underlie this dysfunction. The specific vulnerability of the gastrointestinal system to TD, in the absence of more common signs of wet and dry beriberi, may arise from direct exposure of this system to the gut microbiome and oral drugs, along with the continuous need to redistribute thiamine to other tissues. Cholinergic neurons of the enteric nervous system, which interact closely with gastrointestinal epithelial cells, as well as ACh signaling in non-neuronal cells such as enteric glia and pancreatic cells, depend on the essential role of thiamine in ACh production and the facilitation of ACh signaling. These mechanisms collectively contribute to the unique vulnerability of the gastrointestinal system to TD, even in the absence of classic forms of TD such as dry and wet beriberi. The multitude of proteins involved in mammalian metabolism and function of thiamine is not fully characterized. However, available data point to a much greater complexity of thiamine metabolism and its physiological significance, as commonly considered. This complexity must still be addressed by the identification of all genes involved. Individual differences in genetic variants of the thiamine-dependent proteins, in combination with environmental factors, may underlie personal vulnerabilities to thiamine deficiency, manifesting in an ever-increasing variety of clinical presentations.

Author contributions

EO: Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. AE: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. VB: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

EO is an owner of a nutraceutical company that sells vitamin supplements including thiamine.

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.

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References

1. Wang Y, Huang Y, Chase RC, Li T, Ramai D, Li S, et al. Global burden of digestive diseases: A systematic analysis of the global burden of diseases study, 1990 to 2019. *Gastroenterology*. (2023) 165:773–783.e15. doi: 10.1053/j.gastro.2023.05.050

2. Peery AF, Crockett SD, Murphy CC, Jensen ET, Kim HP, Egberg MD, et al. Burden and cost of gastrointestinal, liver, and pancreatic diseases in the United States: update 2021. *Gastroenterology*. (2022) 162:621–44. doi: 10.1053/j.gastro.2021.10.017

3. Huang Z, Li Y, Park H, Ho M, Bhardwaj K, Sugimura N, et al. Unveiling and harnessing the human gut microbiome in the rising burden of non-communicable diseases during urbanization. *Gut Microbes*. (2023) 15:2237645. doi: 10.1080/19490976.2023.2237645

4. Rome IV. *Criteria*. Rome Found Available at: https://theromefoundation.org/rome-iv/rome-iv-criteria/ (Accessed October 31, 2024).

5. Drossman DA. Functional gastrointestinal disorders: history, pathophysiology, clinical features, and Rome IV. *Gastroenterology*. (2016) 150:1262–1279.e2. doi: 10.1053/j.gastro.2016.02.032

6. Fikree A, Byrne P. Management of functional gastrointestinal disorders. *Clin Med*. (2021) 21:44–52. doi: 10.7861/clinmed.2020-0980

7. Sperber AD, Bangdiwala SI, Drossman DA, Ghoshal UC, Simren M, Tack J, et al. Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome foundation global study. *Gastroenterology*. (2021) 160:99–114.e3. doi: 10.1053/j.gastro.2020.04.014

8. Grover M, Farrugia G, Stanghellini V. Gastroparesis: a turning point in understanding and treatment. *Gut*. (2019) 68:2238–50. doi: 10.1136/gutjnl-2019-318712

9. Mazzone A, Strege PR, Gibbons SJ, Alcaino C, Joshi V, Haak AJ, et al. microRNA overexpression in slow transit constipation leads to reduced NaV1.5 current and altered smooth muscle contractility. *Gut*. (2020) 69:868–76. doi: 10.1136/gutjnl-2019-318747

10. Singh R, Wei L, Ghoshal UC. Micro-organic basis of functional gastrointestinal (GI) disorders: role of microRNAs in GI pacemaking cells. *Indian J Gastroenterol*. (2021) 40:102–10. doi: 10.1007/s12664-021-01159-7

11. Cipriani G, Gibbons SJ, Kashyap PC, Farrugia G. Intrinsic gastrointestinal macrophages: their phenotype and role in gastrointestinal motility. *Cell Mol Gastroenterol Hepatol*. (2016) 2:120–130.e1. doi: 10.1016/j.jcmgh.2016.01.003

12. Wei L, Singh R, Ha SE, Martin AM, Jones LA, Jin B, et al. Serotonin deficiency is associated with delayed gastric emptying. *Gastroenterology*. (2021) 160:2451–2466.e19. doi: 10.1053/j.gastro.2021.02.060

13. Drossman DA, Tack J, Ford AC, Szigethy E, Törnblom H, Oudenhove LV. Neuromodulators for functional gastrointestinal disorders (disorders of gut–brain interaction): A Rome foundation working team report. *Gastroenterology*. (2018) 154:1140–1171.e1. doi: 10.1053/j.gastro.2017.11.279

14. Bunik VI. A challenging interplay between basic research, technologies and medical education to provide therapies based on disease mechanisms. *Front Med*. (2024) 11:672. doi: 10.3389/fmed.2024.1464672

15. Dalayeli N, Hajhashemi V, Talebi A, Minaiyan M. Investigating the impact of selected B vitamins (B1, B2, B6, and B12) on acute colitis induced experimentally in rats. *Int J Prev Med*. (2024) 15:61. doi: 10.4103/ijpvm.ijpvm_232_23

16. Hua-an X, Mei-lin H, Li-ping C, Tai-zhen L, Qi-mei H, Hui-xian L. Comparative study of two methods on promoting the passage of gas by anus on patient after section of gynecology. *Clin Med Eng*. (2009) 16:63–4.

17. Tjong E, Peng Y-Y. Gastrointestinal beriberi and Wernicke's encephalopathy triggered by one session of heavy drinking. *Case Rep Neurol*. (2019) 11:124–31. doi: 10.1159/000499601

18. Shah HN, Bal BS, Finelli FC, Koch TR. Constipation in patients with thiamine deficiency after roux-en-Y gastric bypass surgery. *Digestion*. (2013) 88:119–24. doi: 10.1159/000353245

19. Shah IA, Kawoos Y, Shah AI, Rabyang S, Naqash H, Mir MR. Gastrointestinal beriberi as a prodrome of non-alcoholic Wernicke's encephalopathy: a study of an emerging nutritional deficiency disorder from Kashmir, India. *Int J Res Med Sci*. (2019) 7:1494–9. doi: 10.18203/2320-6012.ijrms20191496

20. Fang-Fang Z, Zhi-Rong F, Yong-Yu X. Effect of vitamin B1 integrated with recovery exercises on the postoperative recovery of gastrointestinal function of patients with transabdominal hysterectomy. *J Qilu Nurs*. (2012) 1:11.

21. Ma Y, Wang C, Elmhadi M, Zhang H, Liu F, Gao X, et al. Dietary supplementation of thiamine enhances colonic integrity and modulates mucosal inflammation injury in goats challenged by lipopolysaccharide and low pH. *Br J Nutr*. (2022) 128:2147–57. doi: 10.1017/S0007114522000174

22. Ma Y, Zhang Y, Elmhadi M, Zhang H, Wang H. Thiamine alleviates high-concentrate-diet-induced oxidative stress, apoptosis, and protects the rumen epithelial barrier function in goats. *Front Vet Sci*. (2021) 8:663698. doi: 10.3389/fvets.2021.663698

23. Nagata M, Sugimoto J. The influences of drugs on the experimental constipation of mice treated with atropine and Papaverine. *J Kansai Med Univ*. (1973) 25:300–21. doi: 10.5361/jkmu1956.25.3_300

24. Bunik VI, Tylicki A, Lukashev NV. Thiamin diphosphate-dependent enzymes: from enzymology to metabolic regulation, drug design and disease models. *FEBS J*. (2013) 280:6412–42. doi: 10.1111/febs.12512

25. Ramamoorthy K, Sabui S, Kim G, Fleckenstein JM, Sheikh A, Said HM. IQGAP-2: A novel interacting partner for the human colonic thiamin pyrophosphate transporter (hcTPPT). *Am J Physiol Cell Physiol*. (2024) 327:C1451–61. doi: 10.1152/ajpcell.00484.2024

26. Patnni C, Reggiani C, Laforenza U, Rindi G. Blood–brain transport of thiamine monophosphate in the rat: A kinetic study in vivo. *J Neurochem*. (1988) 50:90–3. doi: 10.1111/j.1471-4159.1988.tb13234.x

27. Ma J, Shah AM, Wang Z, Fan X. Potential protective effects of thiamine supplementation on the ruminal epithelium damage during subacute ruminal acidosis. *Anim Sci J*. (2021) 92:e13579. doi: 10.1111/asj.13579

28. Sangha V, Hoque MT, Henderson JT, Bendayan R. Novel localization of folate transport systems in the murine central nervous system. *Fluids Barriers CNS*. (2022) 19:92. doi: 10.1186/s12987-022-00391-3

29. Bunik VI, Aleshin VA, Zhou X, Tabakov VY, Karlsson A. Activation of mitochondrial 2-Oxoglutarate dehydrogenase by Cocarboxylase in human lung adenocarcinoma cells A549 is p53/p21-dependent and impairs cellular redox state, mimicking the cisplatin action. *Int J Mol Sci*. (2020) 21:3759. doi: 10.3390/ijms21113759

30. Lång P, Patlaka C, Andersson G. Tartrate-resistant acid phosphatase type 5/ACP5 promotes cell cycle entry of 3T3-L1 preadipocytes by increasing IGF-1/Akt signaling. *FEBS Lett*. (2021) 595:2616–27. doi: 10.1002/1873-3468.14184

31. Smith TJ, Johnson CR, Koshy R, Hess SY, Qureshi UA, Mynak ML, et al. Thiamine deficiency disorders: a clinical perspective. *Ann N Y Acad Sci*. (2021) 1498:9–28. doi: 10.1111/nyas.14536

32. Muley A, Fernandez R, Green H, Muley P. Effect of thiamine supplementation on glycaemic outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. *BMJ Open*. (2022) 12:e059834. doi: 10.1136/bmjopen-2021-059834

33. Mori K, Naganuma T, Kihara A. Role of 2-hydroxy acyl-CoA lyase HACL2 in odd-chain fatty acid production via α-oxidation in vivo. *Mol Biol Cell*. (2023) 34:ar85. doi: 10.1091/mbc.E23-02-0042

34. Tian N, Hu L, Lu Y, Tong L, Feng M, Liu Q, et al. TKT maintains intestinal ATP production and inhibits apoptosis-induced colitis. *Cell Death Dis*. (2021) 12:853–11. doi: 10.1038/s41419-021-04142-4

35. Van Der FLG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol*. (2009) 71:241–60. doi: 10.1146/annurev.physiol.010908.163145

36. Bunik V, Kaehne T, Degtyarev D, Shcherbakova T, Reiser G. Novel isoenzyme of 2-oxoglutarate dehydrogenase is identified in brain, but not in heart. *FEBS J*. (2008) 275:4990–5006. doi: 10.1111/j.1742-4658.2008.06632.x

37. Artiukhov AV, Grabarska A, Gumbarewicz E, Aleshin VA, Kähne T, Obata T, et al. Synthetic analogues of 2-oxo acids discriminate metabolic contribution of the 2-oxoglutarate and 2-oxoadipate dehydrogenases in mammalian cells and tissues. *Sci Rep*. (2020) 10:1886. doi: 10.1038/s41598-020-58701-4

38. Bunik VI, Artiukhov AV, Kazantsev AV, Aleshin VA, Boyko AI, Ksenofontov AL, et al. Administration of Phosphonate Inhibitors of dehydrogenases of 2-Oxoglutarate and 2-Oxoadipate to rats elicits target-specific metabolic and physiological responses. *Front Chem*. (2022) 10:284. doi: 10.3389/fchem.2022.892284

39. Sherrill JD, Kc K, Wang X, Wen T, Chamberlin A, Stucke EM, et al. Whole-exome sequencing uncovers oxidoreductases DHTKD1 and OGDHL as linkers between mitochondrial dysfunction and eosinophilic esophagitis. *JCI Insight*. (2018) 3:922. doi: 10.1172/jci.insight.99922

40. Denton RM, Pullen TJ, Armstrong CT, Heesom KJ, Rutter GA. Calcium-insensitive splice variants of mammalian E1 subunit of 2-oxoglutarate dehydrogenase complex with tissue-specific patterns of expression. *Biochem J*. (2016) 473:1165–78. doi: 10.1042/BCJ20160135

41. Tsepkova PM, Artiukhov AV, Boyko AI, Aleshin VA, Mkrtchyan GV, Zvyagintseva MA, et al. Thiamine induces Long-term changes in amino acid profiles and activities of 2-Oxoglutarate and 2-Oxoadipate dehydrogenases in rat brain. *Biochem Mosc*. (2017) 82:723–36. doi: 10.1134/S0006297917060098

42. Shang S, Liu J, Hua F. Protein acylation: mechanisms, biological functions and therapeutic targets. *Signal Transduct Target Ther*. (2022) 7:396–30. doi: 10.1038/s41392-022-01245-y

43. Fernandes MF, Vinolo MAR. Histone acylations as a mechanism for regulation of intestinal epithelial cells. *Dig Med Res*. (2024) 7:4. doi: 10.21037/dmr-23-3

44. Sutendra G, Kinnaird A, Dromparis P, Paulin R, Stenson TH, Haromy A, et al. A nuclear pyruvate dehydrogenase complex is important for the generation of acetyl-CoA and histone acetylation. *Cell*. (2014) 158:84–97. doi: 10.1016/j.cell.2014.04.046

45. Wang Y, Guo YR, Liu K, Yin Z, Liu R, Xia Y, et al. KAT2A coupled with the α-KGDH complex acts as a histone H3 succinyltransferase. *Nature*. (2017) 552:273–7. doi: 10.1038/nature25003

46. Li W, Long Q, Wu H, Zhou Y, Duan L, Yuan H, et al. Nuclear localization of mitochondrial TCA cycle enzymes modulates pluripotency via histone acetylation. *Nat Commun*. (2022) 13:7414. doi: 10.1038/s41467-022-35199-0

47. Bunik VI, Degtyarev D. Structure-function relationships in the 2-oxo acid dehydrogenase family: substrate-specific signatures and functional predictions for the 2-oxoglutarate dehydrogenase-like proteins. *Proteins*. (2008) 71:874–90. doi: 10.1002/prot.21766

48. Boyko AI, Karlina IS, Zavileyskiy LG, Aleshin VA, Artiukhov AV, Kaehne T, et al. Delayed impact of 2-Oxoadipate dehydrogenase inhibition on the rat brain metabolism is linked to protein Glutarylation. *Front Med*. (2022) 9:263. doi: 10.3389/fmed.2022.896263

49. Aleshin VA, Sibiryakina DA, Kazantsev AV, Graf AV, Bunik VI. Acylation of the rat brain proteins is affected by the inhibition of pyruvate dehydrogenase in vivo. *Biochem Mosc*. (2023) 88:105–18. doi: 10.1134/S0006297923010091

50. Hansen GE, Gibson GE. The α-Ketoglutarate dehydrogenase complex as a hub of plasticity in neurodegeneration and regeneration. *Int J Mol Sci*. (2022) 23:12403. doi: 10.3390/ijms232012403

51. Bunik VI, Raddatz G, Strumilo S. Translating enzymology into metabolic regulation: the case of the 2- Oxoglutarate dehydrogenase multienzyme complex. *Curr Chem Biol*. 7:74–93. doi: 10.2174/2212796811307010008

52. Bunik VI. Redox-driven signaling: 2-Oxo acid dehydrogenase complexes as sensors and transmitters of metabolic imbalance. *Antioxid Redox Signal*. (2019) 30:1911–47. doi: 10.1089/ars.2017.7311

53. Goodwin DW. The Wernicke-Korsakoff syndrome: A clinical and pathological study of 245 patients, 82 with post-mortem examinations. *JAMA*. (1972) 219:389. doi: 10.1001/jama.1972.03190290075032

54. Oudman E, Wijnia JW, Bidesie JR, van Dam MJ, Oey MJ, Smits S, et al. Pediatric Wernicke encephalopathy: A systematic review. *Pediatr Rep*. (2025) 17:15. doi: 10.3390/pediatric17010015

55. Gu Q, Li P, Gu Q, Li P. Biosynthesis of vitamins by probiotic Bacteria In: V Rao and L Rao, editors. Probiotics and prebiotics in human nutrition and health. London: IntechOpen (2016)

56. Wan Z, Zheng J, Zhu Z, Sang L, Zhu J, Luo S, et al. Intermediate role of gut microbiota in vitamin B nutrition and its influences on human health. *Front Nutr*. (2022) 9:1502. doi: 10.3389/fnut.2022.1031502

57. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. *Nature*. (2011) 473:174–80. doi: 10.1038/nature09944

58. Costliow ZA, Degnan PH. Thiamine acquisition strategies impact metabolism and competition in the gut microbe *Bacteroides thetaiotaomicron*. *mSystems*. (2017) 2:e00116–7. doi: 10.1128/mSystems.00116-17

59. Soto-Martin EC, Warnke I, Farquharson FM, Christodoulou M, Horgan G, Derrien M, et al. Vitamin biosynthesis by human gut butyrate-producing Bacteria and cross-feeding in synthetic microbial communities. *MBio*. (2020) 11:e00886–20. doi: 10.1128/mBio.00886-20

60. Magnúsdóttir S, Ravcheev D, de Crécy-Lagard V, Thiele I. Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Front Genet*. (2015) 6:148. doi: 10.3389/fgene.2015.00148

61. Park J, Hosomi K, Kawashima H, Chen Y-A, Mohsen A, Ohno H, et al. Dietary vitamin B1 intake influences gut microbial community and the consequent production of short-chain fatty acids. *Nutrients*. (2022) 14:2078. doi: 10.3390/nu14102078

62. Bermúdez-Sánchez S, Bager P, Dahlerup JF, Baunwall SMD, Licht TR, Mortensen MS, et al. Thiamine-reduced fatigue in quiescent inflammatory bowel disease is linked to *Faecalibacterium prausnitzii* abundance. *Gastro Hep Adv*. (2025) 4:12. doi: 10.1016/j.gastha.2024.08.012

63. Donnino M. Gastrointestinal beriberi: a previously unrecognized syndrome. *Ann Intern Med*. (2004) 141:898–9. doi: 10.7326/0003-4819-141-11-200412070-00035

64. Shah IA, Asimi RP, Kawoos Y, Wani M, Saleem T, Baba WN. Nonalcoholic Wernicke's encephalopathy: A retrospective study from a tertiary Care Center in Northern India. *J Neurosci Rural Pract*. (2017) 8:401–6. doi: 10.4103/jnrp.jnrp_14_17

65. Duca J, Lum CJ, Lo AM. Elevated lactate secondary to gastrointestinal beriberi. *J Gen Intern Med*. (2015) 31:133–6. doi: 10.1007/s11606-015-3326-2

66. Vu TND, Junker MS, Kurjatko A, Albright RC, Heller SF, Rivera M. Gastrointestinal beriberi mimicking a surgical emergency in a well-nourished patient: A case report. *Mayo Clin Proc Innov Qual Outcomes*. (2019) 3:506–9. doi: 10.1016/j.mayocpiqo.2019.08.005

67. Duca J, Lum C. Rare presentation of thiamine deficiency as gastrointestinal syndrome. *Hawaii J Med Public Health*. (2014) 73:46.

68. Prakash S. Gastrointestinal beriberi: a forme fruste of Wernicke's encephalopathy? *BMJ Case Rep*. (2018) 2018:4841. doi: 10.1136/bcr-2018-224841

69. Williams RD. Observations on induced thiamine (vitamin B1) deficiency in man. *Arch Intern Med*. (1940) 66:785. doi: 10.1001/archinte.1940.00190160002001

70. Shimazono N, Katsura E. Review of Japanese literature on beriberi and thiamine. Japan: Vitamin B Research Committee of Japan (1965).

71. Bal B, Finelli F, Shope T, Koch T. Malnutrition-induced dysphagia after roux en Y gastric bypass surgery: 440. *Off J Am Coll Gastroenterol ACG*. (2010) 105:S161. doi: 10.14309/00000434-201010001-00440

72. Machado J, Ministro P, Cancela E, Araújo R, Castanheira A, Silva A. Acute neurologic disorder in Crohn's disease: A rare life-threatening complication. *GE J Port Gastrenterol*. (2014) 21:31–4. doi: 10.1016/j.jpg.2013.10.006

73. Truedsson M, Ohlsson B, Sjöberg K. Wernicke's encephalopathy presenting with severe dysphagia: a case report. *Alcohol Alcohol*. (2002) 37:295–6. doi: 10.1093/alcalc/37.3.295

74. Cornea A, Lata I, Simu M, Rosca EC. Wernicke encephalopathy presenting with dysphagia: A case report and systematic literature review. *Nutrients*. (2022) 14:5294. doi: 10.3390/nu14245294

75. Sparks MI, Collins EN. The role of vitamin B1 in tonus of the large intestine. *Am J Dig Dis*. (1935) 2:618–20. doi: 10.1007/BF03000956

76. Hirose Y, Endoh H, Maruyama M, Ohtake M, Aizawa O, Miida T, et al. False acute abdomen with lactic acidosis in vitamin B1 deficiency. *Nihon Kyukyu Igakukai Zasshi*. (1993) 4:339–43. doi: 10.3893/jjaam.4.339

77. Takai S. Habitual constipation as a sign of infantile Preberiberi and the effect of vitamin B. A clinical experiment and study on a Group of Apparently Healthy lnfants nursed with Arakawa=negative human Milk 66th report of the peroxidase reaction. *Tohoku J Exp Med*. (1935) 27:589–98. doi: 10.1620/tjem.27.589

78. Ruiz M. Dilatation of the digestive organs due to vitamin B1 deficiency. *Rev Clin Esp*. (1954) 53:182–8.

79. Du W, Lu L, Liu Y, Yan Y, La R, Wu Q, et al. The association between dietary vitamin B1 intake and constipation: a population-based study. *BMC Gastroenterol*. (2024) 24:171. doi: 10.1186/s12876-024-03255-2

80. Levin LG, Mal'tsev GI, Gapparov MM. Effect of thiamine deficiency in hydrochloric acid secretion in the stomach. *Vopr Pitan*. (1978) 1:36–40.

81. Konstam G, Sinclair HM. Cardiovascular disturbances caused by deficiency of vitamin B1. *Br Heart J*. (1940) 2:231–40. doi: 10.1136/hrt.2.4.231

82. Wilbur DL. The effects of vitamin deficiency on the gastro-intestinal tract. *Am J Dig Dis*. (1939) 6:610–7. doi: 10.1007/BF02996333

83. Haden RL. Clinical deficiency disease. *Trans Am Clin Climatol Assoc*. (1935) 51:238–46.

84. Hawk EA, Hundley JM. Effect of certain B vitamin deficiencies on gastric secretion in the rat. *Proc Soc Exp Biol Med*. (1951) 78:318–22. doi: 10.3181/00379727-78-19060

85. Dalldorf G, Kellogg M. Incidence of gastric ulcer in albino rats fed diets deficient in vitamin B1. *J Exp Med*. (1932) 56:391–8. doi: 10.1084/jem.56.3.391

86. Iwai W, Abe Y, Iijima K, Koike T, Uno K, Asano N, et al. Gastric hypochlorhydria is associated with an exacerbation of dyspeptic symptoms in female patients. *J Gastroenterol*. (2013) 48:214–21. doi: 10.1007/s00535-012-0634-8

87. Zhang X, Chen J, Fu K, Huang X. Clinical observation on the treatment of functional dyspepsia by injection of vitamin B1 into Zusanli and Hegu acupoints. *Acupunct Res*. (2021) 46:1043–7. doi: 10.13702/j.1000-0607.20210660

88. Ianiro G, Pecere S, Giorgio V, Gasbarrini A, Cammarota G. Digestive enzyme supplementation in gastrointestinal diseases. *Curr Drug Metab*. (2016) 17:187–93. doi: 10.2174/1389200217021601141501 37

89. Prasannan KG, Sundaresan R, Venkatesan D. Thiamine deficency and protein secretion by pancreatic slices in vitro. *Experientia*. (1977) 33:169–70. doi: 10.1007/BF02124046

90. Srinivasan P, Thrower EC, Loganathan G, Balamurugan AN, Subramanian VS, Gorelick FS, et al. Chronic nicotine exposure in vivo and in vitro inhibits vitamin B1 (thiamin) uptake by pancreatic acinar cells. *PLoS One*. (2015) 10:e0143575. doi: 10.1371/journal.pone.0143575

91. Subramanya SB, Subramanian VS, Sekar VT, Said HM. Thiamin uptake by pancreatic acinar cells: effect of chronic alcohol feeding/exposure. *Am J Physiol Gastrointest Liver Physiol*. (2011) 301:G896–904. doi: 10.1152/ajpgi.00308.2011

92. Menendez G, Vazquez E. Carencia de B en patologia digestiva [B deficiency in digestive pathology]. *Rev Clín Esp*. (1944) 14:241–6.

93. Plana GA. Effect of vitamin B1 on the enzymes of the pancreas in children. *Riv Clin Pediatr*. (1939) 1:353–9.

94. Singh M. Effect of thiamin deficiency on pancreatic acinar cell function. *Am J Clin Nutr*. (1982) 36:500–4. doi: 10.1093/ajcn/36.3.500

95. Khoso MS, Saeed A, Fayyaz Z, Goraya A, Anjum MN, Cheema HA. Severe acute pancreatitis complicating with pancreatic encephalopathy in an adolescent girl. *Pak Pediatr J*. (2023) 47:11.

96. Chen L, Shu Y, Liang X, Chen EC, Yee SW, Zur AA, et al. OCT1 is a high-capacity thiamine transporter that regulates hepatic steatosis and is a target of metformin. *Proc Natl Acad Sci*. (2014) 111:9983–8. doi: 10.1073/pnas.1314939111

97. Laforenza U, Patrini C, Alvisi C, Faelli A, Licandro A, Rindi G. Thiamine uptake in human intestinal biopsy specimens, including observations from a patient with acute thiamine deficiency. *Am J Clin Nutr*. (1997) 66:320–6. doi: 10.1093/ajcn/66.2.320

98. Zhang K, Huentelman MJ, Rao F, Sun EI, Corneveaux JJ, Schork AJ, et al. Genetic implication of a novel thiamine transporter in human hypertension. *J Am Coll Cardiol*. (2014) 63:1542–55. doi: 10.1016/j.jacc.2014.01.007

99. Kato K, Mori H, Kito T, Yokochi M, Ito S, Inoue K, et al. Investigation of endogenous compounds for assessing the drug interactions in the urinary excretion involving multidrug and toxin extrusion proteins. *Pharm Res*. (2014) 31:136–47. doi: 10.1007/s11095-013-1144-y

100. Rindi G, Laforenza U. Thiamine intestinal transport and related issues: recent aspects. *Proc Soc Exp Biol Med*. (2000) 224:246–55. doi: 10.1046/j.1525-1373.2000.22428.x

101. Rindi G, Ricci V, Gastaldi G, Patrini C. Intestinal alkaline phosphatase can Transphosphorylate thiamin to thiamin monophosphate during intestinal transport in the rat. *Arch Physiol Biochem*. (1995) 103:33–8. doi: 10.3109/13813459509007560

102. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K-I. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. *Biochem Pharmacol*. (2007) 74:359–71. doi: 10.1016/j.bcp.2007.04.010

103. Laforenza U, Gastaldi G, Rindi G. Thiamine outflow from the enterocyte: a study using basolateral membrane vesicles from rat small intestine. *J Physiol*. (1993) 468:401–12. doi: 10.1113/jphysiol.1993.sp019778

104. Laforenza U, Orsenigo MN, Rindi G. A thiamine/H+ Antiport mechanism for thiamine entry into brush border membrane vesicles from rat small intestine. *J Membr Biol*. (1998) 161:151–61. doi: 10.1007/s002329900322

105. Otsuka M, Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y. A human transporter protein that mediates the final excretion step for toxic

organic cations. *Proc Natl Acad Sci*. (2005) 102:17923–8. doi: 10.1073/pnas.0506483102

106. Komatsu T, Hiasa M, Miyaji T, Kanamoto T, Matsumoto T, Otsuka M, et al. Characterization of the human MATE2 proton-coupled polyspecific organic cation exporter. *Int J Biochem Cell Biol*. (2011) 43:913–8. doi: 10.1016/j.biocel.2011.03.005

107. Said HM, Balamurugan K, Subramanian VS, Marchant JS. Expression and functional contribution of hTHTR-2 in thiamin absorption in human intestine. *Am J Physiol Gastrointest Liver Physiol*. (2004) 286:G491–8. doi: 10.1152/ajpgi.00361.2003

108. Subramanian VS, Marchant JS, Said HM. Targeting and trafficking of the human thiamine transporter-2 in epithelial cells. *J Biol Chem*. (2006) 281:5233–45. doi: 10.1074/jbc.M512765200

109. Gabriel F, Spriestersbach L, Fuhrmann A, Jungnickel KEJ, Mostafavi S, Pardon E, et al. Structural basis of thiamine transport and drug recognition by SLC19A3. *Nat Commun*. (2024) 15:8542. doi: 10.1038/s41467-024-52872-8

110. Gupta A, Thelma BK. Identification of critical variants within SLC44A4, an ulcerative colitis susceptibility gene identified in a GWAS in north Indians. *Genes Immun*. (2016) 17:105–9. doi: 10.1038/gene.2015.53

111. Gupta A, Juyal G, Sood A, Midha V, Yamazaki K, Vich Vila A, et al. A cross-ethnic survey of CFB and SLC44A4, Indian ulcerative colitis GWAS hits, underscores their potential role in disease susceptibility. *Eur J Hum Genet*. (2016) 25:111–22. doi: 10.1038/ejhg.2016.131

112. Wu J, Cheng Y, Zhang R, Shen H, Ma L, Yang J, et al. Evaluating the Association of Common Variants of the SLC44A4 gene with ulcerative colitis susceptibility in the Han Chinese population. *Genet Test Mol Biomark*. (2017) 21:555–9. doi: 10.1089/gtmb.2017.0010

113. Juyal G, Negi S, Sood A, Gupta A, Prasad P, Senapati S, et al. Genome-wide association scan in north Indians reveals three novel HLA-independent risk loci for ulcerative colitis. *Gut*. (2015) 64:571–9. doi: 10.1136/gutjnl-2013-306625

114. Christensen J, El-Gebali S, Natoli M, Sengstag T, Delorenzi M, Bentz S, et al. Defining new criteria for selection of cell-based intestinal models using publicly available databases. *BMC Genomics*. (2012) 13:274. doi: 10.1186/1471-2164-13-274

115. Vomhof-DeKrey EE, Singhal S, Singhal SK, Stover AD, Rajpathy O, Preszler E, et al. RNA sequencing of intestinal enterocytes pre- and post-roux-en-Y gastric bypass reveals alteration in gene expression related to enterocyte differentiation, restitution, and obesity with regulation by Schlafen 12. *Cells*. (2022) 11:3283. doi: 10.3390/cells11203283

116. Bjerrum JT, Hansen M, Olsen J, Nielsen OH. Genome-wide gene expression analysis of mucosal colonic biopsies and isolated colonocytes suggests a continuous inflammatory state in the lamina propria of patients with quiescent ulcerative colitis. *Inflamm Bowel Dis*. (2010) 16:999–1007. doi: 10.1002/ibd.21142

117. Nabokina SM, Inoue K, Subramanian VS, Valle JE, Yuasa H, Said HM. Molecular identification and functional characterization of the human colonic thiamine pyrophosphate transporter. *J Biol Chem*. (2014) 289:4405–16. doi: 10.1074/jbc.M113.528257

118. Dang Y, Zhou D, Du X, Zhao H, Lee C-H, Yang J, et al. Molecular mechanism of substrate recognition by folate transporter SLC19A1. *Cell Discov*. (2022) 8:141–11. doi: 10.1038/s41421-022-00508-w

119. Zhao R, Goldman ID. Folate and thiamine transporters mediated by facilitative carriers (SLC19A1-3 and SLC46A1) and folate receptors. *Mol Asp Med*. (2013) 34:373–85. doi: 10.1016/j.mam.2012.07.006

120. Cohen E, Margalit I, Shochat T, Goldberg E, Krause I. Sex differences in folate levels: A cross sectional study of a large cohort from Israel. *Isr Med Assoc J*. (2021) 23:17–22.

121. Hao L, Ma J, Stampfer MJ, Ren A, Tian Y, Tang Y, et al. Geographical, seasonal and gender differences in folate status among Chinese adults. *J Nutr*. (2003) 133:3630–5. doi: 10.1093/jn/133.11.3630

122. Kim Y-N, Cho Y-O. Folate food source, usual intake, and folate status in Korean adults. *Nutr Res Pract*. (2018) 12:47–51. doi: 10.4162/nrp.2018.12.1.47

123. Wang X, Wang Y, Ma X, Zhou S, Xu J, Guo Y, et al. Gender-specific association of SLC19A1 and MTHFR genetic polymorphism with oxidative stress biomarkers and plasma folate levels in older adults. *Exp Gerontol*. (2023) 178:112208. doi: 10.1016/j.exger.2023.112208

124. Mataix J, Aranda P, Sánchez C, Montellano MA, Planells E, Llopis J. Assessment of thiamin (vitamin B1) and riboflavin (vitamin B2) status in an adult Mediterranean population. *Br J Nutr*. (2003) 90:661–6. doi: 10.1079/BJN2003926

125. O'Brien NL, Quadri G, Lightley I, Sharp SI, Guerrini I, Smith I, et al. SLC19A1 genetic variation leads to altered thiamine diphosphate transport: implications for the risk of developing Wernicke–Korsakoff's syndrome. *Alcohol Alcohol*. (2022) 57:581–8. doi: 10.1093/alcalc/agac032

126. Abdul-Muneer PM, Alikunju S, Schuetz H, Szlachetka AM, Ma X, Haorah J. Impairment of thiamine transport at the GUT-BBB-AXIS contributes to Wernicke's encephalopathy. *Mol Neurobiol*. (2018) 55:5937–50. doi: 10.1007/s12035-017-0811-0

127. Greenwood J, Love ER, Pratt OE. Kinetics of thiamine transport across the blood-brain barrier in the rat. *J Physiol*. (1982) 327:95–103. doi: 10.1113/jphysiol.1982.sp014222

128. Bettendorff L, Wins P. Thiamine triphosphatase and the CYTH superfamily of proteins. *FEBS J*. (2013) 280:6443–55. doi: 10.1111/febs.12498

129. Bettendorff L. Update on thiamine Triphosphorylated derivatives and metabolizing enzymatic complexes. *Biomol Ther*. (2021) 11:1645. doi: 10.3390/biom11111645

130. Hurt JK, Coleman JL, Fitzpatrick BJ, Taylor-Blake B, Bridges AS, Vihko P, et al. Prostatic acid phosphatase is required for the Antinociceptive effects of thiamine and Benfotiamine. *PLoS One*. (2012) 7:e48562. doi: 10.1371/journal.pone.0048562

131. Zylka MJ, Sowa NA, Taylor-Blake B, Twomey MA, Herrala A, Voikar V, et al. Prostatic acid phosphatase is an ectonucleotidase and suppresses pain by generating adenosine. *Neuron*. (2008) 60:111–22. doi: 10.1016/j.neuron.2008.08.024

132. Knyihár-Csillik E, Bezzegh A, Böti S, Csillik B. Thiamine monophosphatase: a genuine marker for transganglionic regulation of primary sensory neurons. *J Histochem Cytochem*. (1986) 34:363–71. doi: 10.1177/34.3.3005391

133. Kim YJ, Lee Y, Kasimoglu Y, Seymen F, Simmer JP, Hu JC-C, et al. Recessive mutations in ACP4 cause Amelogenesis Imperfecta. *J Dent Res*. (2022) 101:37–45. doi: 10.1177/00220345211015119

134. Yousef GM, Diamandis M, Jung K, Diamandis EP. Molecular cloning of a novel human acid phosphatase gene (ACPT) that is highly expressed in the testis. *Genomics*. (2001) 74:385–95. doi: 10.1006/geno.2001.6556

135. Hiroyama M, Takenawa T. Isolation of a cDNA encoding human lysophosphatidic acid phosphatase that is involved in the regulation of mitochondrial lipid biosynthesis. *J Biol Chem*. (1999) 274:29172–80. doi: 10.1074/jbc.274.41.29172

136. Nadler HL, Egan TJ. Deficiency of lysosomal acid phosphatase. *N Engl J Med*. (1970) 282:302–7. doi: 10.1056/NEJM197002052820604

137. Whitfield KC, Bourassa MW, Adamolekun B, Bergeron G, Bettendorff L, Brown KH, et al. Thiamine deficiency disorders: diagnosis, prevalence, and a roadmap for global control programs. *Ann N Y Acad Sci*. (2018) 1430:3–43. doi: 10.1111/nyas.13919

138. Lausch E, Janecke A, Bros M, Trojandt S, Alanay Y, De Laet C, et al. Genetic deficiency of tartrate-resistant acid phosphatase associated with skeletal dysplasia, cerebral calcifications and autoimmunity. *Nat Genet*. (2011) 43:132–7. doi: 10.1038/ng.749

139. Marchetti V, Menghini R, Rizza S, Vivanti A, Feccia T, Lauro D, et al. Benfotiamine counteracts glucose toxicity effects on endothelial progenitor cell differentiation via Akt/FoxO signaling. *Diabetes*. (2006) 55:2231–7. doi: 10.2337/db06-0369

140. Aleshin VA, Mezhenska OA, Parkhomenko YM, Kaehne T, Bunik VI. Thiamine mono- and diphosphate phosphatases in bovine brain Synaptosomes. *Biochem Mosc*. (2020) 85:378–86. doi: 10.1134/S000629792003013X

141. Bunik VI, Aleshin VA. Chapter 11 - analysis of the protein binding sites for thiamin and its derivatives to elucidate the molecular mechanisms of the noncoenzyme action of thiamin (vitamin B1) In: A Rahman, editor. Studies in natural products chemistry. Amsterdam, Netherlands: Elsevier (2017). 375–429.

142. Aleshin VA, Mkrtchyan GV, Bunik VI. Mechanisms of non-coenzyme action of thiamine: protein targets and medical significance. *Biochem Mosc*. (2019) 84:829–50. doi: 10.1134/S0006297919080017

143. Parkhomenko YM, Pavlova AS, Mezhenskaya OA. Mechanisms responsible for the high sensitivity of neural cells to vitamin B1 deficiency. *Neurophysiology*. (2016) 48:429–48. doi: 10.1007/s11062-017-9620-3

144. Gangolf M, Czerniecki J, Radermecker M, Detry O, Nisolle M, Jouan C, et al. Thiamine status in humans and content of phosphorylated thiamine derivatives in biopsies and cultured cells. *PLoS One*. (2010) 5:e13616. doi: 10.1371/journal.pone.0013616

145. Gutiérrez-Rey M, Castellar-Visbal L, Acevedo-Vergara K, Vargas-Manotas J, Rivera-Porras D, Londoño-Juliao G, et al. The weight of bariatric surgery: Wernicke-Korsakoff syndrome after vertical sleeve gastrectomy-A case series. *J Pers Med*. (2024) 14:638. doi: 10.3390/jpm14060638

146. Parrott JM, Parrott AJ, Parrott JS, Williams NN, Dumon KR. Predicting recurrent deficiency and suboptimal monitoring of thiamin deficiency in patients with metabolic and bariatric surgery. *Nutrients*. (2024) 16:2226. doi: 10.3390/nu16142226

147. Kairis S, Andris C, Rakic J-M. Wernicke encephalopathy: a neuro-ophthalmological side effect of bariatric surgery. *Rev Med Liege*. (2024) 79:492–6.

148. Ben-Zvi D, Meoli L, Abidi WM, Nestoridi E, Panciotti C, Castillo E, et al. Time-dependent molecular responses differ between gastric bypass and dieting but are conserved across species. *Cell Metab*. (2018) 28:310–323.e6. doi: 10.1016/j.cmet.2018.06.004

149. Mkrtchyan G, Graf A, Bettendorff L, Bunik V. Cellular thiamine status is coupled to function of mitochondrial 2-oxoglutarate dehydrogenase. *Neurochem Int*. (2016) 101:66–75. doi: 10.1016/j.neuint.2016.10.009

150. Aleshin VA, Artiukhov AV, Oppermann H, Kazantsev AV, Lukashev NV, Bunik VI. Mitochondrial impairment may increase cellular NAD(P)H: Resazurin oxidoreductase activity, perturbing the NAD(P)H-based viability assays. *Cells*. (2015) 4:427–51. doi: 10.3390/cells4030427

151. O'Connell MJ, Walworth NC, Carr AM. The G2-phase DNA-damage checkpoint. *Trends Cell Biol*. (2000) 10:296–303. doi: 10.1016/s0962-8924(00)01773-6

152. Nabokina SM, Reidling JC, Said HM. Differentiation-dependent up-regulation of intestinal thiamin uptake: cellular and molecular mechanisms. *J Biol Chem*. (2005) 280:32676–82. doi: 10.1074/jbc.M505243200

153. Weidinger A, Milivojev N, Hosmann A, Duvigneau JC, Szabo C, Törö G, et al. Oxoglutarate dehydrogenase complex controls glutamate-mediated neuronal death. *Redox Biol*. (2023) 62:102669. doi: 10.1016/j.redox.2023.102669

154. Boyko A, Tsepkova P, Aleshin V, Artiukhov A, Mkrtchyan G, Ksenofontov A, et al. Severe spinal cord injury in rats induces chronic changes in the spinal cord and cerebral cortex metabolism, adjusted by thiamine that improves locomotor performance. *Front Mol Neurosci*. (2021) 14:593. doi: 10.3389/fnmol.2021.620593

155. Artiukhov AV, Solovjeva ON, Balashova NV, Sidorova OP, Graf AV, Bunik VI. Pharmacological doses of thiamine benefit patients with the Charcot–Marie–tooth neuropathy by changing thiamine diphosphate levels and affecting regulation of thiamine-dependent enzymes. *Biochem Mosc*. (2024) 89:1161–82. doi: 10.1134/S0006297924070010

156. Parkhomenko YM, Kudryavtsev PA, Pylypchuk SY, Chekhivska LI, Stepanenko SP, Sergiichuk AA, et al. Chronic alcoholism in rats induces a compensatory response, preserving brain thiamine diphosphate, but the brain 2-oxo acid dehydrogenases are inactivated despite unchanged coenzyme levels. *J Neurochem*. (2011) 117:1055–65. doi: 10.1111/j.1471-4159.2011.07283.x

157. Nixon PF, Diefenbach RJ, Duggleby RG. Inhibition of transketolase and pyruvate decarboxylase by omeprazole. *Biochem Pharmacol*. (1992) 44:177–9. doi: 10.1016/0006-2952(92)90053-L

158. Nemeria NS, Shome B, DeColli AA, Heflin K, Begley TP, Meyers CF, et al. Competence of thiamin diphosphate-dependent enzymes with 2′-Methoxythiamin diphosphate derived from Bacimethrin, a naturally occurring thiamin anti-vitamin. *Biochemistry*. (2016) 55:1135–48. doi: 10.1021/acs.biochem.5b01300

159. Iwadate D, Sato K, Kanzaki M, Komiyama C, Watanabe C, Eguchi T, et al. Thiamine deficiency in metronidazole-induced encephalopathy: A metabolic correlation? *J Neurol Sci*. (2017) 379:324–6. doi: 10.1016/j.jns.2017.06.042

160. Alston TA, Abeles RH. Enzymatic conversion of the antibiotic metronidazole to an analog of thiamine. *Arch Biochem Biophys*. (1987) 257:357–62. doi: 10.1016/0003-9861(87)90577-7

161. Agyei-Owusu K, Leeper FJ. Thiamin diphosphate in biological chemistry: analogues of thiamin diphosphate in studies of enzymes and riboswitches. *FEBS J*. (2009) 276:2905–16. doi: 10.1111/j.1742-4658.2009.07018.x

162. Sheikh A, Tumala B, Vickers TJ, Martin JC, Rosa BA, Sabui S, et al. Enterotoxigenic *Escherichia coli* heat-labile toxin drives enteropathic changes in small intestinal epithelia. *Nat Commun*. (2022) 13:6886. doi: 10.1038/s41467-022-34687-7

163. Anandam KY, Srinivasan P, Yasujima T, Al-Juburi S, Said HM. Proinflammatory cytokines inhibit thiamin uptake by human and mouse pancreatic acinar cells: involvement of transcriptional mechanism(s). *Am J Physiol Gastrointest Liver Physiol*. (2021) 320:G108–16. doi: 10.1152/ajpgi.00361.2020

164. Anthonymuthu S, Sabui S, Manzon KI, Sheikh A, Fleckenstein JM, Said HM. Bacterial lipopolysaccharide inhibits free thiamin uptake along the intestinal tract via interference with membrane expression of thiamin transporters 1 and 2. *Am J Physiol Cell Physiol*. (2024) 327:C1163–77. doi: 10.1152/ajpcell.00570.2024

165. Von Muralt A. The role of thiamine (vitamin B1) in nervous excitation. *Exp Cell Res*. (1958) 14:72–9.

166. Von Muralt A. Thiamine and peripheral neurophysiology In: RS Harris and KV Thimann, editors. Vitamins and Hormones. Cambridge, MA: Academic Press (1947). 93–118.

167. Minz B. Sur la liberation de la vitamine B1 par le trone isole de nerf pneumogastrique soumis a l'exitation electrique. *CRSoc Biol*. (1938) 127:1251–3.

168. Wessler I, Kirkpatrick CJ. Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. *Br J Pharmacol*. (2008) 154:1558–71. doi: 10.1038/bjp.2008.185

169. Caicedo A. Paracrine and autocrine interactions in the human islet: more than meets the eye. *Semin Cell Dev Biol*. (2013) 24:11–21. doi: 10.1016/j.semcdb.2012.09.007

170. Ghoshal UC, Shukla R, Ghoshal U. Small intestinal bacterial overgrowth and irritable bowel syndrome: A bridge between functional organic dichotomy. *Gut Liver*. (2017) 11:196–208. doi: 10.5009/gnl16126

171. Husebye E. Gastrointestinal motility disorders and bacterial overgrowth. *J Intern Med*. (1995) 237:419–27. doi: 10.1111/j.1365-2796.1995.tb01196.x

172. Pimentel M, Soffer EE, Chow EJ, Kong Y, Lin HC. Lower frequency of MMC is found in IBS subjects with abnormal lactulose breath test, suggesting bacterial overgrowth. *Dig Dis Sci*. (2002) 47:2639–43. doi: 10.1023/a:1021039032413

173. Vantrappen G, Janssens J, Hellemans J, Ghoos Y. The interdigestive motor complex of normal subjects and patients with bacterial overgrowth of the small intestine. *J Clin Invest*. (1977) 59:1158–66. doi: 10.1172/JCI108740

174. Kashyap P, Farrugia G. Enteric autoantibodies and gut motility disorders. *Gastroenterol Clin N Am*. (2008) 37:397–410. doi: 10.1016/j.gtc.2008.02.005

175. Therrien A, Bouchard S, Sidani S, Bouin M. Prevalence of small intestinal bacterial overgrowth among chronic pancreatitis patients: A case-control study. *Can J Gastroenterol Hepatol*. (2016) 2016:7424831–7. doi: 10.1155/2016/7424831

176. Liu S, Miriyala S, Keaton MA, Jordan CT, Wiedl C, Clair DKS, et al. Metabolic effects of acute thiamine depletion are reversed by rapamycin in breast and leukemia cells. *PLoS One*. (2014) 9:e85702. doi: 10.1371/journal.pone.0085702

177. Waldenlind L. Possible role of thiamine in neuromuscular transmission. *Acta Physiol Scand*. (1979) 105:1–10. doi: 10.1111/j.1748-1716.1979.tb06309.x

178. Vanuytsel T, Tack J, Farre R. The role of intestinal permeability in gastrointestinal disorders and current methods of evaluation. *Front Nutr*. (2021) 8:717925. doi: 10.3389/fnut.2021.717925

179. Beauchesne E, Desjardins P, Butterworth RF, Hazell AS. Up-regulation of caveolin-1 and blood-brain barrier breakdown are attenuated by N-acetylcysteine in thiamine deficiency. *Neurochem Int*. (2010) 57:830–7. doi: 10.1016/j.neuint.2010.08.022

180. Beauchesne É, Desjardins P, Hazell AS, Butterworth RF. Altered expression of tight junction proteins and matrix metalloproteinases in thiamine-deficient mouse brain. *Neurochem Int*. (2009) 55:275–81. doi: 10.1016/j.neuint.2009.03.014

181. Wen L-M, Jiang W-D, Liu Y, Wu P, Zhao J, Jiang J, et al. Evaluation the effect of thiamin deficiency on intestinal immunity of young grass carp (*Ctenopharyngodon idella*). *Fish Shellfish Immunol*. (2015) 46:501–15. doi: 10.1016/j.fsi.2015.07.001

182. Kunisawa J, Sugiura Y, Wake T, Nagatake T, Suzuki H, Nagasawa R, et al. Mode of bioenergetic metabolism during B cell differentiation in the intestine determines the distinct requirement for vitamin B1. *Cell Rep*. (2015) 13:122–31. doi: 10.1016/j.celrep.2015.08.063

183. Mantis NJ, Rol N, Corthésy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol*. (2011) 4:603–11. doi: 10.1038/mi.2011.41

184. Oudman E, Wijnia JW, Oey MJ, van Dam M, Postma A. Wernicke's encephalopathy in Crohn's disease and ulcerative colitis. *Nutrition*. (2021) 86:111182. doi: 10.1016/j.nut.2021.111182

185. de Sire R, Rispo A, Compare D, Tortora F, Nardone G, Castiglione F. Wernicke encephalopathy in ulcerative colitis. *Inflamm Bowel Dis*. (2022) 28:e70–1. doi: 10.1093/ibd/izab296

186. Yoon SM. Micronutrient deficiencies in inflammatory bowel disease: trivial or crucial? *Intest Res*. (2016) 14:109–10. doi: 10.5217/ir.2016.14.2.109

187. Mańkowska-Wierzbicka D, Michalak S, Karczewski J, Dobrowolska A, Wierzbicka A, Stelmach-Mardas M. Erythrocyte transketolase deficiency in patients suffering from Crohn's disease. *Eur Rev Med Pharmacol Sci*. (2019) 23:8501–5. doi: 10.26355/eurrev_201910_19163

188. Pan X, Ren Z, Liang W, Dong X, Li J, Wang L, et al. Thiamine deficiency aggravates experimental colitis in mice by promoting glycolytic reprogramming in macrophages. *Br J Pharmacol*. (2025). doi: 10.1111/bph.17435

189. Van Welden S, Selfridge AC, Hindryckx P. Intestinal hypoxia and hypoxia-induced signalling as therapeutic targets for IBD. *Nat Rev Gastroenterol Hepatol*. (2017) 14:596–611. doi: 10.1038/nrgastro.2017.101

190. Sabui S, Ramamoorthy K, Romero JM, Simoes RD, Fleckenstein JM, Said HM. Hypoxia inhibits colonic uptake of the microbiota-generated forms of vitamin B1 via HIF-1α-mediated transcriptional regulation of their transporters. *J Biol Chem*. (2022) 298:562. doi: 10.1016/j.jbc.2022.101562

191. Martínez-Moya P, Ortega-González M, González R, Anzola A, Ocón B, Hernández-Chirlaque C, et al. Exogenous alkaline phosphatase treatment complements endogenous enzyme protection in colonic inflammation and reduces bacterial translocation in rats. *Pharmacol Res*. (2012) 66:144–53. doi: 10.1016/j.phrs.2012.04.006

192. Goldberg RF, William G, Austen J, Zhang X, Munene G, Mostafa G, et al. Intestinal alkaline phosphatase is a gut mucosal defense factor maintained by enteral nutrition. *Proc Natl Acad Sci USA*. (2008) 105:3551–6. doi: 10.1073/pnas.0712140105

193. Gagliani N, Palm NW, De ZMR, Flavell RA. Inflammasomes and intestinal homeostasis: regulating and connecting infection, inflammation and the microbiota. *Int Immunol*. (2014) 26:495–9. doi: 10.1093/intimm/dxu066

194. Achamrah N, Déchelotte P, Coëffier M. Glutamine and the regulation of intestinal permeability: from bench to bedside. *Curr Opin Clin Nutr Metab Care*. (2016) 20:86–91. doi: 10.1097/MCO.0000000000000339

195. Mahmood S, Dani HM, Mahmood A. Effect of dietary thiamin deficiency on intestinal functions in rats. *Am J Clin Nutr*. (1984) 40:226–34. doi: 10.1093/ajcn/40.2.226

196. Araújo WL, Trofimova L, Mkrtchyan G, Steinhauser D, Krall L, Graf A, et al. On the role of the mitochondrial 2-oxoglutarate dehydrogenase complex in amino acid metabolism. *Amino Acids*. (2013) 44:683–700. doi: 10.1007/s00726-012-1392-x

197. Trofimova LK, Araújo WL, Strokina AA, Fernie AR, Bettendorff L, Bunik VI. Consequences of the α-ketoglutarate dehydrogenase inhibition for neuronal metabolism and survival: implications for neurodegenerative diseases. *Curr Med Chem*. (2012) 19:5895–906. doi: 10.2174/092986712804143367

198. Culliford A, Markowitz D, Rotterdam H, Green PHR. Scalloping of duodenal mucosa in Crohn's disease. *Inflamm Bowel Dis*. (2004) 10:270–3. doi: 10.1097/00054725-200405000-00015

199. Costantini A, Pala MI. Thiamine and fatigue in inflammatory bowel diseases: an open-label pilot study. *J Altern Complement Med*. (2013) 19:704–8. doi: 10.1089/acm.2011.0840

200. Bager P, Hvas CL, Rud CL, Dahlerup JF. Randomised clinical trial: high-dose oral thiamine versus placebo for chronic fatigue in patients with quiescent inflammatory bowel disease. *Aliment Pharmacol Ther*. (2021) 53:79–86. doi: 10.1111/apt.16166

201. Cristina M-O, Elizabeth B-R, Jose R-AM, Berenice P-G, Diego Z, Luis C-SJ. Mechanisms and therapeutic potential of key anti-inflammatory Metabiotics: trans-

Vaccenic acid, Indole-3-lactic acid, thiamine, and butyric acid. *Probiotics Antimicrob Proteins*. (2025). doi: 10.1007/s12602-025-10475-9

202. Zhang H, Peng AL, Zhao FF, Yu LH, Wang MZ, Osorio JS, et al. Thiamine ameliorates inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis. *J Dairy Sci*. (2020) 103:1931–43. doi: 10.3168/jds.2019-16944

203. Pan XH, Yang L, Beckers Y, Xue FG, Tang ZW, Jiang LS, et al. Thiamine supplementation facilitates thiamine transporter expression in the rumen epithelium and attenuates high-grain-induced inflammation in low-yielding dairy cows. *J Dairy Sci*. (2017) 100:5329–42. doi: 10.3168/jds.2016-11966

204. Sabui S, Anthonymuthu S, Ramamoorthy K, Skupsky J, Jennings TSK, Rahmatpanah F, et al. Effect of knocking out mouse Slc44a4 on colonic uptake of the microbiota-generated thiamine pyrophosphate and colon physiology. *Am J Physiol Gastrointest Liver Physiol*. (2024) 327:G36–46. doi: 10.1152/ajpgi.00065.2024

205. Ma Y, Elmhadi M, Wang C, Zhang H, Wang H. Dietary supplementation of thiamine down-regulates the expression of mitophagy and endoplasmic reticulum stress-related genes in the rumen epithelium of goats during high-concentrate diet feeding. *Ital J Anim Sci*. (2021) 20:2220–31. doi: 10.1080/1828051X.2021.1985944

206. Folda A, Scalcon V, Tonolo F, Rigobello MP, Bindoli A. Thiamine disulfide derivatives in thiol redox regulation: role of thioredoxin and glutathione systems. *Biofactors*. 51:e2121. doi: 10.1002/biof.2121

207. Bonaz B, Sinniger V, Pellissier S. Anti-inflammatory properties of the vagus nerve: potential therapeutic implications of vagus nerve stimulation. *J Physiol*. (2016) 594:5781–90. doi: 10.1113/JP271539

208. Morrisroe K, Baron M, Frech T, Nikpour M. Small intestinal bacterial overgrowth in systemic sclerosis. *J Scleroderma Relat Disord*. (2020) 5:33–9. doi: 10.1177/2397198319863953

209. Kawaguchi Y, Nakamura Y, Matsumoto I, Nishimagi E, Satoh T, Kuwana M, et al. Muscarinic-3 acetylcholine receptor autoantibody in patients with systemic sclerosis: contribution to severe gastrointestinal tract dysmotility. *Ann Rheum Dis*. (2009) 68:710–4. doi: 10.1136/ard.2008.096545

210. Saegusa Y, Takeda H, Muto S, Oridate N, Nakagawa K, Sadakane C, et al. Decreased motility of the lower esophageal sphincter in a rat model of gastroesophageal reflux disease may be mediated by reductions of serotonin and acetylcholine signaling. *Biol Pharm Bull*. (2011) 34:704–11. doi: 10.1248/bpb.34.704

211. Farré R, Sifrim D. Regulation of basal tone, relaxation and contraction of the lower oesophageal sphincter. Relevance to drug discovery for oesophageal disorders. *Br J Pharmacol*. (2007) 153:858–69. doi: 10.1038/sj.bjp.0707572

212. Kato R, Nakajima K, Takahashi T, Miyazaki Y, Makino T, Kurokawa Y, et al. A case of advanced systemic sclerosis with severe GERD successfully treated with acotiamide. *Surg Case Rep*. (2016) 2:36. doi: 10.1186/s40792-016-0162-5

213. Parthasarathy G, Ravi K, Camilleri M, Andrews C, Szarka LA, Low PA, et al. Effect of neostigmine on gastroduodenal motility in patients with suspected gastrointestinal motility disorders. *Neurogastroenterol Motil*. (2015) 27:1736–46. doi: 10.1111/nmo.12669

214. Jayarajah U, Yapa K, Ranaweera K, Rahuman A, Perera P, Weerasekara D. Successful use of neostigmine for resistant gastroparesis following distal gastrectomy: A case report. *Int J Surg Case Rep*. (2023) 106:108166. doi: 10.1016/j.ijscr.2023.108166

215. Ollat H, Laurent B, Bakchine S, Michel B-F, Touchon J, Dubois B. Effets de l'association de la Sulbutiamine à un inhibiteur de l'acétylcholinestérase dans les formes légères à modérées de la maladie d'Alzheimer. *L'Encéphale*. (2007) 33:211–5. doi: 10.1016/S0013-7006(07)91552-3

216. Matsueda K, Hongo M, Tack J, Aoki H, Saito Y, Kato H. Clinical trial: dose-dependent therapeutic efficacy of acotiamide hydrochloride (Z-338) in patients with functional dyspepsia - 100 mg t.i.d. is an optimal dosage. *Neurogastroenterol Motil*. (2010) 22:618–e173. doi: 10.1111/j.1365-2982.2009.01449.x

217. Shrestha DB, Budhathoki P, Subedi P, Khadka M, Karki P, Sedhai YR, et al. Acotiamide and functional dyspepsia: A systematic review and Meta-analysis. *Cureus*. (2021) 13:e20532. doi: 10.7759/cureus.20532

218. Nowlan ML, Scott LJ. Acotiamide: first global approval. *Drugs*. (2013) 73:1377–83. doi: 10.1007/s40265-013-0100-9

219. Szutowicz A, Bielarczyk H, Ronowska A, Gul-Hinc S, Klimaszewska-Łata J, Dyś A, et al. Intracellular redistribution of acetyl-CoA, the pivotal point in differential susceptibility of cholinergic neurons and glial cells to neurodegenerative signals. *Biochem Soc Trans*. (2014) 42:1101–6. doi: 10.1042/BST20140078

220. Bizon-Zygmańska D, Jankowska-Kulawy A, Bielarczyk H, Pawełczyk T, Ronowska A, Marszałł M, et al. Acetyl-CoA metabolism in amprolium-evoked thiamine pyrophosphate deficits in cholinergic SN56 neuroblastoma cells. *Neurochem Int*. (2011) 59:208–16. doi: 10.1016/j.neuint.2011.04.018

221. Mkrtchyan G, Aleshin V, Parkhomenko Y, Kaehne T, Luigi Di Salvo M, Parroni A, et al. Molecular mechanisms of the non-coenzyme action of thiamin in brain: biochemical, structural and pathway analysis. *Sci Rep*. (2015) 5:12583. doi: 10.1038/srep12583

222. Szutowicz A, Bielarczyk H, Jankowska-Kulawy A, Pawełczyk T, Ronowska A. Acetyl-CoA the key factor for survival or death of cholinergic neurons in course of neurodegenerative diseases. *Neurochem Res*. (2013) 38:1523–42. doi: 10.1007/s11064-013-1060-x

223. Szutowicz A, Madziar B, Pawełczyk T, Tomaszewicz M, Bielarczyk H. Effects of NGF on acetylcholine, acetyl-CoA metabolism, and viability of differentiated and non-differentiated cholinergic neuroblastoma cells. *J Neurochem*. (2004) 90:952–61. doi: 10.1111/j.1471-4159.2004.02556.x

224. Madziar B, Tomaszewicz M, Matecki A, Bielarczyk H, Szutowicz A. Interactions between p75 and TrkA receptors in differentiation and vulnerability of SN56 cholinergic cells to beta-amyloid. *Neurochem Res*. (2003) 28:461–5. doi: 10.1023/a:1022800802179

225. Wada-Takahashi S, Tamura K. Actions of reactive oxygen species on AH/type 2 myenteric neurons in guinea pig distal colon. *Am J Physiol Gastrointest Liver Physiol*. (2000) 279:G893–902. doi: 10.1152/ajpgi.2000.279.5.G893

226. Brown IAM, McClain JL, Watson RE, Patel BA, Gulbransen BD. Enteric glia mediate neuron death in colitis through purinergic pathways that require Connexin-43 and nitric oxide. *Cell Mol Gastroenterol Hepatol*. (2016) 2:77–91. doi: 10.1016/j.jcmgh.2015.08.007

227. Karuppagounder SS, Xu H, Shi Q, Chen LH, Pedrini S, Pechman D, et al. Thiamine deficiency induces oxidative stress and exacerbates the plaque pathology in Alzheimer's mouse model. *Neurobiol Aging*. (2009) 30:1587–600. doi: 10.1016/j.neurobiolaging.2007.12.013

228. Campanucci VA, Krishnaswamy A, Cooper E. Mitochondrial reactive oxygen species inactivate neuronal nicotinic acetylcholine receptors and induce Long-term depression of fast nicotinic synaptic transmission. *J Neurosci*. (2008) 28:1733–44. doi: 10.1523/JNEUROSCI.5130-07.2008

229. Stavely R, Abalo R, Nurgali K. Targeting enteric neurons and Plexitis for the Management of Inflammatory Bowel Disease. *Curr Drug Targets*. (2020) 21:1428–39. doi: 10.2174/1389450121666200516173242

230. Lakhan SE, Kirchgessner A. Neuroinflammation in inflammatory bowel disease. *J Neuroinflammation*. (2010) 7:37. doi: 10.1186/1742-2094-7-37

231. Sasa M, Takemoto I, Nishino K, Itokawa Y. The role of thiamine on excitable membrane of crayfish Giant axon. *J Nutr Sci Vitaminol (Tokyo)*. (1976) 22 SUPPL:21–4. doi: 10.3177/jnsv.22.Supplement_21

232. Nghiem H-O, Bettendorff L, Changeux J-P. Specific phosphorylation of Torpedo 43K rapsyn by endogenous kinase(s) with thiamine triphosphate as the phosphate donor. *FASEB J*. (2000) 14:543–54. doi: 10.1096/fasebj.14.3.543

233. Parkhomenko IM, Strokina AA, Pilipchuk SI, Stepanenko SP, Chekhovskaia LI, Donchenko GV. Existence of two different active sites on thiamine binding protein in plasma membranes of synaptosomes. *Ukr Biokhimichnyi Zh 1999*. (2010) 82:34–41.

234. Parkhomenko YM, Vovk AI, Protasova ZS, Pylypchuk SY, Chorny SA, Pavlova OS, et al. Thiazolium salt mimics the non-coenzyme effects of vitamin B1 in rat synaptosomes. *Neurochem Int*. (1999) 178:105791. doi: 10.1016/j.neuint.2024.105791

235. Yamashita A, Shimamoto N, Morita K, Sugiyama H, Kimoto M, Toda K, et al. Thiamine and quinine differently inhibit the early phase of acetylcholine-dependent contraction of mouse ileum in vitro. *Int J Nutr Food Sci*. (2018) 7:94–9. doi: 10.11648/j.ijnfs.20180703.13

236. Yamashita A, Shimamoto N, Morita K, Sugiyama H, Tadakuma S, Kato M, et al. Differential inhibition of the rhythm and amplitude of acetylcholine-dependent contraction in the murine jejunum and ileum in vitro by thiamin and quinine. *J Food Nutr Sci*. (2018) 6:115–22. doi: 10.11648/j.jfns.20180605.11

237. Waldenlind L, Elfman L, Rydqvist B. Binding of thiamine to nicotinic acetylcholine receptor in Torpedo marmorata and the frog end plate. *Acta Physiol Scand*. (1978) 103:154–9. doi: 10.1111/j.1748-1716.1978.tb06202.x

238. Hirsch JA, Parrott J. New considerations on the neuromodulatory role of thiamine. *Pharmacology*. (2012) 89:111–6. doi: 10.1159/000336339

239. Bai L, Mesgarzadeh S, Ramesh KS, Huey EL, Liu Y, Gray LA, et al. Genetic identification of vagal sensory neurons that control feeding. *Cell*. (2019) 179:1129–1143.e23. doi: 10.1016/j.cell.2019.10.031

240. Sinclair R, Bajekal RR. Vagal nerve stimulation and reflux. *Anesth Analg*. (2007) 105:884–5. doi: 10.1213/01.ane.0000269687.74660.45

241. Lu K-H, Cao J, Oleson S, Ward MP, Phillips R, Powley TL, et al. Vagus nerve stimulation promotes gastric emptying by increasing pyloric opening measured with magnetic resonance imaging. *Neurogastroenterol Motil*. (2018) 30:e13380. doi: 10.1111/nmo.13380

242. Paulon E, Nastou D, Jaboli F, Marin J, Liebler E, Epstein O. Proof of concept: short-term non-invasive cervical vagus nerve stimulation in patients with drug-refractory gastroparesis. *Frontline Gastroenterol*. (2017) 8:325–30. doi: 10.1136/flgastro-2017-100809

243. Costantini TW, Bansal V, Krzyzaniak M, Putnam JG, Peterson CY, Loomis WH, et al. Vagal nerve stimulation protects against burn-induced intestinal injury through activation of enteric glia cells. *Am J Physiol*. (2010) 299:G1308–18. doi: 10.1152/ajpgi.00156.2010

244. Rao M, Gershon MD. The bowel and beyond: the enteric nervous system in neurological disorders. *Nat Rev Gastroenterol Hepatol*. (2016) 13:517–28. doi: 10.1038/nrgastro.2016.107

245. Fleming MA II, Ehsan L, Moore SR, Levin DE. The enteric nervous system and its emerging role as a therapeutic target. *Gastroenterol Res Pract*. (2020) 2020:8024171–13. doi: 10.1155/2020/8024171

246. Jakob MO, Murugan S, Klose CSN. Neuro-immune circuits regulate immune responses in tissues and organ homeostasis. *Front Immunol*. (2020) 11:308. doi: 10.3389/fimmu.2020.00308

247. Romanenko AV, Gnatenko VM, Vladimirova IA. Effect of thiamine on neuromuscular transmission in smooth muscles. *Neurophysiology*. (1994) 26:370–7. doi: 10.1007/BF01053581

248. Fujioka F, Choki S, Suzuki T, Sahashi Y. Stimulating effect of thiamine and its derivatives on rat intestinal muscular contraction. *Vitamins*. (1966) 34:390–2. doi: 10.20632/vso.34.4_390

249. Hukuhara T, Fukuda H. The effects of thiamine Tetrahydrofurfuryl disulfide upon the movement of the isolated small intestine. *J Vitaminol (Kyoto)*. (1965) 11:253–60. doi: 10.5925/jnsv1954.11.253

250. Saakyan AG, Arutyunova NL, Nikolaeva EA, Saakyan AG, Arutyunova NL, Nikolaeva EA. Effect of vitamins B1, B6, B12, and C on the motor activity of the stomach, small and large intestines in patients with chronic enterocolitis and colitis. *Vopr Pitan*. (1964) 23:45–50.

251. Tsuchida S, Kimura Y. Electromyography of the intestines by the Intraintestinal method. *Tohoku J Exp Med*. (1966) 89:61–8. doi: 10.1620/tjem.89.61

252. Nakayama S, Nanba R. Effect of thiamine Tetrahydrofurfuryldisulfide on the peristalsis of the small bowel. *Vitamins*. (1963) 28:235–7. doi: 10.20632/vso.28.3_235

253. Hukuhara T, Nanba R, Siina H. Effect of thiamine Tetrahydrofurfuryl disulfide upon the intestinal motility. *J Vitaminol (Kyoto)*. (1965) 11:210–4. doi: 10.5925/jnsv1954.11.210

254. Hills JI, Golub MS, Bettendorff L, Keen CL. The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice. *Neurotoxicol Teratol*. (2012) 34:242–52. doi: 10.1016/j.ntt.2011.07.006

255. Lonsdale D, Shamberger RJ, Audhya T. Treatment of autism spectrum children with thiamine tetrahydrofurfuryl disulfide: a pilot study. *Neuro Endocrinol Lett*. (2002) 23:303–8.

256. Mizutani M, Ihara T, Kaziwara K. Effects of orally administered thiamine tetrahydrofurfuryl disulfide on foetal development of rabbits and monkeys. *Jpn J Pharmacol*. (1972) 22:115–24. doi: 10.1254/jjp.22.115

257. Oi Y, Shishido C, Wada K, Odaka H, Ikeda H, Iwai K. Allylthiamindisulfide and related compounds enhance thermogenesis with increasing noradrenaline and adrenaline secretion in rats. *J Nutr Sci Vitaminol (Tokyo)*. (1999) 45:643–53. doi: 10.3177/jnsv.45.643

258. Narat JK, Loef JA. Effects of a vitamin B1 concentrate. *Arch Intern Med*. (1937) 60:449–53. doi: 10.1001/archinte.1937.00180030066006

259. Romanenko AV. Effect of thiamine on various types of synaptic junctions. *Neirofiziol Neurophysiol*. (1986) 18:621–9.

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The advanced lung cancer inflammation index has an L-shaped association with prognosis in American adults with metabolic dysfunction-associated fatty liver disease: a cohort study

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Background: Regular monitoring and follow-up of patients with metabolic dysfunction-associated fatty liver disease (MAFLD) are of paramount importance in ensuring effective management of the condition. The ALI was assessed as a composite measure reflecting nutritional status and systemic inflammation. It was calculated as body mass index (BMI) (kg/m^2) × serum albumin (g/dL)/neutrophil-to-lymphocyte ratio (NLR). Our study aims to find the relationship between advanced lung cancer inflammation index (ALI) levels and the prognosis of patients with MAFLD and to determine the predictive value of ALI in this context.

Methods: Multivariate-adjusted Cox regression models were used to analyze the association between ALI and all-cause, cardiovascular, cancer, and diabetes-related mortalities in patients with MAFLD. Kaplan–Meier curves showed the association of ALI with all-cause and cardiovascular mortalities in patients with MAFLD. Follow-up time for this study was calculated from the date of examination to the date of death or to 31 December 2019, and mortality was ascertained using the International Classification of Diseases, 10th Revision codes. Restricted cubic spline (RCS) analysis was conducted to assess the potential non-linear relationship between ALI level and MAFLD prognosis. The predictive ability of ALI was observed using receiver operating characteristic (ROC) curves. Stratified and sensitivity analyses were used to enhance the reliability and robustness.

Results: This study included 2,908 patients with MAFLD from the National Health and Nutrition Examination Survey (NHANES) database between 2003 and 2018. The median follow-up period for the 2,908 participants was 10.3 years, during which 636 deaths occurred. In the Cox regression model, the HRs (95%CIs) for all-cause, cardiovascular, cancer, and diabetes-related mortalities in the last quartile compared to the first quartile of ALI levels were 0.62 (0.44–0.85), 0.25 (0.14–0.45), 0.96 (0.51–1.81), and 0.69 (0.25–1.92), respectively. RCS analysis demonstrated a L-shaped non-linear association between ALI levels and both all-cause and cardiovascular mortalities in participants with MAFLD. Subgroup analyses highlighted population heterogeneity in the relationship between ALI and MAFLD prognosis. ROC curve analysis showed that ALI had strong predictive power for all-cause and cardiovascular mortalities, with area under the curve values of 0.80 (0.77–0.83) and 0.82 (0.74–0.89), respectively.

Conclusion: There was an L-shaped nonlinear association of the protective effect of ALI: when the indicators are below specific thresholds (all-cause mortality 71.48, cardiovascular mortality 68.54), a higher ALI was significantly associated with reduced mortality risks in MAFLD patients; otherwise the protective effect tended to be consistent. ALI exhibits a robust predictive capability for all-cause and cardiovascular mortalities among participants with MAFLD, providing a valuable prognostic tool for optimizing patient management. We recommend early surveillance and management of patients with MAFLD to improve patient survival.

KEYWORDS

MAFLD, ALI, FLI, NHANES, mortality

1 Introduction

Originating in 2020, metabolic dysfunction-associated fatty liver disease (MAFLD) was conceptualized to supersede the traditional term: non-alcoholic fatty liver disease (NAFLD). In 2023, metabolic dysfunction-associated steatotic liver disease (MASLD) was introduced as an updated nomenclature. A study showed (1) significant concordance across NAFLD, MAFLD, and MASLD classifications.

MAFLD is a widespread liver disease of significant global concern, affecting approximately one-third of the global population, with an increasing prevalence (2). In addition to liver-specific morbidity and mortality, MAFLD is associated with an elevated risk of extrahepatic conditions, including type 2 diabetes mellitus (T2DM), extrahepatic cancers (notably colorectal cancer), cardiovascular disease and chronic kidney disease. Therefore, this disease places a heavy burden on global health (3, 4). Identifying reliable prognostic markers for MAFLD is critical for enabling early intervention and effective disease management.

Chronic inflammatory responses have been identified as pivotal pathophysiological mechanisms that drive MAFLD initiation and progression, potentially through oxidative stress and insulin resistance (5–9). Persistent inflammation can exacerbate progressive extracellular matrix (ECM) deposition, a process closely associated with adverse outcomes such as liver failure, hepatocellular carcinoma, and death (10). Serum albumin is a key marker of nutritional status. Serum albumin level represents a potential biomarker for early hepatic failure and a possible predictor (11). Therefore, the prognosis of MAFLD patients is related to multiple factors. The advanced lung cancer inflammation index (ALI) combines the status of inflammation, albumin levels, and BMI levels, and can comprehensively assess their impact on the outcomes of MAFLD patients. Previous studies have also confirmed the prognostic value of ALI in chronic diseases.

Initially developed for evaluating the prognosis of lung cancer (12, 13), ALI has since been applied to other conditions, including hypertension (14), coronary artery disease (15), and various malignancies (16). Despite ALI's widespread use, no study to date has explored its relationship with the prognosis of those with MAFLD. And our research has worked on this.

2 Materials and methods

2.1 Study population

This cohort study used data from the National Health and Nutrition Examination Survey (NHANES), which employs a stratified, multistage probability sample strategy to perform cross-sectional assessments of the health and nutritional status of the American people. An ethics review board from the National Center for Health Statistics authorized the NHANES protocol. Our analysis collected data from eight survey cycles, spanning 16 years, from 2003 to 2018. The procedure for including and excluding data is shown in Figure 1.

2.2 ALI scoring

ALI is a composite score that reflects both inflammation levels and nutritional status. A high value of ALI suggests a low level of inflammation and good nutritional. The formula for ALI is provided in Figure 2. The formula includes neutrophil-to-lymphocyte ratio (NLR), which is an indicator of inflammation.

2.3 MAFLD diagnosis

Liver biopsy is the best way to diagnose MAFLD. However, its invasiveness, high cost, and potential complications limit its wide application (17, 18). Thus, non-invasive markers provide a more practical diagnostic approach. In this study, we employed the United States fatty liver index (US-FLI), a non-invasive marker developed and validated using the NHANES database, with a diagnostic area under the curve (AUC) of 0.80 (19). A US-FLI score of ≥30 was considered to have hepatic steatosis. The third equation in the Figure 2 shows the equation of the US-FLI. To verify the robustness of our results, we performed a sensitivity analysis using a widely validated marker: the Fatty Liver Index (FLI), with a diagnostic accuracy of 0.84 (20). An FLI score ≥60 was considered indicative of hepatic steatosis. The fourth equation in Figure 2 demonstrates the equation of the FLI.

2.4 Follow-up and outcomes

The mortality outcomes and classification were determined by linking to the National Death Index (NDI) records. The NDI provides detailed mortality data using standardized codes based on the International Classification of Diseases, Tenth Revision (ICD-10). The follow-up period was calculated from the date of examination to the date of death or the end of the follow-up period. The follow-up of participants in this study continued until December 31, 2019.

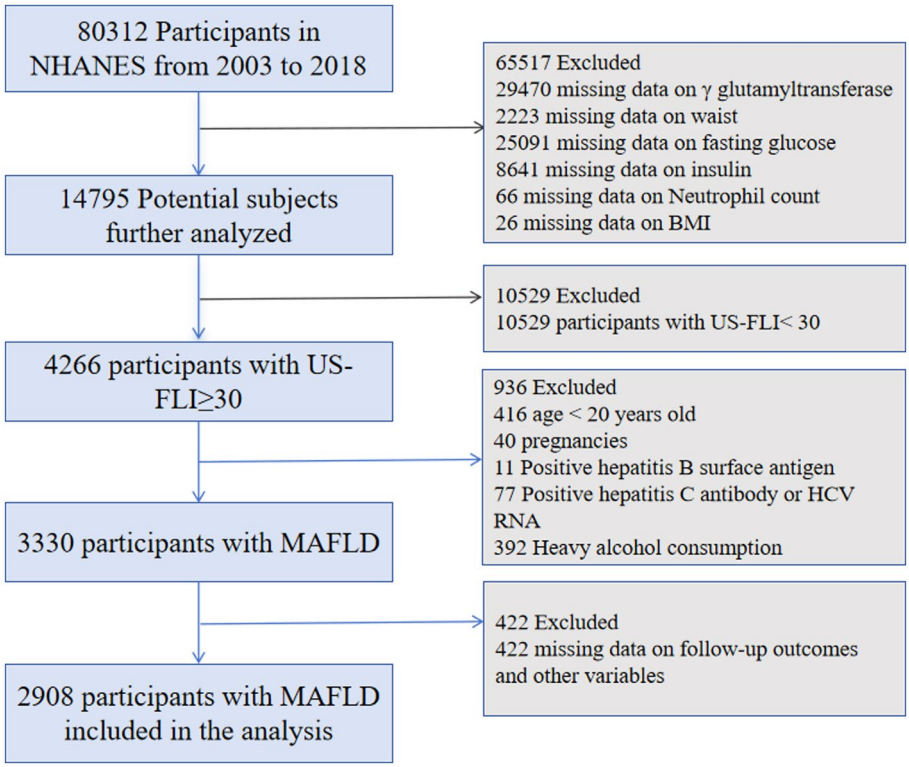

FIGURE 1
Flowchart depicting the process of selecting the study population.

$$ALI = \frac{\text{(Body mass index) (kg/m\^2)} \times \text{serum albumin (g/dL)}}{\text{NLR}}$$

$$NLR = \frac{\text{neutrophil count } (\times 10^9)}{\text{lymphocyte count } (\times 10^9)}$$

$$\text{US-FLI} = \frac{e^{-0.8073 \times \text{non-Hispanic black} + 0.3458 \times \text{Mexican American} + 0.0093 \times \text{age} + 0.6151 \times \ln(\gamma\text{-glutamyltransferase}) + 0.0249 \times \text{waist circumference} + 1.1792 \times \ln(\text{insulin}) + 0.8242 \times \ln(\text{glucose}) - 14.7812}}{1 + e^{-0.8073 \times \text{non-Hispanic black} + 0.3458 \times \text{Mexican American} + 0.0093 \times \text{age} + 0.6151 \times \ln(\gamma\text{-glutamyltransferase}) + 0.0249 \times \text{waist circumference} + 1.1792 \times \ln(\text{insulin}) + 0.8242 \times \ln(\text{glucose}) - 14.7812}} \times 100$$

$$\text{FLI} = \frac{e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times (\text{Body mass index}) + 0.718 \times \ln(\gamma\text{-glutamyltransferase}) + 0.053 \times \text{waist circumference} - 15.745}}{1 + e^{0.953 \times \ln(\text{triglycerides}) + 0.139 \times (\text{Body mass index}) + 0.718 \times \ln(\gamma\text{-glutamyltransferase}) + 0.053 \times \text{waist circumference} - 15.745}} \times 100$$

FIGURE 2
The formula for ALI, NLR, US-FLI and FLI.

2.5 Covariates

Covariates were selected based on previous studies (21). These covariates included demographic characteristics [race, sex, age, education, and poverty-income ratio (PIR)], laboratory tests [high-density lipoprotein (HDL)-C levels, alanine transaminase (ALT), aspartate aminotransferase (AST), triglycerides (TG), and total cholesterol (TC)], other diseases [diabetes (DM), cardiovascular disease (CVD), and high blood pressure (HBP)], and behavioral factors (physical activity and smoking). Daily energy intake and Body mass index (BMI) were also included. Data from laboratory tests were analyzed as continuous variables, and the descriptions of the remaining covariates are shown in Figure 3. Age and PIR were grouped according to previous studies (22, 23). The cut-off values of BMI were determined based on the criteria of the World Health Organization (WHO).

2.6 Statistical analysis

Categorical variables were represented as percentages (%), while continuous variables were summarized using the mean

(standard error) or median (interquartile range). For data analysis, we used the chi-square test for categorical variables, t-test for normally distributed data, and Wilcoxon rank-sum test for skewed data. Weighted Cox proportional hazards models were employed to evaluate the association between ALI and mortality outcomes, including all-cause, cardiovascular, cancer, and diabetes-related mortalities. Kaplan–Meier survival curves were generated to visually depict the link between ALI and all-cause and cardiovascular mortalities.

To investigate if ALI and cardiovascular and all-cause deaths have a non-linear connection among people with MAFLD, we performed restricted cubic spline (RCS) testing. Subgroup analyses, stratified by education, race, sex, age, diabetes, hypertension, and cardiovascular disease, were conducted to examine the association between ALI and all-cause and cardiovascular mortalities in specific populations. For visual clarity, ALI values were rescaled to ALI/10 in the subgroup analyses. Finally, sensitivity analyses were performed to view the stability of the findings under various data processing scenarios.

The data was processed and analyzed using R version 4.3.0 and the Storm Statistical Platform. The threshold for statistical significance was $p < 0.05$.

Covariates	Explanation
age	The age was divided into less than 40, between 40 and 59, or 60 and above.
gender	The gender was divided into "Male" or "Female".
race	The race was divided into "Mexican American", "Other", "Non-Hispanic White" or "Non-Hispanic Black".
education	The education was divided into "Less than high school", "high school" or "Above high school".
PIR	The PIR was divided into "less than 1(low income)", "between 1 and 3(middle income)", or "more than 3 (higher level of income)".
HBP	A "YES" answer in questionnaires asking whether the participant had been diagnosed with this disease by a doctor.This covariate was analyzed using "YES" or "NO".
DM	A "YES" answer in questionnaires asking whether the participant had been diagnosed with this disease by a doctor.This covariate was analyzed using "YES" or "NO".
CVD	Cardiovascular disease history was determined by questionnaire responses regarding congestive heart failure, coronary heart disease, angina/angina pectoris, or heart attack.This covariate was analyzed using "YES" or "NO".
smoke	Participants were categorized into former smokers, current smokers and never smokers based on their responses to the questionnaire. Former smokers were those who had smoked more than 100 cigarettes in their lifetime but were not current smokers. Current smokers were defined as those who had previously smoked >100 cigarettes and were currently smoking. Non-smokers were defined as those who denied having smoked 100 cigarettes in their lifetime.
activity	Participants who had engaged in vigorous or moderate physical activity in the past 30 days were considered physically active; otherwise, they were considered physically inactive. Participants who answered no clear were categorized as "Unknown".
BMI	Body mass index (BMI) was categorized as less than 25, 25–29.9, or 30 and above.
daily energy intake	Dietary data in NHANES were based on a 24-hour recall method and energy values were calculated by trained staff. Energy intake was analyzed using continuous variables.

FIGURE 3
The details of the covariates.

3 Results

3.1 Baseline characteristics

The initial dataset included 80,312 participants. Those with missing data on ALI and US-FLI (n = 65,517), US-FLI scores <30 (n = 10,529), minors (n = 416), pregnant women (n = 40), individuals with hepatitis B (n = 11) or hepatitis C (n = 77), excessive alcohol consumption (n = 392), and missing survival outcomes or covariates (n = 422) were excluded, leaving 2,908 patients with MAFLD (Figure 1).

Participants were stratified into four quartiles based on ALI values: Q1 (ALI ≤ 49.53), Q2 (49.53 < ALI ≤ 68.54), Q3 (68.54 < ALI ≤ 90.99), and Q4 (ALI > 90.99). In Table 1, we can see that compared with participants in the lower ALI quartiles, those in the higher ALI quartiles were older, less likely to be obese, and had fewer non-Hispanic White individuals. They also had higher education levels, greater energy intake, and a lower prevalence of hypertension, diabetes, and cardiovascular diseases. Participants who had never smoked were more likely to have higher ALI values. The levels of HDL-C, ALT, AST, TG, and TC, as well as physical activity, were similar across the ALI quartiles, and the poverty-income ratio showed no significant differences among the groups.

Continuous variables not conforming to normal distribution were expressed as median (IQR) and compared using the Wilcoxon rank sum test.

3.2 ALI and mortality in patients with MAFLD

The median follow-up period for the 2,908 participants was 10.3 years, during which 636 deaths occurred, leading to a mortality rate of 21.87%. Among these, there were 166 cardiovascular, 154 cancer, and 49 diabetes-related deaths.

As shown in the Table 2, after adjusting for all covariates, compared with the Q1 group, the hazard ratios (HRs) (95% CI) for

TABLE 1 Baseline characteristics of the participants (N = 2,908).

Characteristics	ALI				p
	Quantile 1	Quantile 2	Quantile 3	Quantile 4	
	<49.53	49.53–68.54	68.54–90.99	>90.99	
Participants, n	727	727	727	727	
Energy intake, median (IQR), kcal/day	1780 (1364.50–2409.50)	1908 (1419.50–2534.50)	1951 (1397–2,533)	1992 (1460–2,624)	0.006
HDL-C, median (IQR), mg/dL	46 (39–54)	44 (38–52)	44 (38–51)	44 (38–52)	<0.001
ALT, median (IQR) U/L	23 (18–31)	25 (20–34)	27 (21–37)	27 (21–39)	<0.001
AST, median (IQR) U/L	24 (20–28)	24 (20–29)	25 (21–30)	26 (22–32)	0.004
TG, median (IQR), mg/dL	132 (94–193)	144 (105–202.5)	151 (108–202)	147 (101–218.5)	0.004
TC, median (IQR), mg/dL	186 (161–220.5)	196 (170–222)	195 (170–221)	199 (173–226)	<0.001
Gender, n (%)					<0.001
Male	58.84	63.37	58.40	49.81	
Female	41.16	36.63	41.60	50.19	
Race, n (%)					<0.001
Mexican American	8.65	11.20	13.90	14.91	
Other	9.91	8.51	10.00	8.94	
Non-Hispanic White	78.89	76.48	70.28	64.05	
Non-Hispanic Black	2.55	3.81	5.81	12.10	
Education, n (%)					0.023
Less than high school	23.35	21.19	21.21	23.19	
High school	29.59	22.93	26.43	23.58	
Above high school	47.06	55.88	52.36	53.23	
Hypertension, n (%)					0.011
Yes	55.38	50.36	47.60	47.73	
No	44.62	49.64	52.40	52.27	
CVD, n (%)					<0.001
Yes	19.62	10.92	10.42	9.35	
No	80.38	89.08	89.58	90.65	
DM, n (%)					0.046

(Continued)

TABLE 1 (Continued)

Characteristics	ALI				*p*
	Quantile 1	Quantile 2	Quantile 3	Quantile 4	
	<49.53	49.53–68.54	68.54–90.99	>90.99	
Yes	19.70	19.54	17.64	14.21	
No	76.48	76.98	79.75	81.36	
Unknown	3.82	3.48	2.61	4.43	
Smoke status, *n* (%)					<0.001
Former	37.23	32.18	28.19	29.00	
Never	41.36	49.90	53.63	57.33	
Current	21.41	17.92	18.19	13.67	
Age, *n* (%)					<0.001
≤39	47.42	33.88	27.36	24.43	
40–59	35.93	43.71	42.85	42.88	
≥60	16.65	22.41	29.80	32.69	
PIR, *n* (%)					0.327
<1	44.19	46.38	47.17	42.01	
1–3	40.85	41.63	39.37	43.56	
>3	14.97	12.00	13.47	14.43	
BMI, *n* (%)					<0.001
<25	54.14	66.79	80.08	80.87	
25–29.9	35.75	30.00	17.74	17.86	
≥30	10.12	3.21	2.18	1.27	
Physical activity, *n* (%)					0.008
Inactive	60.54	51.22	56.34	59.06	
Active	38.79	48.30	43.31	40.77	
Unknown	0.67	0.48	0.35	0.18	

all-cause mortality in the Q2, Q3, and Q4 groups were 0.70 (0.54–0.91), 0.59 (0.45–0.78), and 0.62 (0.44–0.85), respectively. This indicated that with each unit increase in ALI, the risk of all-cause mortality decreased by 30, 41, and 38% in the Q2 to Q4 groups. For cardiovascular mortality, the HRs (95% CI) were 0.48 (0.28–0.81), 0.46 (0.27–0.77), and 0.25 (0.14–0.45), indicating reductions of 52, 54, and 75%, respectively, compared with Q1. ALI exhibited a stronger positive effect on cardiovascular mortality than on all-cause mortality. ALI level association with cancer or diabetes-related mortalities was not statistically significant. Consequently, subsequent analyses focused on all-cause mortality and cardiovascular mortality.

In Figures 4A,B show the Kaplan–Meier survival analysis curves for all-cause and cardiovascular mortalities, respectively. The values 0–3 represent the four ALI quartiles (Q1, Q2, Q3, Q4). All-cause and cardiovascular mortalities were reduced among participants with MAFLD when the ALI was greater than the Q1 level.

3.3 Non-linear relationship analysis

RCS analysis revealed a distinct L-shaped non-linear relationship between ALI and both all-cause and cardiovascular mortalities (Figure 5). The inflection points for all-cause and cardiovascular mortalities were 71.48 and 68.54, respectively.

3.4 Subgroup analysis

Subgroup analysis showed that in patients with MAFLD, 55.95% were male, 49.28% were aged 20–39 years, 41.95% were non-Hispanic White, and 43.71% had an education level above high school (Figure 6). Stratified analyses by sex, race, education, age, diabetes status, hypertension, and cardiovascular disease revealed significant interactions between ALI and all-cause mortality in all strata, except for age. This finding suggests heterogeneity in the association between ALI levels and all-cause mortality across populations. The correlation between ALI and all-cause mortality in patients with MAFLD was more pronounced in women. In the interaction test for cardiovascular mortality, no interaction *p*-values reached statistical significance, except for education level.

3.5 Sensitivity analysis

To confirm the reliability of the results, three additional cox regression analyses were performed (Table 3). Firstly, after excluding extreme ALI values, the association between ALI and both all-cause and cardiovascular mortalities remained significant, with HRs for the Q4 group being 0.62 and 0.25, respectively. Secondly, after excluding participants who died within 2 years of the end of the follow-up

period, the results from the Cox regression analyses remained consistent. Lastly, when FLI ≥ 60 was used as the diagnostic criterion, expanding the cohort to 5,397 participants with MAFLD, the results also remained robust. Sensitivity analyses confirmed that higher ALI levels were associated with a reduced risk of all-cause and cardiovascular mortalities in people with MAFLD, whereas no significant effect on cancer or diabetes-related mortalities was observed.

TABLE 2 The relationship between ALI and all-cause, CVD, cancer, and DM-related mortality.

ALI	Model 1	Model 2	Model 3
	HR (95%CI)	HR (95%CI)	HR (95%CI)
All-cause mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.51 (0.39, 0.66)	0.61 (0.47, 0.79)	0.70 (0.54, 0.91)
Quantile 3	0.34 (0.26, 0.46)	0.53 (0.4, 0.7)	0.59 (0.45, 0.78)
Quantile 4	0.32 (0.24, 0.43)	0.53 (0.39, 0.71)	0.62 (0.44, 0.85)
Cardiovascular mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.38 (0.23, 0.63)	0.46 (0.28, 0.76)	0.48 (0.28, 0.81)
Quantile 3	0.33 (0.2, 0.55)	0.49 (0.3, 0.82)	0.46 (0.27, 0.77)
Quantile 4	0.18 (0.1, 0.33)	0.27 (0.15, 0.5)	0.25 (0.14, 0.45)
Cancer-related mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.79 (0.47, 1.3)	0.96 (0.59, 1.59)	1.09 (0.65, 1.83)
Quantile 3	0.51 (0.29, 0.91)	0.83 (0.47, 1.48)	0.94 (0.52, 1.69)
Quantile 4	0.44 (0.24, 0.77)	0.83 (0.46, 1.48)	0.96 (0.51, 1.81)
Diabetes-related mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.33 (0.14, 0.8)	0.36 (0.15, 0.88)	0.49 (0.16, 1.47)
Quantile 3	0.23 (0.08, 0.68)	0.28 (0.09, 0.83)	0.38 (0.11, 1.33)
Quantile 4	0.37 (0.14, 0.96)	0.41 (0.15, 1.14)	0.69 (0.25, 1.92)

HR, hazard ratio; CI, confidence interval; Ref: reference. Model I: adjusted for no covariates. Model II: adjusted for age, gender, race. Model III: adjusted for age, gender, race, education, BMI, PIR, HDL-C, ALT, AST, TG, TC, HBP, DM, CVD, smoking, activity, daily energy intake.

3.6 ROC curve analysis

ROC curves do not account for time, whereas time-dependent ROC curves evaluate the predictive performance of factors across various time points. These time-dependent ROC curves demonstrated that ALI had excellent predictive accuracy for both all-cause and cardiovascular mortalities in people with MAFLD, with AUC values of 0.80 (0.77–0.83) and 0.82 (0.74–0.89), respectively (Figure 7).

4 Discussion

To our knowledge, this is the first large-scale cohort investigation to explore the prognostic relationship between ALI and MAFLD. Our findings demonstrated a significant inverse association between ALI levels and all-cause and cardiovascular mortalities in patients with MAFLD, whereas no statistically significant relationship was observed for cancer or diabetes-related mortalities. RCS analysis revealed a distinct L-shaped non-linear relationship between ALI levels and mortality outcomes. Subgroup analyses revealed population heterogeneity in ALI levels and all-cause mortality among patients with MAFLD. There was no interaction between the subgroups for cardiovascular mortality, except for education level. ROC curve analysis confirmed the strong predictive ability of ALI for all-cause and cardiovascular mortality, with AUC values of 0.80 and 0.82, respectively.

The roles of inflammation and diet in MAFLD pathogenesis have been widely discussed. Gong et al. (24) discovered that systemic inflammation is strongly associated with hepatic steatosis risk. Pro-inflammatory diets are implicated in increased MAFLD risk, prompting public health initiatives to prioritize nutrient-dense food availability (6). Nutritional deficiencies, including protein-energy malnutrition and subclinical micronutrient deficiencies, are linked to hepatic steatosis (25). Moreover, supplementation with vitamins E and D has shown the potential to improve liver function and lipid metabolism and reduce hepatic steatosis risk (26). Among various dietary patterns, the Mediterranean diet stands out for its benefits in

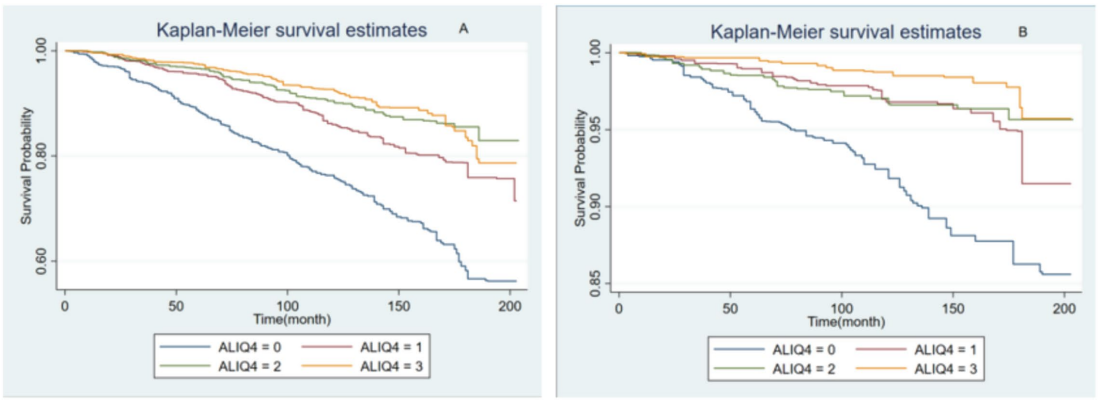

FIGURE 4
The Kaplan–Meier survival analysis curves for all-cause **(A)** and cardiovascular mortalities **(B)**.

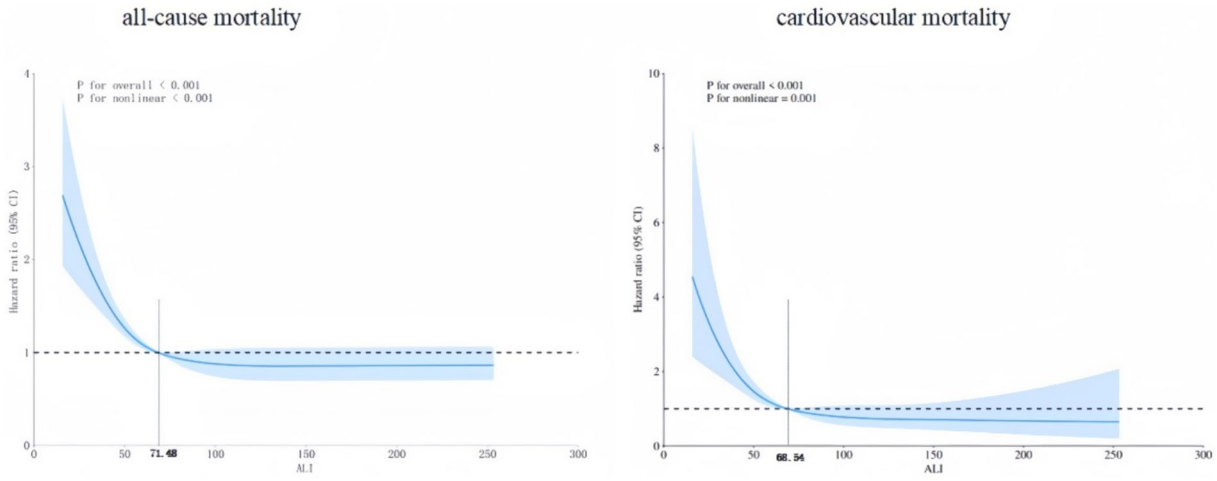

FIGURE 5
The RCS analysis for all-cause and cardiovascular mortalities.

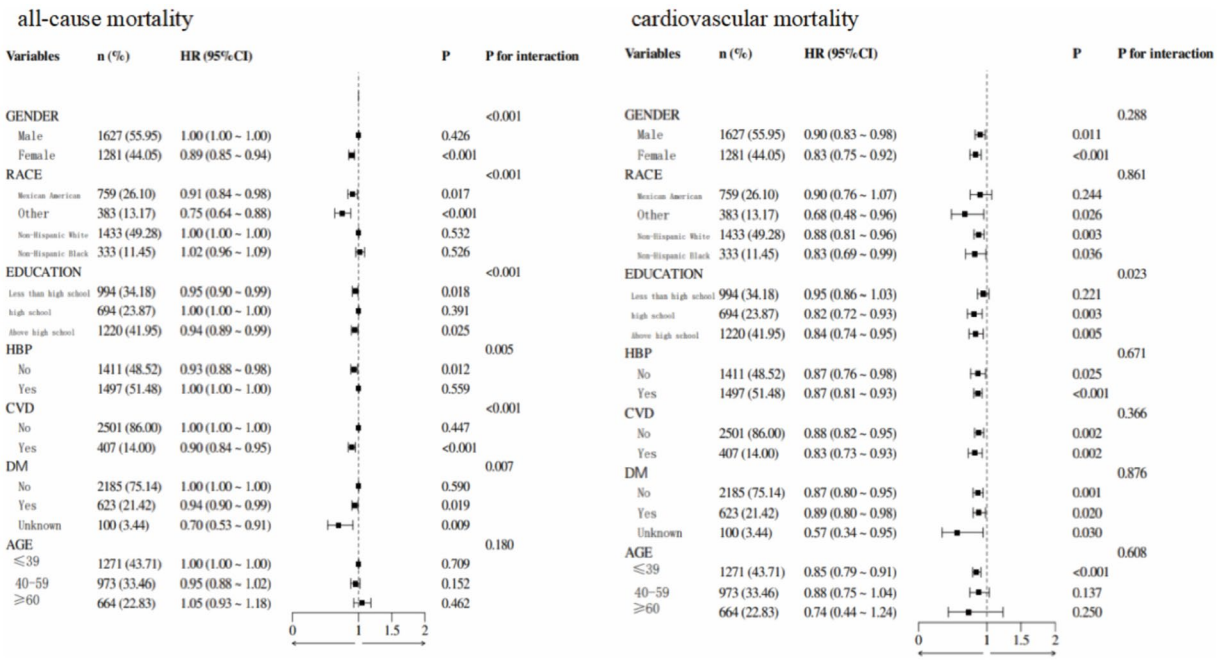

FIGURE 6
Forest plot demonstrated the relationship between ALI and all-cause and cardiovascular mortalities.

reducing cardiovascular risk, hepatic fat accumulation, and fibrosis progression (26). Our study builds upon this foundation, providing further evidence for the interplay between inflammation, nutrition, and prognosis in patients with MAFLD.

Oxidative stress plays a critical role in MAFLD pathogenesis by inducing endoplasmic reticulum stress, increasing pro-inflammatory cytokine release, and activating hepatic stellate cells, leading to fibrosis (26, 27). Approximately 33% of the inflammation-related effects in MAFLD are mediated through the triglyceride-glucose index, highlighting the exacerbating role of insulin resistance in inflammation (28, 29). In contrast, insulin resistance promotes hepatic fat accumulation by increasing free fatty acid flux and hyperinsulinemia-driven anabolic pathways (30). Nutrient-rich foods may play a role in MAFLD prognosis through antioxidant, anti-inflammatory, lipid, and insulin metabolism. Nutrient-rich foods prevent oxidative stress in the liver by reducing glutathione disulfide/glutathione and thiobarbituric acid reactive species in the liver, which can reduce the expression levels of the gene associated with pro-oxidant activity (26). Diet can also balance redox activity by regulating mitochondrial function (31). Nutrient-rich foods may reduce hepatic levels of interleukin (IL)-2,

TABLE 3 Result of sensitivity analysis.

ALI	Model 1	Model 2	Model 3
	HR (95%CI)	HR (95%CI)	HR (95%CI)
① After excluding extreme ALI values			
All-cause mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.51 (0.39, 0.66)	0.61 (0.47, 0.79)	0.70 (0.54, 0.91)
Quantile 3	0.34 (0.26, 0.46)	0.53 (0.4, 0.7)	0.60 (0.45, 0.78)
Quantile 4	0.32 (0.24, 0.43)	0.53 (0.39, 0.72)	0.62 (0.44, 0.86)
Cardiovascular mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.38 (0.23, 0.63)	0.46 (0.28, 0.76)	0.48 (0.28, 0.81)
Quantile 3	0.33 (0.2, 0.55)	0.49 (0.3, 0.82)	0.46 (0.27, 0.77)
Quantile 4	0.18 (0.1, 0.33)	0.28 (0.15, 0.5)	0.25 (0.14, 0.46)
Cancer-related mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.79 (0.47, 1.3)	0.96 (0.59, 1.59)	1.09 (0.65, 1.83)
Quantile 3	0.51 (0.29, 0.91)	0.83 (0.47, 1.48)	0.94 (0.52, 1.69)
Quantile 4	0.44 (0.24, 0.76)	0.82 (0.45, 1.49)	0.95 (0.51, 1.81)
Diabetes-related mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.33 (0.14, 0.8)	0.36 (0.15, 0.88)	0.49 (0.16, 1.47)
Quantile 3	0.23 (0.08, 0.68)	0.28 (0.09, 0.83)	0.38 (0.11, 1.33)
Quantile 4	0.37 (0.14, 0.96)	0.42 (0.15, 1.15)	0.69 (0.25, 1.92)
② Excluding participants who died within 2 years			
All-cause mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.52 (0.40, 0.69)	0.63 (0.48, 0.83)	0.73 (0.55, 0.96)
Quantile 3	0.33 (0.25, 0.45)	0.52 (0.38, 0.69)	0.58 (0.43, 0.78)
Quantile 4	0.33 (0.24, 0.45)	0.55 (0.40, 0.76)	0.65 (0.45, 0.92)
Cardiovascular mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.38 (0.22, 0.65)	0.47 (0.28, 0.79)	0.48 (0.28, 0.85)
Quantile 3	0.28 (0.16, 0.48)	0.42 (0.24, 0.73)	0.39 (0.22, 0.69)
Quantile 4	0.16 (0.09, 0.31)	0.25 (0.13, 0.46)	0.23 (0.12, 0.43)
Cancer-related mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.83 (0.47, 1.46)	1.02 (0.59, 1.76)	1.16 (0.66, 2.05)
Quantile 3	0.55 (0.30, 1.01)	0.91 (0.49, 1.70)	1.06 (0.562, 2.00)
Quantile 4	0.54 (0.30, 0.97)	1.03 (0.55, 1.93)	1.26 (0.64, 2.51)
Diabetes-related mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.34 (0.13, 0.88)	0.37 (0.14, 0.97)	0.51 (0.15, 1.75)
Quantile 3	0.26 (0.09, 0.79)	0.31 (0.10, 0.96)	0.42 (0.11, 1.63)
Quantile 4	0.41 (0.15, 1.12)	0.46 (0.16, 1.33)	0.77 (0.26, 2.30)
③ FLI ≥60 was used as the diagnostic criterion			
All-cause mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.45 (0.34, 0.59)	0.53 (0.40, 0.70)	0.55 (0.42, 0.73)
Quantile 3	0.36 (0.27, 0.49)	0.48 (0.36, 0.65)	0.53 (0.40, 0.71)
Quantile 4	0.33 (0.25, 0.44)	0.48 (0.36, 0.64)	0.56 (0.42, 0.74)
Cardiovascular mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.33 (0.20, 0.55)	0.40 (0.24, 0.66)	0.4 (0.24, 0.67)
Quantile 3	0.36 (0.22, 0.60)	0.48 (0.29, 0.81)	0.52 (0.31, 0.87)
Quantile 4	0.23 (0.13, 0.41)	0.32 (0.18, 0.58)	0.36 (0.21, 0.65)
Cancer-related mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.56 (0.33, 0.97)	0.69 (0.40, 1.20)	0.80 (0.45, 1.42)
Quantile 3	0.38 (0.21, 0.68)	0.52 (0.28, 0.96)	0.62 (0.34, 1.13)
Quantile 4	0.52 (0.31, 0.87)	0.82 (0.47, 1.43)	1.10 (0.62, 1.93)
Diabetes-related mortality			
Quantile 1	Ref.	Ref.	Ref.
Quantile 2	0.37 (0.15, 0.95)	0.41 (0.16, 1.05)	0.44 (0.16, 1.21)
Quantile 3	0.45 (0.16, 1.26)	0.54 (0.19, 1.49)	0.62 (0.22, 1.72)

Model I: adjusted for no covariates. Model II: adjusted for age, gender, race. Model III: adjusted for age, gender, race, education, BMI, PIR, HDL-C, ALT, AST, TG, TC, HBP, DM, CVD, smoking, activity, daily energy intake.

IL-6, and tumor necrosis factorα, alleviating inflammatory injury (32). In addition, nutrient-rich foods may regulate glucose metabolism and modulate and optimize energy metabolism (26).

Several studies have explored the predictive roles of inflammatory and nutritional indicators in the prevalence and prognosis of MAFLD. Zhang et al. (33) identified novel systemic inflammation markers, including the platelet-to-lymphocyte ratio (PLR), NLR, and systemic immune-inflammation index (SII), as predictors of cardiovascular mortalities in MAFLD. This study found that the AUC values of NLR, SII, and PLR were 0.69, 0.60, and 0.52, respectively. Therefore, in comparison, ALI has a higher predictive value, which was also confirmed by another study (34). Serum albumin level represents a potential biomarker for early hepatic failure and a possible predictor (11). Patients with high albumin levels have a lower risk of death or the need for *in situ* liver transplantation (35). ALI, which includes BMI, serum albumin level, and NLR, reflects the interplay between nutrition, immunity, and systemic inflammation. It has demonstrated prognostic value in diverse chronic conditions, including T2DM, hypertension, and kidney disease (36–38). Interestingly, BMI in ALI is theoretically protective against MAFLD, contrary to the belief that obesity adversely affects MAFLD. First, the effect of BMI may explain the L-shaped non-linear correlation observed in the RCS plot. Second, components of ALI, such as inflammation and BMI, interact with each other. Inflammation reduces appetite and leads to malnutrition, which in turn can lead to weight loss (39). Finally, there may be an obesity paradox in the population with MAFLD, where higher BMI may confer survival advantages in terms of nutritional and cardiovascular reserves (40, 41). Some studies have suggested that although overweight patients or patients with obesity are at a higher risk of developing liver disease, they have a survival advantage over lean patients (42, 43). However, we think that high ALI scores resulting from high BMI do not have the same mortality risk reduction benefit as high ALI

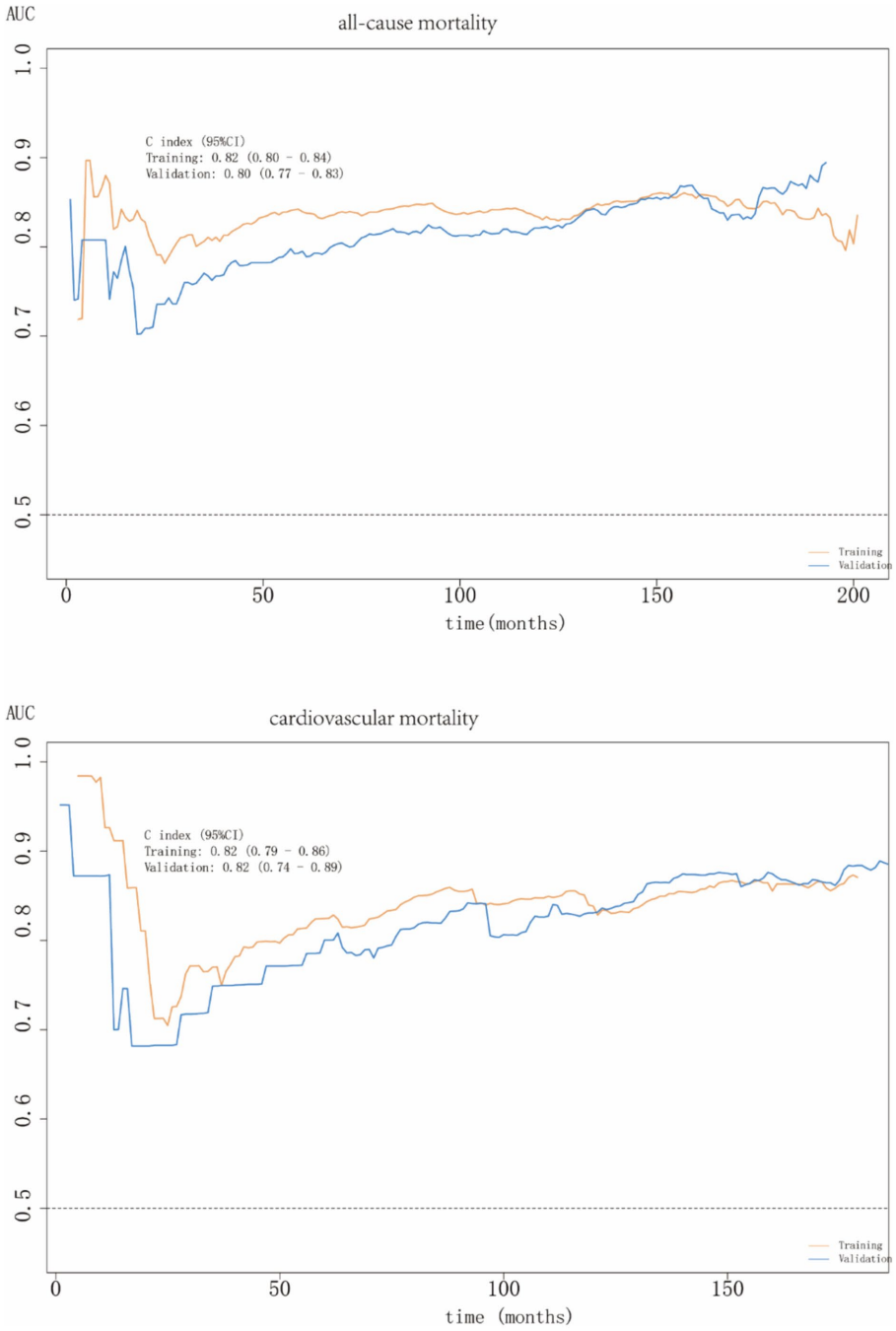

FIGURE 7
ROC curves of all-cause and cardiovascular mortalities.

achieved through moderate BMI. Merely relying on increasing BMI to enhance ALI is not advisable. Weight loss measures should still be reserved for obese patients with high metabolic risks. Sufficient nutritional reserves, low levels of inflammation, and the absence of metabolic burdens brought about by severe obesity better align with the obesity paradox observed in advanced chronic diseases. Future research could stratify ALI by BMI to identify the optimal BMI range.

The L-shape non-linear relationship of RCS analysis has meaningful points: Below the thresholds (71.48 for all-cause mortality and 68.54 for cardiovascular mortality), a higher ALI was significantly associated with reduced mortality risks in MAFLD patients. Above these thresholds, this protective effect plateaued. Specifically, in MAFLD patients, increasing ALI below 71.48 was associated with a significant reduction in all-cause mortality. Similarly, increasing ALI below 68.54 was associated with a significant reduction in cardiovascular mortality. However, when ALI exceeded 71.48 and 68.54, respectively, further increases conferred limited additional benefit for reducing either all-cause or cardiovascular mortality.

Beyond its prognostic value, ALI provides actionable guidance for risk-stratified management of MAFLD: the inflection point for

all-cause mortality is ALI = 71.48, and the inflection point for cardiovascular mortality is ALI = 68.54. Considering the actual situation, ALI ≤ 68.54 can be used as a comprehensive high-risk cut-off point. When ALI ≤ 68.54, it is recommended to strengthen follow-up. Secondly, in terms of comorbidity management: the high-risk group (ALI ≤ 68.54) needs to actively carry out secondary prevention of cardiovascular diseases; priority should be given to controlling inflammation. The calculation of ALI is convenient and has a low cost, making it practical for risk monitoring in the MAFLD population. However, it should be noted that: ALI is not an independent decision-making tool and needs to be used in combination with other indicators (such as FIB-4 for assessing liver fibrosis). Secondly, dynamic monitoring of ALI changes is more important than a single value.

Interestingly, the inverse association between ALI levels and all-cause mortality was more pronounced in females. This phenomenon may be related to the inherent biological differences between genders, which profoundly influence the disease process and prognosis. Mechanistically, estrogen exerts a protective effect on liver diseases by reducing oxidative stress, insulin resistance, lipid metabolism, and fibrosis (44–46). The liver is not only a key regulatory organ for metabolic homeostasis but also a major target for estrogen signaling - estrogen receptors α (estrogen receptor α, ERα) and β (estrogen receptor β, ERβ) bind to estrogen response element (estrogen response element, ERE) to directly regulate lipid and glucose metabolism in the liver (45). Studies have confirmed that a decline in estrogen levels promotes the development of extensive hepatic steatosis and the progression of liver fibrosis (47). Additionally, estrogen has anti-inflammatory and antioxidant effects, and a decrease in estrogen levels exacerbates oxidative stress and systemic inflammation (46). Estrogen may enhance the protective effect of a low NLR in ALI, leading to a more significant association with prognosis in female patients.

Our research has a number of advantages. First of all, it is the first study to show that ALI has predictive significance for MAFLD prognosis. Second, the large, nationally representative cohort strengthens the generalizability of our findings. Third, our results' robustness is reinforced by thorough comprehensive covariate adjustment and sensitivity analyses. However, the study has certain drawbacks. Because this was an observational study, a causal relationship between ALI and mortality cannot be established. Additionally, residual confounding factors cannot be entirely excluded. Thirdly, although sensitivity analyses expanding the study population through another.

MAFLD diagnostic criteria were performed, the high exclusion rate due to missing data may raise concerns about possible selection bias. Large-scale interventional research is required in the future to confirm these results and elucidate the causative mechanisms.

5 Conclusion

Our study provides an effective approach for predicting and managing patients with MAFLD. Low ALI levels were associated with poor prognosis, highlighting the utility of ALI in identifying populations at high risk of MAFLD and facilitating timely interventions. The observed L-shaped non-linear relationship shows that: when the indicators are below specific thresholds (all-cause mortality 71.48, cardiovascular mortality 68.54), a higher ALI was significantly associated with reduced mortality risks in MAFLD patients; however, once the thresholds are exceeded, the protective effect will stabilize. This underscores the importance of maintaining an optimal ALI level to improve survival outcomes in patients with MAFLD. Future cohort studies of ALI in MAFLD populations in other countries are needed. Explore intervention measures for ALI to promote more effective disease prevention and treatment.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics ethics review board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YL: Writing – original draft, Writing – review & editing. SY: Writing – original draft, Writing – review & editing. HX: Investigation, Methodology, Software, Writing – review & editing. JO: Conceptualization, Data curation, Formal analysis, Validation, Writing – review & editing. JD: Investigation, Methodology, Software, Supervision, Writing – review & editing. XJ: Writing – review & editing, Visualization. XS: Writing – review & editing, Visualization. RZ: Funding acquisition, Project administration, Resources, Validation, Visualization, Writing – review & editing.

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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.

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References

1. Zou H, Ma X, Pan W, Xie Y. Comparing similarities and differences between NAFLD, MAFLD, and MASLD in the general U.S. population. *Front Nutr*. (2024) 11:1411802. doi: 10.3389/fnut.2024.1411802

2. Chan WK, Chuah KH, Rajaram RB, Lim LL, Ratnasingam J, Vethakkan SR. Metabolic dysfunction-associated Steatotic liver disease (MASLD): a state-of-the-art review. *J Obes Metab Syndr*. (2023) 32:197–213. doi: 10.7570/jomes23052

3. Mantovani A, Scorletti E, Mosca A, Alisi A, Byrne CD, Targher G. Complications, morbidity and mortality of nonalcoholic fatty liver disease. *Metabolism*. (2020) 111s:154170. doi: 10.1016/j.metabol.2020.154170

4. Beygi M, Ahi S, Zolghadri S, Stanek A. Management of Metabolic-Associated Fatty Liver Disease/metabolic dysfunction-associated Steatotic liver disease: from medication therapy to nutritional interventions. *Nutrients*. (2024) 16:2220. doi: 10.3390/nu16142220

5. Tauil RB, Golono PT, de Lima EP, de Alvares Goulart R, Guiguer EL, Bechara MD, et al. Metabolic-associated fatty liver disease: the influence of oxidative stress, inflammation, mitochondrial dysfunctions, and the role of polyphenols. *Pharmaceuticals (Basel)*. (2024) 17:1354. doi: 10.3390/ph17101354

6. Yan J, Zhou J, Ding Y, Tu C. Dietary inflammatory index is associated with metabolic dysfunction-associated fatty liver disease among United States adults. *Front Nutr*. (2024) 11:1340453. doi: 10.3389/fnut.2024.1340453

7. Jung CH, Lee WY, Kim BY, Park SE, Rhee EJ, Park CY, et al. The risk of metabolic syndrome according to the white blood cell count in apparently healthy Korean adults. *Yonsei Med J*. (2013) 54:615–20. doi: 10.3349/ymj.2013.54.3.615

8. Pan X, Lv J, Liu M, Li Y, Zhang Y, Zhang R, et al. Chronic systemic inflammation predicts long-term mortality among patients with fatty liver disease: data from the National Health and nutrition examination survey 2007-2018. *PloS One*. (2024) 19:e0312877. doi: 10.1371/journal.pone.0312877

9. Hu W, Luo L, Li M, Xiong X, Huang W, Huang Y, et al. Anti-inflammatory diet reduces risk of metabolic dysfunction-associated fatty liver disease among US adults: a nationwide survey. *Scand J Gastroenterol*. (2024) 59:1192–201. doi: 10.1080/00365521.2024.2395851

10. Yang L, Zheng SG. Role of regulatory T cells in inflammatory liver diseases. *Autoimmun Rev*. (2025) 24:103806. doi: 10.1016/j.autrev.2025.103806

11. Sun L, Yin H, Liu M, Xu G, Zhou X, Ge P, et al. Impaired albumin function: a novel potential indicator for liver function damage? *Ann Med*. (2019) 51:333–44. doi: 10.1080/07853890.2019.1693056

12. Jafri SH, Shi R, Mills G. Advance lung cancer inflammation index (ALI) at diagnosis is a prognostic marker in patients with metastatic non-small cell lung cancer (NSCLC): a retrospective review. *BMC Cancer*. (2013) 13:158. doi: 10.1186/1471-2407-13-158

13. Song M, Zhang Q, Song C, Liu T, Zhang X, Ruan G, et al. The advanced lung cancer inflammation index is the optimal inflammatory biomarker of overall survival in patients with lung cancer. *J Cachexia Sarcopenia Muscle*. (2022) 13:2504–14. doi: 10.1002/jcsm.13032

14. Tu J, Wu B, Xiu J, Deng J, Lin S, Lu J, et al. Advanced lung cancer inflammation index is associated with long-term cardiovascular death in hypertensive patients: national health and nutrition examination study, 1999-2018. *Front Physiol*. (2023) 14:1074672. doi: 10.3389/fphys.2023.1074672

15. Fan W, Zhang Y, Liu Y, Ding Z, Si Y, Shi F, et al. Nomograms based on the advanced lung Cancer inflammation index for the prediction of coronary artery disease and calcification. *Clin Appl Thromb Hemost*. (2021) 27:10760296211060455. doi: 10.1177/10760296211060455

16. Feng JF, Huang Y, Chen QX. A new inflammation index is useful for patients with esophageal squamous cell carcinoma. *Onco Targets Ther*. (2014) 7:1811–5. doi: 10.2147/ott.S68084

17. Yip TC, Lyu F, Lin H, Li G, Yuen PC, Wong VW, et al. Non-invasive biomarkers for liver inflammation in non-alcoholic fatty liver disease: present and future. *Clin Mol Hepatol*. (2023) 29:S171–83. doi: 10.3350/cmh.2022.0426

18. Huo C, Liu Y, Xie F, Zhao L, Huang H, Feng Q. Advanced lung cancer inflammation index predicts the outcomes of patients with non-metastatic gastric cancer after radical surgical resection. *J Gastrointest Oncol*. (2023) 14:1653–4. doi: 10.21037/jgo-23-315

19. Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States National Health and nutrition examination survey. *Aliment Pharmacol Ther*. (2015) 41:65–76. doi: 10.1111/apt.13012

20. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The fatty liver index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol*. (2006) 6:33. doi: 10.1186/1471-230x-6-33

21. Dong J, Li Z, Wang C, Zhang R, Li Y, Liu M, et al. Dietary folate intake and all-cause mortality and cardiovascular mortality in American adults with non-alcoholic fatty liver disease: data from NHANES 2003 to 2018. *PloS One*. (2024) 19:e0314148. doi: 10.1371/journal.pone.0314148

22. Li S, Chen F, Huang C, Huang G, Cheng Y, Li T, et al. Relationships between antibiotic exposure and asthma in adults in the United States: results of the National Health and nutrition examination survey between 1999 and 2018. *Front Public Health*. (2023) 11:1123555. doi: 10.3389/fpubh.2023.1123555

23. Xu W, Zhang L, Yang Q, Cao Y, Rao R, Lv L, et al. Associations of prognostic nutritional index with cardiovascular all-cause mortality among CVD patients with diabetes or prediabetes: evidence from the NHANES 2005-2018. *Front Immunol*. (2025) 16:1518295. doi: 10.3389/fimmu.2025.1518295

24. Gong H, He Q, Zhu L, Feng Z, Sun M, Jiang J, et al. Associations between systemic inflammation indicators and nonalcoholic fatty liver disease: evidence from a prospective study. *Front Immunol*. (2024) 15:1389967. doi: 10.3389/fimmu.2024.1389967

25. Bauer KC, Littlejohn PT, Ayala V, Creus-Cuadros A, Finlay BB. Nonalcoholic fatty liver disease and the gut-liver Axis: exploring an undernutrition perspective. *Gastroenterology*. (2022) 162:1858–75.e2. doi: 10.1053/j.gastro.2022.01.058

26. Seidita A, Cusimano A, Giuliano A, Meli M, Carroccio A, Soresi M, et al. Oxidative stress as a target for non-pharmacological intervention in MAFLD: could there be a role for EVOO? *Antioxidants (Basel)*. (2024) 13:731. doi: 10.3390/antiox13060731

27. Kisseleva T, Brenner DA. Role of hepatic stellate cells in fibrogenesis and the reversal of fibrosis. *J Gastroenterol Hepatol*. (2007) 22:S73–8. doi: 10.1111/j.1440-1746.2006.04658.x

28. Sedibe A, Maswanganyi K, Mzimela NC, Gamede M. Prevalence of metabolic dysfunction-associated steatotic liver disease in people living with HIV and on antiretroviral treatment: a systematic review and meta-analysis protocol. *Health Sci Rep*. (2024) 7:e70071. doi: 10.1002/hsr2.70071

29. Chen Y, Zhao X. The mediating role of insulin resistance in the association between inflammatory score and MAFLD: NHANES 2017-2018. *Immun Inflamm Dis*. (2024) 12:e70035. doi: 10.1002/iid3.70035

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

31. Cai L, Ming D, Chen W, Zhao Y, Li Y, Sun W, et al. Silybin alleviated hepatic injury by regulating redox balance, inflammatory response, and mitochondrial function in weaned piglets under paraquat-induced oxidative stress. *Antioxidants (Basel)*. (2024) 13:324. doi: 10.3390/antiox13030324

32. Ma M, Long Q, Chen F, Zhang T, Wang W. Active vitamin D impedes the progression of non-alcoholic fatty liver disease by inhibiting cell senescence in a rat model. *Clin Res Hepatol Gastroenterol*. (2020) 44:513–23. doi: 10.1016/j.clinre.2019.10.007

33. Zhang Z, Zhang W, Liu Z, Ou J, Sun Y, Zhang L, et al. Association between systemic inflammation markers and cardiovascular mortality in adults with metabolic dysfunction-associated steatotic liver disease. *Nutr Metab Cardiovasc Dis*. (2024):103781. doi: 10.1016/j.numecd.2024.10.019

34. Zhang Y, Zhang AH, Li RL, Li WJ, Liu Y, Li T. Association of neutrophil-to-lymphocyte ratio and risk of all-cause and cardiovascular mortality in adults with

metabolic dysfunction-associated steatotic liver disease: a population-based cohort study. *Front Med*. (2024) 11:1499524. doi: 10.3389/fmed.2024.1499524

35. Takahashi H, Kawanaka M, Fujii H, Iwaki M, Hayashi H, Toyoda H, et al. Association of Serum Albumin Levels and Long-Term Prognosis in patients with biopsy-confirmed nonalcoholic fatty liver disease. *Nutrients*. (2023) 15:2014. doi: 10.3390/nu15092014

36. Chen Y, Guan M, Wang R, Wang X. Relationship between advanced lung cancer inflammation index and long-term all-cause, cardiovascular, and cancer mortality among type 2 diabetes mellitus patients: NHANES, 1999-2018. *Front Endocrinol (Lausanne)*. (2023) 14:1298345. doi: 10.3389/fendo.2023.1298345

37. Zhang Y, Pan Y, Tu J, Liao L, Lin S, Chen K, et al. The advanced lung cancer inflammation index predicts long-term outcomes in patients with hypertension: national health and nutrition examination study, 1999-2014. *Front Nutr*. (2022) 9:989914. doi: 10.3389/fnut.2022.989914

38. Zhou J, Liu W, Liu X, Wu J, Chen Y. Independent and joint influence of depression and advanced lung cancer inflammation index on mortality among individuals with chronic kidney disease. *Front Nutr*. (2024) 11:1453062. doi: 10.3389/fnut.2024.1453062

39. Stumpf F, Keller B, Gressies C, Schuetz P. Inflammation and nutrition: friend or foe? *Nutrients*. (2023) 15:1159. doi: 10.3390/nu15051159

40. Ding Y, Xu X, Tian T, Yu C, Ge X, Gao J, et al. Weight change across adulthood in relation to non-alcoholic fatty liver disease among non-obese individuals. *Nutrients*. (2022) 14:2140. doi: 10.3390/nu14102140

41. Patel N, Elsaid O, Shenoy A, Sharma A, McFarlane SI. Obesity paradox in patients undergoing coronary intervention: a review. *World J Cardiol*. (2017) 9:731–6. doi: 10.4330/wjc.v9.i9.731

42. Inagaki K, Tawada N, Takanashi M, Akahori T. The association between body mass index and all-cause mortality in Japanese patients with incident hemodialysis. *PloS One*. (2022) 17:e0269849. doi: 10.1371/journal.pone.0269849

43. Karagozian R, Bhardwaj G, Wakefield DB, Baffy G. Obesity paradox in advanced liver disease: obesity is associated with lower mortality in hospitalized patients with cirrhosis. *Liver Int*. (2016) 36:1450–6. doi: 10.1111/liv.13137

44. Nagral A, Bangar M, Menezes S, Bhatia S, Butt N, Ghosh J, et al. Gender differences in nonalcoholic fatty liver disease. *Euroasian J Hepatogastroenterol*. (2022) 12:S19–25. doi: 10.5005/jp-journals-10018-1370

45. Mahboobifard F, Pourgholami MH, Jorjani M, Dargahi L, Amiri M, Sadeghi S, et al. Estrogen as a key regulator of energy homeostasis and metabolic health. *Biomed Pharmacother*. (2022) 156:113808. doi: 10.1016/j.biopha.2022.113808

46. Liu W, Xu Y, Xiao L, Li K, Liu Q. Composite dietary antioxidant index is associated with the prevalence of metabolic syndrome in females: results from NHANES 2011-2016. *Front Nutr*. (2025) 12:1529332. doi: 10.3389/fnut.2025.1529332

47. Turola E, Petta S, Vanni E, Milosa F, Valenti L, Critelli R, et al. Ovarian senescence increases liver fibrosis in humans and zebrafish with steatosis. *Dis Model Mech*. (2015) 8:1037–46. doi: 10.1242/dmm.019950

Glossary

ALI - Advanced lung cancer inflammation index

NAFLD - on-alcoholic fatty liver disease

MAFLD - Metabolic dysfunction-associated fatty liver disease

MASLD - metabolic dysfunction-associated steatotic liver disease

NHANES - National Health and Nutrition Examination Survey

T2DM - Type 2 diabetes mellitus

NDI - National Death Index

ICD-10 - International Classification of Diseases, Tenth Revision

RCS - Restricted cubic spline

ROC - Receiver operating characteristic

AUC - Area under the curve

BMI - Body mass index

PIR - poverty-income ratio

HRs - hazard ratios

ALT - Alanine transaminase

AST - aspartate aminotransferase

HDL - high-density lipoprotein

TC - total cholesterol

TG - triglycerides

DM - diabetes mellitus

CVD - cardiovascular disease

HBP - high blood pressure

FLI - Fatty liver index

US-FLI - United States fatty liver index

PLR - platelet-to-lymphocyte ratio

SII - systemic immune-inflammation index

NLR - neutrophil-to-lymphocyte ratio

IL - interleukin

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The impact of high-salt diet and diuretics on the development of the aestival phenomenon in patients with chronic heart failure

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Background: Chronic heart failure (CHF) often requires the use of high-dose loop diuretics to achieve decongestion. However, such therapy may lead to adverse effects, including deterioration of renal function and electrolyte imbalance. Recent evidence suggests that diuretic-induced dehydration may trigger metabolic responses resembling aestivation—a survival mechanism involving a shift from inorganic to organic osmolytes, particularly increased urea synthesis, to conserve water.

Methods: This prospective, single-center cohort study included 102 patients (median age 75 years, 57.8% female) hospitalized with CHF from January to July 2023. The diuretic group received average daily doses of furosemide 39.1 ± 22.1 mg, torasemide 7.4 ± 3 mg, and spironolactone 42 ± 12.4 mg. Biochemical parameters—including sodium, potassium, glucose, urea, and estimated plasma osmolality (eOSM)—were assessed on days 1 and 7 of hospitalization. Plasma osmolyte ratios (PropUrea/eOSM, PropNa/eOSM) were calculated. Propensity score matching (PSM) was used to adjust for confounders such as age, ejection fraction, and renal function.

Results: By day 7, plasma osmolality in the diuretic group increased from 300 [297; 304] to 302.2 [298.3; 305.8] mOsm/L (p = 0.039), while no significant change occurred in the non-diuretic group. Urea levels rose to 7.95 [5.65; 9.90] mmol/L in the diuretic group versus 5.90 [5.05; 7.50] mmol/L in the control group (p = 0.012). The PropUrea/eOSM increased to 2.63% [1.89; 3.28] in the diuretic group compared to 2.00% [1.70; 2.50] (p = 0.011). Conversely, PropNa/eOSM decreased to 46.46% [46.02; 46.74] versus 46.68% [46.33; 46.89] (p = 0.050). Multivariate logistic regression confirmed that diuretic therapy was independently associated with these changes: PropUrea/eOSM (OR = 3.52, 95% CI: 1.94–7.26, p < 0.001), and PropNa/eOSM (OR = 0.16, 95% CI: 0.06–0.39, p < 0.001). These effects were most pronounced in patients consuming >10 g/day of salt.

Conclusion: This study demonstrated that in patients with chronic heart failure (CHF), intensive loop diuretic therapy—especially when combined with high sodium intake—is associated with a shift in plasma osmolytes, marked by increased urea and reduced sodium contributions to osmolality. These changes suggest activation of water-conservation mechanisms and are independent of CHF severity or renal dysfunction, as confirmed by propensity score matching. Clinically, the urea-to-osmolality ratio may serve as an early marker of metabolic

stress and muscle catabolism. Patients consuming >10 g/day of salt appear especially susceptible to this aestivation-like response. Early identification of these changes may guide adjustments in diuretic regimens, as well as prompt nutritional and physical interventions to mitigate sarcopenia and functional decline. These findings support a personalized approach to diuretic therapy in CHF, emphasizing the role of dietary sodium in shaping metabolic responses and highlighting metabolic aestivation as a potential contributor to fatigue and weakness in this population.

KEYWORDS

chronic heart failure, diuretics, sodium intake, aestivation, osmolytes, urea, plasma osmolality

1 Introduction

Decompensated heart failure often requires the use of high doses of diuretics. However, research indicates that the administration of large doses of diuretics can lead to several side effects, including worsening renal function and electrolyte disturbances. The ADHERE study showed that patients receiving lower doses of furosemide (<160 mg) had a reduced risk of hospital mortality, shorter stays in the ICU, and fewer prolonged hospitalizations or renal side effects compared to patients receiving more than 160 mg of furosemide (1). The use of higher doses of diuretics was associated with increased diuresis and more favorable outcomes at some secondary endpoints, but also posed a higher risk of deterioration of renal function (2). Obese patients who received high-intensity diuretic therapy had an increased risk of deterioration of renal function within 72 h of treatment compared to a control group. Furthermore, high-intensity diuretic treatment was associated with a higher frequency of decline in renal function, which was similar in both obese and non-obese patients (3).

A recently described novel pathophysiological mechanism of muscle mass loss related to sodium metabolism in patients with chronic heart failure (CHF) involves active conservation of total body water during acute changes in sodium balance. This mechanism resembles the state of aestivation observed in animals for water conservation, resulting in metabolic regulation aimed at increasing the contribution of nonionic osmolytes, such as urea and glucose (4). In a study by Nihlén S. et al. (5), it was found that during treatment in the intensive care unit (ICU), diuretic-induced iatrogenic dehydration is associated with a shift toward the intensive production of organic osmolytes, mainly urea." (Use "toward" instead of "towards" for consistency with American English). This adaptation is part of a universal response to water deficiency, observed in a wide range of organisms, from aestivating worms to higher animals, including mammals (6). This aligns with scientific data indicating that fluid loss can trigger protein breakdown in various cell types (7). Manifestations of aestivation in mammals include the synthesis of amino acid osmolytes, which contribute to increased plasma urea concentration, reduced urine output (oliguria), peripheral hypoperfusion, and muscle mass loss. The interplay of these mechanisms with varying levels of sodium intake in patients is intriguing (8), considering that individuals on a high-salt diet typically have significant depots of osmotically neutral sodium, including in muscle tissue (9, 10). These mechanisms may explain the high prevalence of sarcopenia in patients with CHF (11).

Importantly, patients with chronic HF often enter the hospital on chronically high salt diets; their interstitial tissues can store millimolar quantities of osmotically neutral Na^+ (11). We hypothesized that intensive loop diuresis "unmasks" this hidden sodium, simultaneously triggering an $Na^+ \rightarrow$ urea osmolyte switch and accelerating muscle loss—an effect that may be exaggerated when dietary salt is unrestricted.

Understanding this switch is clinically actionable for several reasons:

A rising urea-to-osmolality ratio during the first treatment week may signal that further loop-diuretic escalation will yield diminishing natriuretic returns while promoting catabolism; it can also help the clinician choose the optimal delivery strategy—high-dose intermittent boluses vs. lower-dose continuous (infusion) therapy—so that decongestion is achieved without exposing the patient to unnecessary metabolic stress. Because increased urea production often precedes measurable loss of lean body mass, tracking this ratio could identify individuals who would benefit from nutritional or exercise interventions. Furthermore, recognition of aestivation-like metabolic changes may guide clinical decision-making regarding the timing and intensity of diuretic therapy, particularly in patients with concurrent high sodium intake. Clinicians should consider monitoring the urea-to-osmolality ratio as a biomarker for early detection of catabolic stress, potentially prompting earlier implementation of protein supplementation, physical therapy interventions, or adjustment of diuretic dosing strategies to minimize muscle wasting while maintaining effective decongestion.

Objective of the Study: To evaluate the impact of varying levels of sodium intake and diuretic therapy on biochemical changes and the activation of aestivation mechanisms in patients with chronic heart failure (CHF).

2 Materials and methods

2.1 Study design

The study was carried out in the Cardiology Department of GBUZ GVV No. 3 DZM, with patient recruitment taking place from January 2023 to July 2023. This was a prospective single-center open-label cohort study that aimed to investigate the impact of intensive diuretic therapy on the development of aestivation in patients with chronic heart failure (CHF) on diets with varying sodium content. The study

adhered to the principles of the Helsinki Declaration. The study design is presented in Figure 1.

Upon admission, patients were divided into two groups according to the required level of diuretic therapy with loop diuretics. Biochemical analyses were performed on the first day of hospitalization before the initiation of diuretic therapy and after 7 days of therapy. The concentrations of sodium, potassium, glucose, urea, albumin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, cholesterol, creatinine, and C-reactive protein (CRP) were measured.

2.2 Inclusion and exclusion criteria

Participants: Patients with a confirmed diagnosis of chronic heart failure, who had been on stable therapy (ACE inhibitors/ARBs, beta-blockers at more than 50% of the maximum dose) for more than 3 weeks, were included in the study. According to current clinical guidelines and evidence from major randomized trials, a dose of ≥50% is necessary to achieve the proven clinical benefits of beta-blocker therapy, including reduced mortality and hospitalization. Patients receiving lower doses may represent a less stable population (e.g., undergoing dose titration or with poor tolerance), which could introduce confounding variability in the assessment of metabolic and osmotic adaptations. None of the patients were receiving neprilysin/angiotensin receptor inhibitors (ARNI, such as sacubitril/valsartan) or sodium-glucose co-transporter 2 inhibitors (SGLT2 inhibitors, such as empagliflozin or dapagliflozin) at the time of inclusion in the study. This was due to the limited availability of these medications in the inpatient setting during the study period and their absence from standard therapy regimens for the majority of patients in the observed cohort. The main inclusion and exclusion criteria are presented in Table 1. The study was conducted in accordance with the Helsinki Declaration and approved by the ethics committee.

2.3 Laboratory analyses

Biochemical parameters—including sodium, potassium, glucose, urea, creatinine, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and C-reactive protein (CRP)—were measured using standard laboratory methods in the hospital's certified clinical diagnostic laboratory.

The following equipment was used:

- Beckman Coulter DxC700 AU (Ireland)—for biochemical analysis
- CL-2000i (Mindray, China)—chemiluminescent immunoassay analyzer
- Cobas e 411 (Roche Diagnostics, Japan)—immunoassay analyzer
- XN-Series (Sysmex, Japan)—for hematology analysis

CRP levels were assessed using an immunoturbidimetric assay. All laboratory procedures were performed according to the manufacturers'

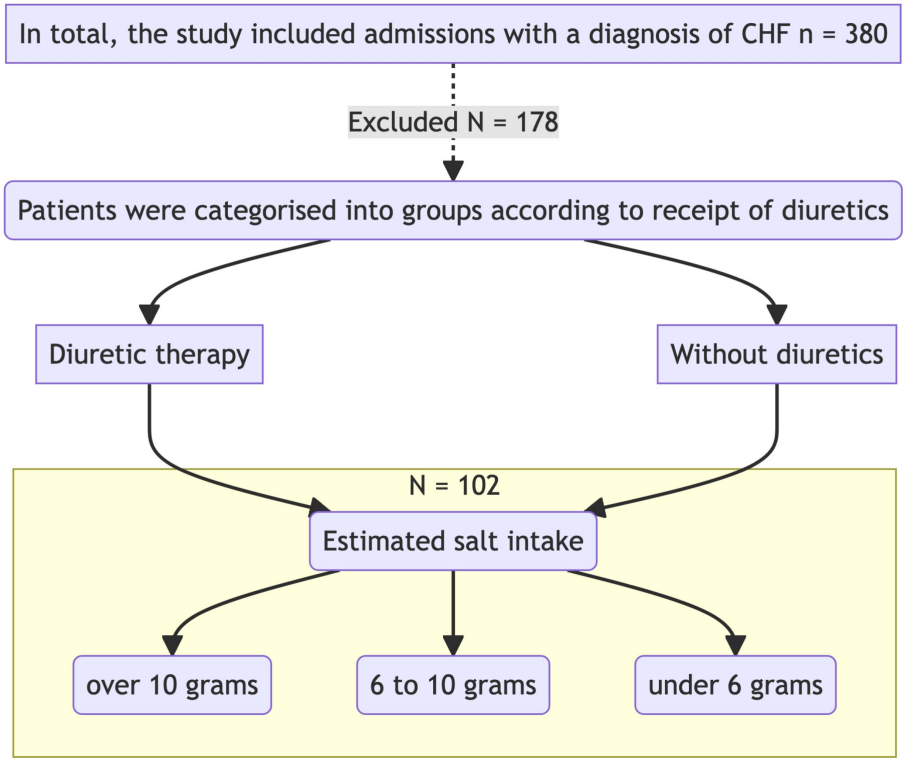

FIGURE 1
Research design.

TABLE 1 Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Presence of an established diagnosis of CHF (NYHA I- IV Functional class)	Unlikely cooperation with the patient during the trial, low adherence to therapy for social, psychological, economic, and other reasons, incapacity
Stable therapy for 3 months prior to inclusion in the study	Patients with cancer who are on radiation therapy or chemotherapy
Stable therapy with ACE inhibitors/ARBs, beta-blockers for 3 weeks prior to hospitalization	Need to be in the intensive care unit at the time of admission
Signing voluntary informed consent	Presence of any severe, decompensated, or unstable chronic somatic disease that, in the opinion of the investigators, could affect study outcomes or patient safety. These included, but were not limited to: • Severe anemia (hemoglobin < 90 g/L) • Decompensated or poorly controlled endocrine disorders (e.g., uncontrolled diabetes mellitus with HbA1c > 9%, untreated hypothyroidism or hyperthyroidism, adrenal insufficiency) • Systemic autoimmune diseases (e.g., systemic lupus erythematosus, vasculitis, rheumatoid arthritis with high inflammatory activity) • Advanced liver disease (e.g., liver cirrhosis Child-Pugh class B or C, active hepatitis) • Severe chronic pulmonary diseases (e.g., COPD with frequent exacerbations or requiring oxygen therapy) • Advanced neurological disorders impairing mobility or cognitive function (e.g., Parkinson's disease, recent stroke with residual deficit)
Constant salt intake according to the food diary during the week before hospitalization	Abuse of alcohol, drugs, or medicines
No loop diuretic therapy prior to inclusion in the study for 2 weeks	Taking thiazide or thiazide-like diuretics

protocols with proper calibration and internal quality control procedures in place.

2.4 Calculation of plasma Osmolarity

$$eOSM = 2 * Na^{+} + 2 * K^{+} + U + Gl \quad (1)$$

In Equation 1, where U_n is plasma urea, Gl_n is plasma glucose, and eOSM is the estimated plasma osmolality.

Plasma osmolality was assessed according to Equation 1. We then calculated the proportions of each osmolyte relative to the estimated plasma osmolality, obtaining the following ratios: Prop $_{Na/eOSM}$, Prop $_{K/eOSM}$, Prop $_{Urea/eOSM}$, and Prop $_{Glucose/eOSM}$, respectively.

2.5 Methods for determining salt intake

Salt intake was evaluated using a diet questionnaire in which patients recorded their dietary information for any two weekdays and one weekend day during the week prior to hospitalization. The electronic questionnaire was published on the website http://www.saltquest.ru/Sodium_project/. The electronic questionnaire contained a pre-formed database of foods produced in Russia with known sodium content per 100 grams of product or dish. All individual products and recipes were grouped into similar types of food (e.g., popcorn, potato chips, crackers) and then combined into broader categories (e.g., snacks), which formed the foundation of the food diary.

When filling out the food diary, the type of meal (breakfast, lunch, dinner), its volume, and the fact that the dish was additionally salted were taken into account. The volume of additional salting was calculated based on 0.1 grams of salt per additional salting.

Patients with a consistent level of sodium intake were included in the study. Weekly intake fluctuations were allowed within ±2 grams per day, provided that they did not exceed the salt intake categories: (1) less than 6 grams, (2) 6 to 10 grams, (3) more than 10 grams per day (12).

2.6 Definition of heart failure

The presence of heart failure in patients was determined based on the National Clinical Guidelines for the Diagnosis and Treatment of Chronic Heart Failure (CHF) (13). The stage and functional class of CHF for each patient included in the final analysis were determined by two independent experienced cardiologists. If their assessments differed, the final decision was made after joint discussion.

Ejection fraction (EF) for all patients was determined using the Simpson's method in the apical four-chamber and two-chamber views, and the average EF was calculated:

- Heart failure with preserved ejection fraction (HFpEF): EF ≥ 50%
- Heart failure with mildly reduced ejection fraction (HFmrEF): EF 41–49%
- Heart failure with reduced ejection fraction (HFrEF): EF ≤ 40%

These thresholds were used in combination with clinical signs and symptoms, natriuretic peptide levels, and echocardiographic

parameters (such as left atrial volume index and left ventricular mass index), as recommended in the guidelines.

Lifestyle characteristics were assessed at baseline using a structured questionnaire. The questionnaire included information on:

- Physical activity: categorized as low (sedentary), moderate (regular walking or light activity), or high (structured exercise ≥3 times/week);
- Smoking status: current smoker, former smoker, or never smoked;
- Alcohol consumption: categorized as none, occasional (≤2 times/week), or regular (>2 times/week)

2.7 Determination of kidney function and verification of chronic kidney disease

The glomerular filtration rate (GFR) was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) 2011 formula (14), according to the National Guidelines for Chronic Kidney Disease (CKD). Albuminuria was determined using test strips and the albumin/creatinine ratio was measured in a morning urine sample. The diagnosis of CKD was verified according to the guidelines (15), based on the following criteria:

- The presence of any clinical markers of kidney damage was confirmed twice, with a time interval of at least 3 months between tests.
- Detected decrease in GFR (>60 mL/min/1.73 m^2), albuminuria, or any other clinical markers of kidney damage confirmed over a period of 3 months.
- Persistent GFR < 60 mL/min/1.73 m^2 regardless of the dynamics of other markers.
- Diagnosis of irreversible markers (signs) of structural kidney changes confirmed by biopsy or imaging studies.

2.8 Sample size calculation

The sample size for the study was calculated based on the results of the study by Nihlen et al. (5). We assumed that the expected mean difference between the diuretic groups receiving and those not receiving them would be around 4% in the proportion of urea, with a standard deviation not exceeding 5%. Given the limited number of studies that have investigated changes in urea levels during diuretic therapy, a coefficient of variation level of 10% was adopted. This provided sufficient confidence that the calculated confidence limits would be reliable enough to confirm the study results at a power of 90%. This corresponded to a minimum sample size of 34 patients to assess the possible change in urea levels during diuretic therapy.

Taking into account the potential exclusion of respondents due to incomplete adherence to the study protocol and the possible reduction in the representative sample after the analysis of propensity matching, the sample size was increased by 50–70% from the calculated size. Therefore, the required sample size was determined to be at least 51 individuals. This number was sufficient to obtain statistically significant results. Therefore, an adequate sample size was formed to carry out the study.

2.9 Statistical analysis

Statistical analysis of the data obtained was performed using R, version 4.3.2, in the RStudio development environment (packages: ggplot2, ggpubr, dplyr, tidyverse, gtsummary, rstatix). The normality of the distribution was determined using the Shapiro–Wilk test, as well as the Kolmogorov–Smirnov test. We also examined the values of skewness and kurtosis and constructed QQ plots and distribution histograms.

Quantitative data were presented as mean (M) ± standard deviation (SD) or median with the 25th and 75th percentiles. Both parametric and nonparametric statistical methods were used to describe the results. The Kruskal-Wallis test or analysis of variance (ANOVA) was used to compare multiple groups. To compare two groups, Student's *t*-test was applied for normally distributed data and the Wilcoxon rank sum test was used for non-normally distributed data.

For categorical variables, frequency tables were constructed and checked using the Chi-square test with Yates correction. Fisher's exact test was used when the group size was less than 5, followed by *post hoc* analysis with Holm's correction for multiple comparisons. The Spearman correlation coefficient was used to study the relationship between variables. Logistic regression (both univariate and multivariate) was applied to examine the association between categorical dependent variables with multiple categories.

To adjust for predefined confounding factors in our analysis, we used the matching of propensity score (16). The cohort of interest was matched using the MatchIt library (17) with the 'full' matching method, which provided the best match based on standardized mean differences. The matching model included the following covariates: Age, Left ventricular ejection fraction (EF), Estimated glomerular filtration rate (eGFR).

Missing data handling: Only patients with complete data for both day 1 and day 7 laboratory assessments were included in the main comparative analyses. Cases with missing values for key variables (electrolytes, urea, glucose, eGFR) were excluded from the respective comparisons. No imputation techniques were applied.

Statistical hypotheses were tested with the null hypothesis rejected at a significance level of less than 0.05.

3 Results

3.1 Clinical characteristics of the group

A total of 102 individuals were included in the study, with a median age of 75 years (minimum age 43, maximum age 93 years). The number of women was slightly higher than that of men: 59 (57.8%).

The predominant etiologies of chronic heart failure (CHF) among enrolled patients were ischemic heart disease, including a history of myocardial infarction (67%, $n = 68$), and arterial hypertension without significant coronary artery disease (33%, $n = 34$). Other causes of CHF were not represented in the study cohort. The clinical characteristics of each group, based on whether they received diuretic therapy, are presented in Table 2. For most parameters, the patients did not differ significantly. However, significant differences were observed in some parameters; for example, the patients in the diuretic

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TABLE 2 Clinical characteristics of patients in the group who received diuretics and those who did not received diuretics.

Characteristic	Patients receiving diuretics, N = 59[1]	Patients not receiving diuretics, N = 43[1]	p-value[2]
Age in years, median [Q25; Q75]	81 (72, 84)	73 (67, 79)	0.011
Gender			>0.9
Female (%)	34 (58%)	25 (58%)	
Male (%)	25 (42%)	18 (42%)	
Sodium 1 day (mMol/L)	138.7 ± 3.1	138.6 ± 4.3	>0.9
Potassium 1 day (mMol/L)	4.07 ± 0.43	4.00 ± 0.41	0.3
Urea 1 day (mMol/L)	8.4 ± 4.3	7.1 ± 2.6	0.15
Glucose 1 day (mMol/L)	6.58 ± 2.21	6.46 ± 1.68	0.7
Sodium day 7 (mMol/L)	139.32 ± 2.64	139.51 ± 3.78	0.9
Potassium day 7 (mMol/L)	4.34 ± 0.53	4.15 ± 0.47	0.042
Urea day 7 (mMol/L)	9.2 ± 4.1	6.4 ± 1.8	<0.001
Glucose day 7 (mMol/L)	6.04 ± 1.66	5.75 ± 1.39	0.5
Functional class of CHF (NYHA)			<0.001
NYHA I	5 (8.5%)	18 (42%)	
NYHA II	27 (46%)	25 (58%)	
NYHA III	26 (44%)	0 (0%)	
NYHA IV	1 (1.7%)	0 (0%)	
Ejection fraction (%)	48 ± 8	51 ± 4	0.049
Estimated osmolarity on day 1 (mOsm/L)	301 ± 8	299 ± 9	0.5
Estimated osmolarity on day 7 (mOsm/L)	302.5 ± 6.8	299.5 ± 7.8	0.039
Estimated glomerular filtration rate (ml/min/1.73 m2)	50 ± 15	57 ± 14	0.021
Albumin (g/l)	39.2 (37.1, 40.8)	39.2 (37.0, 41.4)	0.8
Total protein (g/l)	67.8 (65.1, 71.0)	68.5 (64.8, 71.5)	>0.9
ALT (U/L)	18 (12, 29)	17 (13, 23)	0.5
AST (U/L)	20 (16, 29)	20 (18, 25)	0.8
HDL (mMol/L)	1.31 (1.17, 1.45)	1.26 (1.06, 1.34)	0.2
LDL (mMol/L)	2.80 (2.18, 3.29)	2.63 (2.20, 3.27)	>0.9
Triglycerides (mMol/L)	1.30 (0.91, 1.71)	1.27 (0.85, 1.84)	0.9
Cholesterol (mMol/L)	4.50 (3.64, 5.40)	4.60 (3.50, 5.45)	>0.9
CRP (mg/L)	5 (3, 10)	5 (2, 8)	0.3
Diabetes mellitus			0.7
No (%)	39 (66%)	30 (70%)	
Yes (%)	20 (34%)	13 (30%)	
CKD (stage)			0.025
C1	0 (0%)	1 (2.3%)	
C2	1 (1.7%)	5 (12%)	
C3a	24 (41%)	23 (53%)	
C3b	16 (27%)	4 (9.3%)	
C4	5 (8.5%)	1 (2.3%)	
No	13 (22%)	9 (21%)	
Physical activity: Low (%)	26 (44%)	18 (42%)	0.8
Physical activity: Moderate (%)	24 (41%)	17 (40%)	0.6
Physical activity: High (%)	9 (15%)	8 (18%)	0.7

(Continued)

TABLE 2 (Continued)

Characteristic	Patients receiving diuretics, N = 59[1]	Patients not receiving diuretics, N = 43[1]	p-value[2]
Smoking status: Current smoker (%)	6 (10%)	5 (12%)	0.7
Smoking status: Former smoker (%)	18 (31%)	16 (37%)	0.9
Smoking status: Never smoked (%)	30 (50%)	18 (42%)	0.9
Alcohol consumption: None (%)	16 (27%)	14 (33%)	0.6
Alcohol consumption: Occasional (%)	13 (22%)	11 (26%)	0.7

[1]Median (IQR); n (%).
[2]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test.

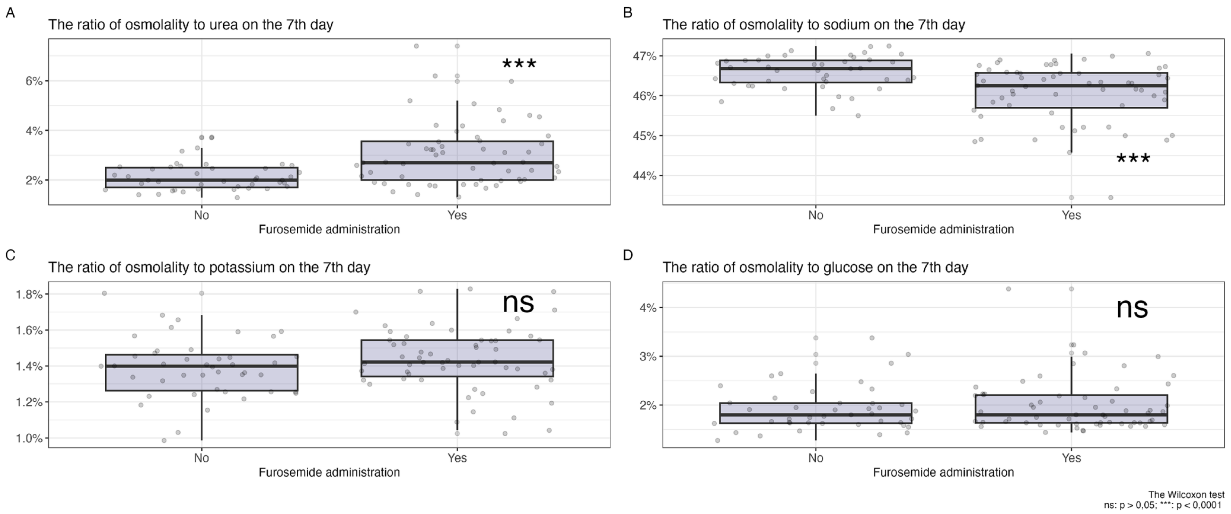

FIGURE 2
Changes in the ratio of osmolytes in plasma on the 7th day of diuretic therapy are detailed as follows: **(A)** osmolarity to urea ratio, **(B)** osmolarity to sodium ratio, **(C)** osmolarity to potassium ratio, **(D)** osmolarity to glucose ratio.

therapy group were older than those in the non-diuretic therapy group. Patients requiring diuretics generally had higher stages and functional classes of CHF. Consequently, this group had more patients with lower ejection fractions, and due to the interrelationship between CHF and chronic kidney disease (CKD), patients in the diuretic group often had higher stages of CKD.

The average doses of diuretics during the 7-day observation period were as follows: furosemide – 39.1 ± 22.1 mg, torasemide—7.4 ± 3 mg, and spironolactone—42 ± 12.4 mg. Patients receiving thiazide or thiazide-like diuretics were not included in the study.

The diuretic dosages for patients with different stages of CKD were distributed as follows: for CKD stage 3a, the average dose of furosemide was 34.0 mg ± 13.5 mg, torasemide—7.3 mg ± 2.9 mg, and spironolactone—37.5 mg ± 14.4 mg. For CKD stage 3b, the average dose of furosemide was 40.0 mg ± 16.3 mg, torasemide—7.3 mg ± 3.0 mg, and spironolactone—35.7 mg ± 13.4 mg. In patients with CKD stage 4, the average dose of furosemide increased to 60.0 mg ± 52.9 mg.

On the first day of observation, the mean estimated osmolality in the group of patients receiving treatment (n = 59) was 300 [297; 304] mOsm/L, which was comparable to the group of patients not receiving treatment (n = 43), where the value was 299 [296; 302] mOsm/L with a p-value > 0.5, indicating that there was no statistically significant difference. However, on the seventh day, a statistically significant increase in osmolality was observed in the treatment group, reaching 302.2 [298.3; 305.8] mOsm/L compared to the nontreatment group, which had a value of 300.2 [295.9; 303.5] mOsm/L, with a p-value of 0.039. These data highlight the potential impact of therapeutic interventions on plasma osmolality as part of the treatment approach for chronic heart failure.

3.2 Changes in plasma osmolyte ratios on day 7 of diuretic therapy

On the first day (Figure 2) of observation, for patients who did not receive diuretics, no statistically significant differences were identified in the proportions of osmolytes relative to plasma osmolality between the groups planned for diuretic treatment and those who did not receive such treatment (glucose 2[1.8; 2.3] vs. 2[1.8; 2.4] p = 0.66, potassium 1.3[1.3; 1.4] vs. 1.3[1.3; 1.4] p = 0.35, sodium 46.3[45.9; 46.7] vs. 46.4[46.1; 46.8] p = 0,14, urea 2.5[1.9; 3.4] vs. 2.2[1.8; 2.6] p = 0.15).

In Figure 2, a comparison of the changes in the proportions of key plasma osmolytes is shown after 7 days of loop diuretic treatment. Changes in potassium and glucose levels did not show statistically significant differences, while an increase in the proportion of urea and

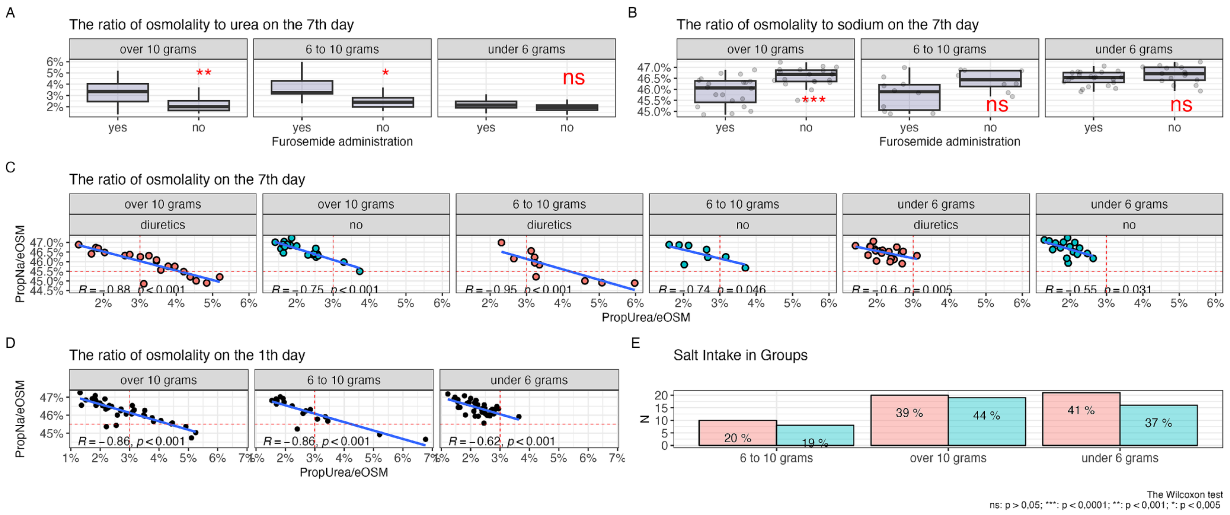

FIGURE 3
Relationship of osmolytes with the level of salt intake, **(A)** ratio of urea to plasma osmolarity among patients with varying levels of salt intake, **(B)** ratio of sodium to plasma osmolarity among patients with varying levels of salt intake, **(C)** correlation of the ratios of urea and sodium to plasma osmolarity among patients with different levels of salt intake undergoing diuretic therapy over 7 days, **(D)** correlation of the ratios of urea and sodium to plasma osmolarity among patients with different levels of salt intake on the first day of the study without diuretics, **(E)** salt intake in the diuretic therapy group (red column) and without diuretics (green column).

a decrease in the proportion of sodium in plasma osmolality were observed in the group receiving diuretic therapy.

Therefore, after 7 days of diuretic treatment, a statistically significant increase in the proportion of urea and a decrease in the proportion of sodium were observed.

3.3 Relationship between plasma osmolytes and sodium intake levels

In Figure 3A, it is shown that during diuretic therapy, patients who consumed 10 grams of salt per day experienced a statistically significant increase in the proportion of urea compared to other osmolytes. A similar effect was observed with a diet of 6 to 10 grams of salt per day. Meanwhile, on a low-salt diet, no statistically significant relationship was observed. PropNa/eOSM (Figure 3B) showed statistically significant changes only with a diet of 10 grams of salt per day; with a salt intake of 6 to 10 grams per day, a similar trend was observed, but it was statistically non-significant, and no changes were observed with a diet of less than 6 grams per day.

Figure 3C shows a negative linear relationship between the proportions of urea and sodium relative to other osmolytes, with the most pronounced correlation seen with diets consuming 10 grams and 6 to 10 grams of salt per day during diuretic therapy. Furthermore, there was a shift in the proportion of urea to values above 3% and a decrease in the proportion of sodium below 45.5%, while without diuretics, this observation was not present in diets of 10 grams and 6–10 grams of salt per day, and the correlation level remained almost the same. With a salt intake of less than 6 grams per day, the linear relationship was less pronounced and almost all observations were concentrated in the range with a proportion of urea less than 3% and a proportion of sodium more than 45.5%.

Figure 3D demonstrates a negative linear relationship between the proportions of urea and sodium relative to other osmolytes before the start of diuretic therapy, with a more pronounced negative linear trend with diets of 10 grams and 6 to 10 grams of salt per day, and a less pronounced trend with a diet of less than 6 grams of salt per day.

In Figure 3E, the level of salt intake among groups of patients receiving diuretic therapy and those not receiving it is shown. The largest number of patients were observed in the groups with a diet that included a salt intake of 10 grams and less than 6 grams per day, and to a lesser extent in the 6–10 grams per day group. There were no statistically significant differences between the groups ($p = 0.6$).

3.4 Propensity score matching

Taking into account the presence of statistically significant differences between the compared groups (with and without diuretics) in terms of age, CHF stage, ejection fraction, glomerular filtration rate (GFR) and chronic kidney disease (CKD), pseudorandomisation was performed using the propensity score matching (PSM) method to eliminate the potential influence of CHF severity or significant contributions from renal disease and function in patients. After the pseudorandomisation procedure, the total number of patients was reduced to 71. However, biases in the aforementioned parameters were eliminated and the results are presented in Table 3.

In Table 4, the results of the impact of various osmolytes on osmolality after diuretics application, performed following the propensity score matching procedure, are presented. The table indicates that despite the balance of the sample for renal function, there are still statistically significant differences in the levels of urea, PropUrea/eOSM, and PropNa/eOSM on the seventh day of diuretic therapy. These changes suggest that the alterations in the contribution of urea to osmolality are not related to changes in renal function or a higher degree of CHF in patients, but are directly caused by the impact of diuretic therapy on muscle tissue, leading to increased urea synthesis and a reduction in the sodium proportion.

TABLE 3 Group differences in key clinical characteristics after adjustment using propensity score matching (PSM).

Characteristic	Patients receiving diuretics, *N* = 31[1]	Patients not receiving diuretics, *N* = 43[1]	*p*-value[2]
Age (years)	73 (65, 77)	73 (67, 79)	0.6
Ejection fraction (%)	52.0 (50.0, 53.0)	52.0 (50.0, 52.0)	0.8
Estimated glomerular filtration rate (ml/min/1.73 m2)	55 (49, 66)	56 (48, 65)	0.7
CKD (stage)			0.2
C1	0 (0%)	1 (2.3%)	
C2	1 (3.2%)	5 (12%)	
C3a	13 (42%)	23 (53%)	
C3b	4 (13%)	4 (9.3%)	
C4	0 (0%)	1 (2.3%)	
нет	13 (42%)	9 (21%)	

[1]Median (IQR); n (%).
[2]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test.

TABLE 4 Relationship of plasma osmolyte concentrations and their ratios to estimated plasma osmolality (eOSM) in patients receiving and not receiving diuretics after adjustment using propensity score matching (PSM).

Characteristic	Patients receiving diuretics, *N* = 28[1]	Patients not receiving diuretics, *N* = 43[1]	*p*-value[2]
Sodium day 1 (mMol/L)	138.90 (137.33, 140.53)	138.80 (137.20, 140.70)	>0.9
Potassium day 1 (mMol/L)	3.97 ± 0.38	4.00 ± 0.41	>0.9
Glucose day 1 (mMol/L)	5.81 (5.40, 6.67)	5.91 (5.39, 7.09)	0.6
Urea day 1 (mMol/L)	6.45 (5.15, 8.10)	6.60 (5.50, 7.95)	0.6
Sodium day 7 (mMol/L)	139.40 (138.58, 140.93)	139.80 (138.45, 141.20)	0.8
Potassium day 7 (mMol/L)	4.17 ± 0.48	4.15 ± 0.47	0.7
Glucose day 7 (mMol/L)	5.26 (4.78, 6.52)	5.33 (4.87, 6.25)	0.9
Urea day 7 (mMol/L)	7.95 (5.65, 9.90)	5.90 (5.05, 7.50)	0.012
Urea to osmolarity ratio (%)	2.63 (1.89, 3.28)	2.00 (1.70, 2.50)	0.011
Sodium to osmolarity ratio (%)	46.46 (46.02, 46.74)	46.68 (46.33, 46.89)	0.050
Potassium to osmolarity ratio (%)	1.40 (1.32, 1.49)	1.40 (1.26, 1.46)	0.8
Glucose to osmolarity ratio (%)	1.74 (1.59, 2.19)	1.80 (1.63, 2.04)	0.9

[1]Median (IQR).
[2]Wilcoxon rank sum test; Wilcoxon rank sum exact test.

In Figures 4A,B, the impact of salt intake and diuretic therapy on the contribution of key electrolytes to plasma osmolality is illustrated, following the PSM procedure.

In graph A, it is shown that with high salt intake (more than 10 grams and from 6 to 10 grams per day), the use of diuretics is associated with a higher percentage contribution of urea to the total plasma osmolality. For salt intake of less than 6 grams per day, there is no statistically significant increase in the contribution of urea.

In graph B, a significant reduction in the sodium proportion in plasma osmolality is observed with a salt intake of more than 10 grams per day during diuretic therapy. Although statistical significance is not reached for the 6–10 grams range and less than 6 grams of salt per day, a trend toward a reduction in the proportion of sodium is still observed for the 6–10 gram group. The contribution of potassium and glucose to osmolality remains statistically insignificant regardless of the level of salt intake and diuretic use.

The box plots illustrate the distribution of median values, interquartile ranges, and individual measurements, providing a visual representation of changes in plasma electrolyte composition under the influence of these factors, adjusted using the PSM method.

3.5 Modeling results

In Table 5, the results of the modeling of the use of diuretics against the background of a diet with varying levels of sodium intake and the changes in the contributions of various osmolytes to plasma osmolality, such as urea, glucose, sodium, and potassium, and their impact on the likelihood of diuretic use in patients are presented.

Model 1: Analysis of PropK/eOSM on the seventh day and various levels of salt intake yielded an odds ratio (OR) of 6.45 with a 95% confidence interval (CI) of [0.62; 78.3] and a *p*-value of 0.12. This indicates that there is no statistically significant effect of osmolality relative to potassium on the likelihood of diuretic use.

Model 2: Evaluation of PropGlucose/eOSM on day 7 and various levels of salt intake showed an OR of 1.32 with a 95% CI of [0.60; 3.13]

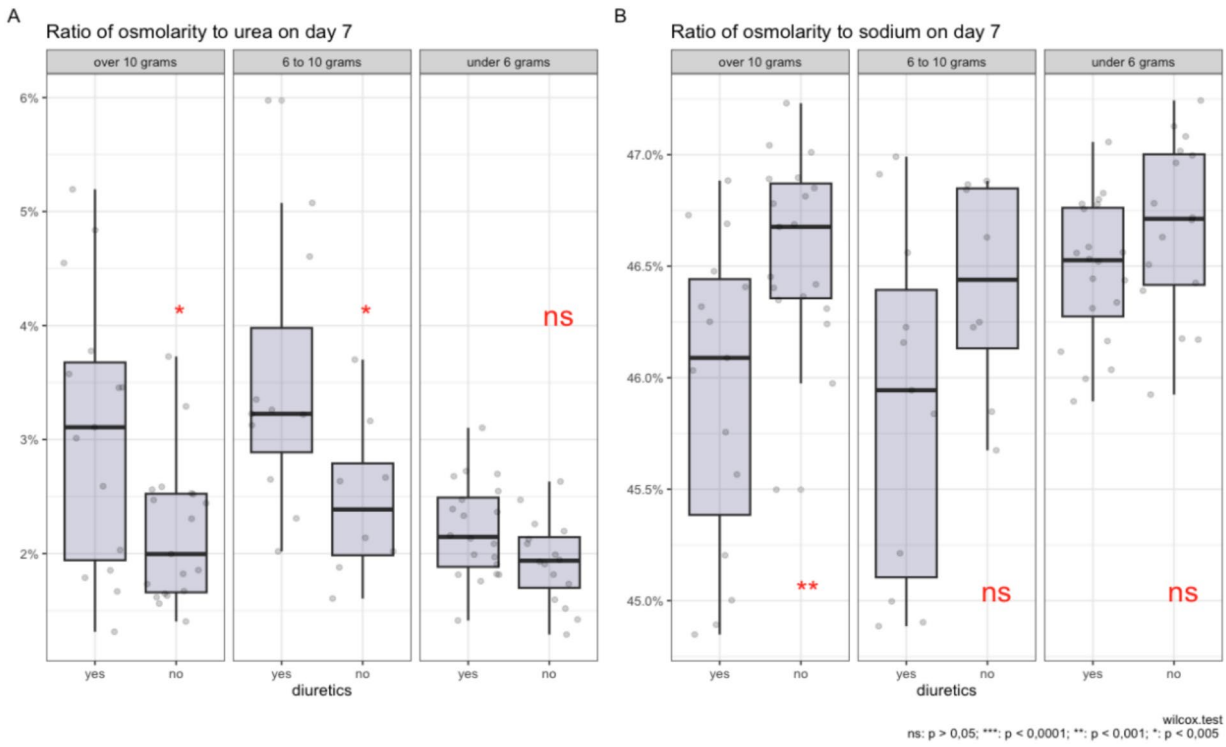

FIGURE 4
Comparison of the ratio of plasma osmolality to urea **(A)** and sodium **(B)** on day 7 of diuretic therapy across groups with varying levels of salt intake, after data adjustment using propensity score matching.

TABLE 5 **Multivariate logistic regression models evaluating the association between the use of diuretics and plasma osmolyte ratios on day 7, with adjustment for salt intake.**

Переменные	Model 1			Model 2			Model 3			Model 4		
	OR[1]	95% CI[1]	*p*-value	OR[1]	95% CI[1]	*p*-value	OR[1]	95% CI[1]	*p*-value	OR[1]	95% CI[1]	*p*-value
PropK/eOSM	6.45	0.62, 78.3	0.12									
Salt intake			0.5			0.6			0.2			0.3
Over 10 grams	—	—		—	—		—	—		—	—	
6 to 10 grams	1.68	0.68, 4.20		1.51	0.62, 3.71		2.65	0.97, 7.71		2.29	0.86, 6.39	
Under 6 grams	1.70	0.57, 5.30		1.54	0.53, 4.69		1.39	0.40, 4.88		1.54	0.46, 5.38	
PropGlucose/eOSM.				1.32	0.60, 3.13	0.5						
PropUrea/eOSM							3.52	1.94, 7.26	**<0.001**			
PropNa/eOSM										0.16	0.06, 0.39	**<0.001**
AIC	143			145			124			126		

[1]OR, Odds Ratio; CI, Confidence Interval. Bold values indicate $p < 0.001$.

and a *p*-value of < 0.5. Here, too, no statistically significant results were obtained, indicating that there was no dependence between diuretic use and changes in PropGlucose/eOSM.

Models 3 and 4: These models provide estimates for PropUrea/eOSM and PropNa/eOSM on the seventh day. In Model 4, the OR estimate for PropUrea/eOSM on day 7 is 3.52 with a 95% CI of [1.94;

7.26] and a p-value of < 0.001. This indicates a significant association between diuretic use and changes in PropUrea/eOSM. In contrast, an increase in osmolality compared to sodium on day 7 is associated with a significant reduction in the likelihood of diuretic use (OR = 0.16, 95% CI: 0.06–0.39, $p < 0.001$).

Thus, the results confirm that the use of diuretics in combination with a diet containing varying amounts of salt induces different changes in PropUrea/eOSM and PropNa/eOSM.

When constructing univariate models that evaluate the use of diuretics, PropNa/eOSM on day 7 and PropUrea/eOSM on day 7 without considering salt intake, statistically significant results are obtained. However, they are less robust compared to models that include the level of salt intake. For example, in the PropUrea / eOSM model, the odds ratio (OR) is 3.01 (95% CI 1.74, 5.89; $p < 0.001$), and for the PropNa / eOSM model, the OR is 0.19 (95% CI 0.07, 0.43; $p < 0.001$).

4 Discussion

The main finding of the present study is that patients with chronic heart failure (CHF) exhibit metabolic features resembling aestivation in response to loop diuretic therapy, particularly under conditions of high dietary sodium intake. This adaptation is characterized by a shift in plasma osmolyte composition—namely, an increase in the contribution of urea and a relative decrease in sodium—to maintain plasma osmolality and promote water conservation. The rise in the urea-to-osmolality ratio suggests enhanced proteolysis and nitrogen conservation, potentially indicating muscle catabolism. These mechanisms may contribute to the development of sarcopenia and reduced functional status commonly observed in CHF.

This study expands upon previously published findings in several important ways. It is the first clinical investigation to demonstrate a relationship between excessive sodium intake and the use of diuretics in patients with chronic heart failure, leading to dehydration. The results are consistent with prior studies conducted in cohorts of critically ill patients in intensive care units receiving diuretic therapy (5), where similar shifts in osmolyte composition—primarily an increased contribution of urea—were observed by day seven. These findings support the generalizability of the concept of metabolic aestivation as a universal adaptive response occurring under conditions of critical illness and fluid loss–associated therapy.

Aestivation can be characterized as a series of physiological adaptations aimed at preventing dehydration and death. The key goal of aestivation is the conservation of water in the body. The need to conserve water under conditions of high salt intake induces a state similar to aestivation in experimental mice (18).

Homer Smith was the first to study changes in osmolytes and body hydration in aestivating 'lungfish' (19). These fish, living in underground mud cocoons, enter a state of aestivation when in water that is hyperosmotic relative to their body fluids, which promotes water loss. To prevent dehydration, aestivating lungfish increase the level of urea in their plasma and tissues.

Urea and its transporters play a crucial role in the urine concentration processes in the kidneys. Studies show that in cases of protein deficiency, the ability to concentrate urine decreases, which can be restored by adding urea. Genetically modified mice lacking specific urea transporters demonstrate similar reductions in urine concentration capacity (18).

There is a hypothesis that a high concentration of urea in the interstitial space of the renal medulla is necessary for effective urine concentration. This is achieved by reabsorption of urea through specific protein transporters, such as UT-A1 and UT-A3. The UT-B1 transporter, present in erythrocytes, also plays an important role in this process, facilitating efficient countercurrent exchange and urine concentration. The absence of this transporter in humans and mice leads to a reduced urine concentration capacity (18).

The principle of water conservation in urine concentration through accumulation of urea has been previously established and confirmed in mice with impaired renal urea transporter function (20). According to previous data that indicate opposing effects of urea and NaCl osmolytes on urea transport managed by the UT-A1 transporter under conditions of acute osmotic diuresis, it is demonstrated that this principle of water conservation, dependent on urea, is used to maintain kidney concentration processes, possibly compensating for the osmotic diuretic effect of salt excretion. As a result, endogenous water accumulation ensures consistent urine volume despite intense natriuresis (20). These mechanisms are particularly relevant in patients with chronic heart failure undergoing diuretic therapy. The present study offers a novel perspective on intensive diuretic treatment, suggesting that it may activate distinct metabolic effects associated with the aestivation response.

Intense natriuresis can occur for various reasons and can be related to external factors that affect patients or the use of medications that increase natriuresis. Bankir et al. (21) previously noted that during the study 'Dietary approaches to stop hypertension (DASH)', increased salt excretion led to higher Na^+ concentrations in patient urine without increasing urine volume. These results underscore the importance of controlling kidney concentrating function and water conservation regulation as key factors in urine formation and extracellular volume homeostasis in humans with high salt intake.

Water conservation in the body through modification of urea osmolyte synthesis mechanisms in response to increased salt intake involves not only the kidney urea recirculation process but also activation of urea osmolyte synthesis in the liver and muscle tissue, as shown in a study in mice subjected to a high-salt diet (6). The study demonstrated that the urea content in the kidneys, liver, and muscles explains 87% of the variability in plasma urea levels in mice, with the liver contributing the most and the muscles the least. This indicates that the transport of urea osmolyte by the kidneys and the production of urea osmolyte in the liver and skeletal muscles are integrated physiological components. Considering the energy-intensive nature of urea synthesis, it has significant implications for energy metabolism with high salt intake, which can be observed under conditions of intensive diuretic therapy. Ishikawa et al. (22) showed that prolonged use of loop diuretics is associated with muscle wasting in patients with renal insufficiency.

The strengths of the present study are that it is the first to analyze the presence of an aestivation-like response in patients with chronic heart failure (CHF), depending on different levels of salt intake and the use of diuretic therapy. This expands upon prior findings by providing clinical evidence outside of the ICU setting. In the work of S. Nihlen et al. (5), the hypothesis of a similarity was presented between the human body's response to fluid loss and the aestivation

response in animals. The study included 241 post-intensive care patients and showed that the increase in total osmolality during the period of reducing body hyperhydration is due to loss of free water and changes in osmolyte balance: a decrease in the sodium contribution and an increase in the urea contribution. These results suggest an aestivation mechanism similar to the body's response to prolonged dehydration, influencing patient survival. In an intensive care unit study from the third to the seventh day, 177 patients (73%) received furosemide. The analysis showed that the correlation between the proportion of urea and effective osmolality on the seventh day after the adjusted cumulative dose of furosemide was $r = 0.55$. Among patients with and without renal replacement therapy, this figure was $r = 0.54$ and $r = 0.55$, respectively. In the subgroup without furosemide and renal replacement therapy ($n = 17$), the correlation reached $r = 0.92$. For patients treated with furosemide but without renal replacement therapy, the figure was $r = 0.61$. There was a shift in the osmolytes from sodium (Na+) and potassium (K+) to urea, i.e., from inorganic to organic osmolytes. This phenomenon was also present in patients with normal estimated glomerular filtration rate (eGFR), even after adjusting the model for eGFR. Finally, although the level and dynamics of osmolality during the 3–7 days of ICU stay were similar between survivors and nonsurvivors, the urea-to-effective osmolality ratio was higher in patients who did not survive 90 days after ICU admission.

In 2022, Hultström et al. (23) conducted a study analyzing the effects of dehydration in patients with COVID-19, particularly examining the response to aestivation in the body and its impact on long-term disease outcomes. The results showed that metabolic aestivation in response to dehydration in patients with COVID-19 is associated with the severity of the disease. Metabolomic analysis identified amino acids with an aestivation profile, indicating muscle protein breakdown and the use of released amino acids for urea synthesis in the liver (24).

A study by C. Baumgartner et al. (25) examined the relationship between glycerophosphocholine (GPC), an organic osmolyte, and surrogate parameters of hydration status and osmolality in healthy individuals using non-invasive ^{31}P-magnetic resonance spectroscopy of the calf and thigh muscles. In a sample of 30 volunteers, significant correlations were found between GPC levels and markers of fluid and electrolyte balance, such as uric acid ($r = 0.437$, $p = 0.018$) and urea ($r = 0.387$, $p = 0.035$). Multiple regression analysis revealed that GPC concentrations could predict changes in uric acid levels ($R^2 = 0.462$, adjusted $R^2 = 0.421$, $p < 0.001$), suggesting that the GPC content in skeletal muscles adapts in response to changes in fluid status.

The results of the present study demonstrated that changes in the contribution of individual osmolytes to plasma osmolality—particularly the increase in the proportion of urea—reflect the activation of water-conserving mechanisms, which play a key role in urine formation and the maintenance of extracellular fluid homeostasis in patients with chronic heart failure (CHF), especially under conditions of high salt intake and intensive diuretic therapy. Furthermore, the findings suggest that the altered contribution of urea to osmolality is not related to changes in renal function or more advanced CHF, but rather is a direct consequence of the effects of diuretic therapy on muscle tissue, leading to increased urea synthesis and a reduction in the proportion of sodium.

A study by G. Rossitto et al. (26) demonstrated that in patients with hypertension, a high sodium intake (>5 g/day) is associated with an increased glomerular filtration rate (127.5 mL/min/1.73 m^2 vs. 94.1 mL/min/1.73 m^2 with low sodium intake, $p = 0.001$) and increased renal energy expenditure for sodium reabsorption (difference of 18 kcal/day, $p < 0.001$), despite high fractional sodium excretion (0.81% vs. 0.39%, $p < 0.001$) and low water excretion (0.89% vs. 1.13%, $p = 0.015$). This leads to a catabolic shift and an increase in protein metabolism byproducts, which may elevate cardiovascular risk independently of blood pressure.

We hypothesized that the degree of aestivation might differ in patients on a diet with varying salt content undergoing intensive diuretic therapy. This is because patients on a long-term high-salt diet may have osmotically inactive sodium in muscle tissue (27). In our previous work (28), we found a relationship between Na + and negatively charged glycosaminoglycan (GAG) structures in rats on a high-salt diet. Therefore, we decided to investigate how excessive salt intake and intensive diuretic therapy in patients with CHF without ICU would affect the development of aestivation. In intensive diuretic therapy with sodium loss, partial replenishment can occur due to osmotically neutral sodium, so in our study, under a high-salt diet, the transition from inorganic (high sodium proportion) to organic osmolytes (high urea proportion) is more pronounced with a high-salt diet, whereas the effect is statistically insignificant with a diet containing less than 6 grams of salt per day. In our study, after 7 days of diuretic therapy, a statistically significant increase in the proportion of urea was observed (from 2.2% [1.8; 2.6] to 2.5% [1.9; 3.4], $p = 0.15$) and a decrease in the sodium proportion in plasma (from 46.4% [46.1; 46.8] to 46.3% [45.9; 46.7], $p = 0.14$) were observed. At the initial observation stage (first day), no statistically significant differences in plasma osmolality were found between the diuretic-receiving and nondiuretic-recipient groups.

To our knowledge, this is the first study to show that in patients who consume more than 10 grams of salt per day and undergo intensive diuretic therapy, urea increases significantly ($p < 0.05$) compared to other groups. However, with diets consuming 6 to 10 grams of salt per day and less than 6 grams, changes in the proportion of urea and sodium were statistically non-significant ($p > 0.05$). It is important to note that this is relevant in the context of hemodynamic changes, which are frequently observed and worsen the prognosis of patients with chronic heart failure (CHF). For instance, in the study by Sonaglioni A. et al. (29), heart failure patients exhibiting the "cold-dry" phenotype had significantly worse survival outcomes compared to other hemodynamic subtypes ($p < 0.001$), partly due to high sodium levels (HR 1.03, 95% CI 1.01–1.04). Consequently, the administration of diuretics to patients with the "cold-dry" hemodynamic profile should be approached with particular caution, and treatment should primarily aim to improve cardiac output and tissue perfusion.

5 Conclusion

The present study demonstrated that in patients with chronic heart failure (CHF), intensive loop diuretic therapy—particularly against the background of excessive sodium intake—is associated with changes in the plasma osmotic profile characteristic of metabolic

aestivation. The key observation was a statistically significant increase in the contribution of urea and a decrease in the proportion of sodium to calculated plasma osmolality, indicating activation of water conservation mechanisms. These changes were not attributable to the severity of CHF or impaired renal function, as shown by the post–propensity score matching (PSM) analysis, and likely reflect osmolyte redistribution resulting from diuretic therapy and enhanced proteolysis.

From a clinical perspective, these findings suggest that monitoring the urea-to-osmolality ratio during the initial days of diuretic therapy may serve as an early biomarker for metabolic stress and muscle catabolism risk. Clinicians managing CHF patients should consider implementing individualized diuretic strategies that account for baseline dietary sodium intake, with particular attention to patients consuming >10 g/day of salt who may be at increased risk for aestivation-like metabolic responses. Early recognition of elevated urea-to-osmolality ratios should prompt consideration of nutritional support, physical therapy interventions, and potentially modified diuretic dosing regimens to achieve optimal fluid balance while minimizing catabolic consequences.

Thus, the findings offer a novel perspective on therapeutic strategies in the management of CHF. In particular, they highlight the importance of an individualized approach to prescribing diuretics that accounts for dietary sodium intake: high sodium consumption may exacerbate the adverse metabolic consequences of therapy by promoting a shift from inorganic to organic osmolytes and triggering skeletal muscle catabolism. This, in turn, may contribute to the development of sarcopenia and reduced functional capacity. The concept of metabolic aestivation may provide a new pathophysiological explanation for fatigue and weakness syndromes in such patients and warrants further investigation in clinical settings.

6 Limitations of the study

The present study has several limitations. It was conducted within a single medical center, which limits the generalizability of the findings to the broader population of patients with chronic heart failure (CHF). Despite the use of the propensity score matching (PSM) method, the final sample size was relatively small, reducing the statistical power of the analysis and precluding subgroup stratification. We did not perform direct measurements of muscle catabolism; thus, conclusions regarding increased proteolysis are based on indirect indicators (i.e., an increase in the proportion of urea in plasma osmolality). The study lacks long-term follow-up, so clinical outcomes such as changes in muscle mass, physical activity, and survival were not evaluated. The influence of other medications prescribed for heart failure—many of which may also induce diuresis and electrolyte disturbances—should also be investigated. Finally, the study did not assess direct markers of the hormonal stress response (e.g., cortisol, vasopressin, or ACTH), which could further clarify the underlying pathophysiological mechanisms.

Additionally, this study did not evaluate the clinical utility of the urea-to-osmolality ratio as a monitoring tool in routine clinical practice, nor did it assess the potential impact of implementing aestivation-aware diuretic strategies on patient outcomes such as length of stay, readmission rates, or functional status at discharge. Future prospective studies should investigate whether routine monitoring of osmolyte ratios and implementation of targeted interventions based on these findings can improve clinical outcomes and reduce the incidence of diuretic-associated muscle wasting in CHF patients.

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 humans were approved by Ethics Committee of the Cardiology Department of GBUZ GVV No. 3 DZM (protocol code 4, 04.07. 2022). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

DD: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. AS: Investigation, Methodology, Resources, Validation, Writing – original draft. VM: Supervision, Validation, Writing – original draft, Writing – review & editing. GA: Conceptualization, Project administration, Supervision, Validation, Writing – review & editing.

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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.

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References

1. Peacock WF, Colucci WS, De Marco T. Impact of intravenous loop diuretics on outcomes of patients hospitalized with acute decompensated heart failure: insights from the ADHERE registry. *Cardiology*. (2008) 113:12–9. doi: 10.1159/000164149

2. Felker GM, Lee KL, Bull DA, Redfield MM, Stevenson LW, Goldsmith SR, et al. Diuretic strategies in patients with acute decompensated heart failure. *N Engl J Med*. (2011) 364:797–805. doi: 10.1056/NEJMoa1005419

3. Gupta A, Fonarow GC, Butler J. Obesity and the response to intensified diuretic treatment in decompensated heart failure: a DOSE trial substudy. *J Card Fail*. (2012) 18:837–44. doi: 10.1016/j.cardfail.2012.09.005

4. Storey KB, Storey JM. Aestivation: signaling and hypometabolism. *J Exp Biol*. (2012) 215:1425–33. doi: 10.1242/jeb.054403

5. Nihlén S, Walther SM, Palmér K. The contribution of plasma urea to total osmolality during iatrogenic fluid reduction in critically ill patients. *Function*. (2021) 3:zqab055. doi: 10.1093/function/zqab055

6. Delles C, Rossitto G. Of snails, earthworms, and men: insights into strategies to preserve water. *Function*. (2021) 3:zqab071. doi: 10.1093/function/zqab071

7. Haussinger D, Lang F, Gerok W. Regulation of cell function by the cellular hydration state. *Am J Physiol Endocrinol Metab*. (1994) 267:E343–55.

8. Lerchl K, Rakova N, Dahlmann A, Rauh M, Goller U, Basner M, et al. Agreement between 24-hour salt ingestion and sodium excretion in a controlled environment. *Hypertension*. (2015) 66:850–7. doi: 10.1161/HYPERTENSIONAHA.115.05851

9. Titze J, Shakibaei M, Schafflhuber M. Reduced osmotically inactive Na storage capacity and hypertension in the dahl model. *Am J Physiol Ren Physiol*. (2002) 283:F134–41. doi: 10.1152/ajprenal.00323.2001

10. Titze J, Lang R, Ilies C, Schwind KH, Kirsch KA, Dietsch P, et al. Osmotically inactive skin Na+ storage in rats. *Am J Physiol Ren Physiol*. (2003) 285:F1108–17. doi: 10.1152/ajprenal.00200.2003

11. Zuo X, Zhang Z, Li B. Sarcopenia and cardiovascular diseases: a systematic review and meta-analysis. *J Cachexia Sarcopenia Muscle*. (2023) 14:1183–98. doi: 10.1002/jcsm.13221

12. Dragunov DO, Sokolova AV, Arutjunov GP. Development and validation of a questionnaire to assess the level of salt intake in the adult population of the Russian Federation using machine learning methods. *Russian Archives Internal Med*. (2024) 14:284–97. doi: 10.20514/2226-6704-2024-14-4-284-297

13. Russian Society of Cardiology (RSC). 2020 clinical practice guidelines for chronic heart failure. Russ. *J Cardiol*. (2020) 25:4083. doi: 10.15829/1560-4071-2020-4083

14. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. CKD-EPI (chronic kidney disease epidemiology collaboration)a new equation to estimate glomerular filtration rate. *Ann Intern Med*. (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006

15. Clinical recommendations. Chronic kidney disease (CKD). *Nephrology (Saint-Petersburg)*. (2021) 25:10–82.

16. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. *Multivar Behav Res*. (2011) 46:399–424. doi: 10.1080/00273171.2011.568786

17. Ho DE, Imai K, King G. MatchIt: nonparametric preprocessing for parametric causal inference. *J Stat Softw*. (2011) 42:1–28. doi: 10.18637/jss.v042.i08

18. Kovarik JJ, Fuchs D, Exner M. Adaptive physiological water conservation explains hypertension and muscle catabolism in experimental chronic renal failure. *Acta Physiol (Oxford)*. (2021) 232:e13629. doi: 10.1111/apha.13629

19. Wilkie MP, Wright PA, Wood CM. The African lungfish (*Protopterus dolloi*): ionoregulation and osmoregulation in a fish out of water. *Physiol Biochem Zool*. (2007) 80:99–112. doi: 10.1086/508837

20. Kitada K, Daub S, Zhang Y, Klein JD, Nakano D, Pedchenko T, et al. High salt intake reprioritizes osmolyte and energy metabolism for body fluid conservation. *J Clin Invest*. (2017) 127:1944–59. doi: 10.1172/JCI88532

21. Perucca J, Bouby N, Sandford R. Sex difference in urine concentration across differing ages, sodium intake, and level of kidney disease. *Am J Phys Regul Integr Comp Phys*. (2007) 292:R700–5. doi: 10.1152/ajpregu.00500.2006

22. Ishikawa S, Kaizu K, Osawa H. Loop diuretics are associated with greater risk of sarcopenia in patients with non-dialysis-dependent chronic kidney disease. *PLoS One*. (2018) 13:e0192990. doi: 10.1371/journal.pone.0192990

23. Hultström M, Lipcsey M, Morrison DR, Nakanishi T, Butler-Laporte G, Chen Y, et al. Dehydration is associated with production of organic osmolytes and predicts physical long-term symptoms after COVID-19: a multicenter cohort study. *Crit Care*. (2021) 26:322. doi: 10.1186/s13054-022-04203-w

24. Barrueta Tenhunen A, Butler-Laporte G, Yoshiji S, Morrison DR, Nakanishi T, Chen Y, et al. Metabolomic pattern associated with physical sequelae in patients presenting with respiratory symptoms validates the aestivation concept in dehydrated patients. *Physiol Genomics*. (2024) 56:483–91. doi: 10.1152/physiolgenomics.00021.2024

25. Baumgartner C, Wolf P, Beiglböck H, Pfleger L, Fellinger P, Heitzinger G, et al. Potential role of skeletal muscle glycerophosphocholine in response to altered fluid balance in humans: an in vivo nuclear magnetic resonance study. *Am J Physiol Endocrinol Metab*. (2023) 324:E339–46. doi: 10.1152/ajpendo.00286.2022

26. Rossitto G, Maiolino G, Lerco S, Ceolotto G, Blackburn G, Mary S, et al. High sodium intake, glomerular hyperfiltration, and protein catabolism in patients with essential hypertension. *Cardiovasc Res*. (2020) 117:1372–81. doi: 10.1093/cvr/cvaa205

27. Wiig H, Luft FC, Titze JM. The interstitium conducts extrarenal storage of sodium and represents a third compartment essential for extracellular volume and blood pressure homeostasis. *Acta Physiol (Oxford)*. (2018) 222:e13006. doi: 10.1111/apha.13006

28. Artyukov I, Melnikova A, Ivanov A. The first observation of osmotically neutral sodium accumulation in the myocardial interstitium. *Sci Rep*. (2021) 11:22025. doi: 10.1038/s41598-021-01443-8

29. Sonaglioni A, Lonati C, Tescaro L. Prevalence and clinical outcome of main echocardiographic and hemodynamic heart failure phenotypes in a population of hospitalized patients 70 years old and older. *Aging Clin Exp Res*. (2022) 34:1081–94. doi: 10.1007/s40520-021-02025-4

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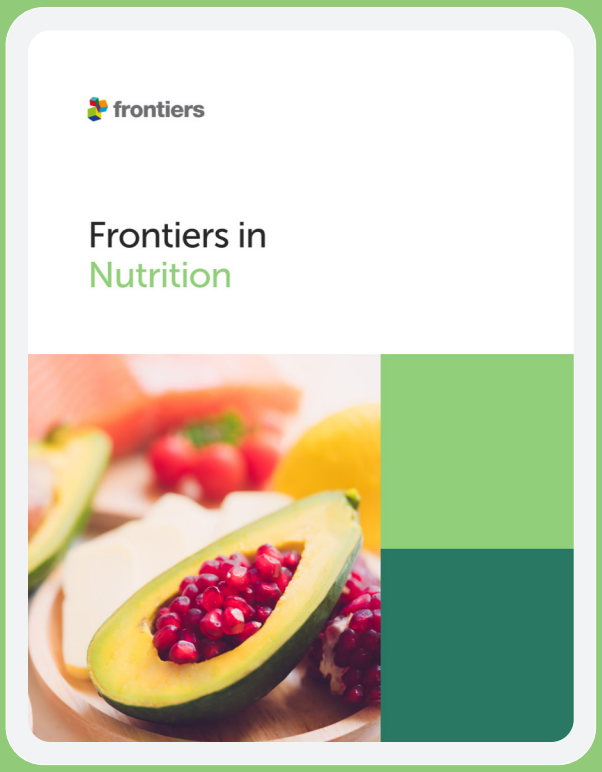

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