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Value-based nutritional intervention to reduce the progression of chronic human diseases

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Value-based nutritional intervention to reduce the progression of chronic human diseases

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Editorial: Value-based nutritional intervention to reduce the progression of chronic human diseases

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KEYWORDS

magnesium, phosphate, selenium, COVID-19, vitamin D

Editorial on the Research Topic
Value-based nutritional intervention to reduce the progression of chronic human diseases

Nutritional imbalances, including in various vitamins and minerals, are associated with initiation and progression of numerous chronic disorders, including systemic and metabolic diseases (1–10). Value-based nutritional care is an approach that links the incentive for healthcare providers to the value and outcomes they deliver to patients in terms of quality, equity, and cost of care. Providing value-based nutritious foods and increasing the awareness of benefits of healthy eating habits can prevent the pathogenesis and progression of chronic human diseases. The intervention with value-based nutrition can also reduce the disease burden of patients with chronic diseases and thereby decrease overall care costs (11–15). However, before implementing the value-based nutritional intervention, the role(s) and regulation(s) of essential nutritional components in various chronic diseases need to be determined. This "Research Topic" is intended to bring together experts to share their experiences in nutritional manipulation to reduce the burden of chronic human diseases. A total of 13 articles by 82 authors have been published on this "Research Topic" to efficiently accomplish the intended objectives.

Analyzing data from the U.S. National Health and Nutrition Examination Survey (NHANES), Wang J. et al. reported that dietary magnesium intake levels and the risk of osteoporosis are negatively correlated, particularly among individuals with 55 years or older. Studies have shown that maintaining optimal magnesium and vitamin D balance improves overall skeletal functions in elderly individuals (16). In a separate study, using the same NHANES database, Wang H. et al. found that a diet with higher inflammatory potential, as measured by the Dietary Inflammatory Index, is associated with increased hyperuricemia risk, implicating dietary modification as a potential approach for hyperuricemia's prevention and control. Mironov et al. detailed the possible toxic effects of consuming a high phosphate diet for a prolonged period of time, with potential adverse effects on various organs. Phosphate-based additives and preservatives are commonly used in our daily consumed foods and drinks. Since the FDA does not mandate food industries to list phosphate content on labels, it is not easy to monitor the amount of consumed phosphate (17). Sun et al. reviewed the benefits of maintaining adequate selenium on health and disease. Selenium has claimed to have anti-oxidant, anti-tumorigenic, anti-diabetic, and immune

boosting functions. The references value of selenium level varies among various populations. Moreover, selenium has a very narrow range between deficiency and toxicity. Safe modes and amounts of selenium ingestion as a supplement need to be determined. In a multi-center case-control study, conducted in the Guangdong province of southern China, a known endemic region for nasopharyngeal carcinoma, Ge et al. observed an association between levels of various serum trace elements and the risk of nasopharyngeal carcinoma. Serum levels of cadmium and manganese were positively related to nasopharyngeal carcinoma risk, which might be of clinical relevance and importance in early diagnosis of cancer patients. Li et al. proposed a prognostic significance of a novel nutritional metabolism-related scoring system (NMRS) for patients with newly diagnosed osteosarcoma. Through iterative LASSO cox analysis, an NMRS was also constructed to calculate the prognosis of osteosarcoma patients. The investigators found that NMRS can faithfully reflect patients' nutritional and metabolic status, and they predicted that by combining NMRS, patients could be further risk stratified based on existing clinical characteristics. Another skeletal lesion, spinal tuberculosis accounts for about 50% of osteoarthritis tuberculosis. Jiang et al. proposed a scoring scale to assist physicians in evaluating whether patients with thoracic and lumbar tuberculosis would develop hypoalbuminemia following surgery. The scale is simple, reliable, and has clinical guiding significance to avoid postoperative hypoalbuminemia effectively.

In a systematic review and meta-analysis, Zhang et al. elaborated on the effects of vitamin D supplementation on COVID-19 patients. They found that vitamin D supplementation reduced mortality in COVID-19 patients in cohort studies but not in randomized controlled trials (RCTs), raising an important issue of vitamin D's utility in treating COVID-19. Using umbrella meta-analysis, Musazadeh et al. detected that supplementation with L-carnitine can improve lipid profile, and they recommended L-carnitine as an adjuvant anti-hyperlipidemic agent; further clinical studies with large-scale RCTs are needed to have better therapeutic insights.

Loss of masticatory function, as a result of tooth loss is linked to the changes in food choices and inadequate nutritional intake. Using a protocol for a factorial randomized clinical trial, Qian et al. hypothesized that receiving rehabilitation of masticatory function with fixed implant dentures together with nutritional awareness is the most efficient intervention for enhancing nutrient intake in community-dwelling elderly individuals with extensive tooth loss. The results of this study have both therapeutic and policy-making implications. In a separate cross-sectional study that enrolled 3,706 participants, Qu reported a negative association between the Oxidative Balance Score (OBS) and periodontitis, suggesting that managing OBS in dietary intake and modifying lifestyle may alleviate the occurrence of periodontitis. However, such negative associations differed in the elderly and diabetes groups. Yang et al. showed that N-linoleyltyrosine ameliorates high-fat diet-induced obesity in C57BL/6 mice via regulating cannabinoid receptors, demonstrating the anti-obesity potential of N-linoleyltyrosine. As most children, who are overweight, tend to remain overweight in their adult life, causing a substantial financial burden on society. Therefore, it is crucial to target unhealthy dietary patterns in early life. Charneca et al. documented that the dietary intake of key components of a healthy diet in Portuguese preschool children is inadequate, with high intake of sugary foods and low intake of vegetables and legumes, suggesting the urgent need for nutrition education and communication strategies, including the use of technology to improve the feeding practices and develop healthy eating habits in the young children as an anti-obesity measure.

In summary, the aforementioned articles, published in this "Research Topic" highlight the nutritional aspects of various chronic diseases. These articles also identified the value of selective nutritional components and the importance of providing nutritional balance to prevent or delay the emergence of chronic diseases. Together, this "Research Topic" highlighted the need for conversation among the healthcare-providing communities to develop effective value-based nutritional strategies to promote healthier dietary habits and provide compassionate care to reduce the burden of chronic diseases and improve general health for all age groups (18).

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MR: Writing—original draft, Writing—review & editing. AA: Writing—original draft, Writing—review & editing.

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ORIGINAL RESEARCH
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What Level Should Preoperative Albumin of Thoracic and Lumbar Tuberculosis Patients Be Reached: A Case-Controlled Study

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Objective: To explore the risk factors of hypoalbuminemia in patients with thoracic and lumbar tuberculosis and develop a scoring scale, according to which the patients with thoracic and lumbar tuberculosis were divided into 2 groups to, respectively calculate the perioperative albumin changes and to find out the preoperative albumin recommended value.

Methods: A total of 166 patients with thoracic and lumbar tuberculosis, who underwent spinal focus debridement between January 2012 to May 2020, were identified into 2 groups: with and without postoperative hypoalbuminemia (n = 131 and n = 35, respectively), recording and analyzing clinical characteristics by multivariate analysis to establish a scoring scale. Using this scale, patients with spinal tuberculosis were divided into a high-risk group and a low-risk group, and then, calculated the average decrease of postoperative albumin in both groups. Combined with the diagnostic threshold of hypoalbuminemia, we proposed the preoperative albumin safe values of the patients with thoracic and lumbar tuberculosis.

Results: A total of 131 of 166 patients experienced postoperative hypoalbuminemia after spinal focus debridement. Multivariate binary logistic regression analysis identified pulmonary tuberculosis (adjusted odds ratio = 0.270, p = 0.012), pre-operative serum albumin value (adjusted odds ratio = 0.754, $p < 0.001$), and operation time (adjusted odds ratio = 1.017, p = 0.002) as independent risk factors for the occurrence of postoperative hypoalbuminemia in patients with thoracic and lumbar tuberculosis. According to the OR value, the risk factors are assigned to make the scoring scale, the receiver operating characteristic (ROC) curve indicates that postoperative hypoalbuminemia rises when the score is greater than or equal to 4 points. The scoring scale is tested in the derivation set (166 patients) showed: sensitivity-51.9%, specificity-91.4%, and in the validation set (102 patients) showed: sensitivity-63.6% and specificity-86.1%. The perioperative albumin decreased value is 4.71 ± 2.66 g/L in the low-risk group and 8.99 ± 3.37 g/L in the high-risk group ($p < 0.001$).

Conclusion: Complicated with pulmonary tuberculosis, low preoperative albumin value and long operation time can lead to postoperative hypoalbuminemia in patients

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with thoracic and lumbar tuberculosis. The scoring scale can effectively assist physicians to evaluate whether patients with thoracic and lumbar tuberculosis develop hypoalbuminemia after surgery. The scale is simple and reliable and has clinical guiding significance. For low-risk patients and high-risk patients, preoperative albumin values should reach 40 and 44 g/L, respectively, to effectively avoid postoperative hypoalbuminemia.

Keywords: spinal tuberculosis, postoperative hypoalbuminemia, risk factors, scoring scale, recommended value

INTRODUCTION

Spinal tuberculosis (STB) is common extrapulmonary tuberculosis, accounting for about 50% of osteoarthritis tuberculosis (1). At present, antituberculous drug therapy, combined with surgical treatment, is considered to be the gold standard for the treatment of spinal tuberculosis (2). The debridement of lesions is the key point of spinal tuberculosis therapy, which enhances the control of tuberculosis changes, improves the efficacy of anti-tuberculosis drugs, promotes bone graft fusion, and reduces the risk of recurrence of spinal tuberculosis (3, 4). However, STB debridement has the disadvantages of larger trauma, longer operation time, and more bleeding, considering that 42% of the patients over 60 years old who underwent spinal surgery are malnourished before the operation (5, 6). The possibility of postoperative hypoalbuminemia in patients with STB is significantly higher than in those with a spinal degenerative disease (7–9). Hypoalbuminemia defined as serum albumin less than 35 g/L is commonly considered a representation of malnutrition (10, 11). The ensuing poorer clinical outcomes have been associated with an increased incidence of postoperative complications in a variety of orthopedic surgeries, ranging from spinal fusions to hip fractures, which are closely related to hypoalbuminemia (12–14). In conclusion, patients with spinal tuberculosis are prone to hypoproteinemia after surgery, which can easily lead to various complications and even adverse clinical outcomes. For clinicians, how to avoid postoperative hypoalbuminemia in spinal tuberculosis patients is of great clinical significance. It has been found that preoperative albumin value in patients with total knee arthroplasty is a protective factor for postoperative hypoalbuminemia and the postoperative loss value of serum albumin is closely related to preoperative serum albumin level (15, 16), thus we speculated that the preoperative albumin value of patients with STB may be an important factor in the prevention of postoperative hypoalbuminemia. The objective of our study was to pertinently define the recommended preoperative serum albumin value based on the risk factors of postoperative hypoalbuminemia in patients with STB. In this study, we retrospectively analyzed the clinical characteristics of patients who underwent spinal tuberculosis surgery with or without postoperative hypoalbuminemia and identified three characteristics as independent risk factors of postoperative hypoalbuminemia then established a scoring scale to estimate patients with STB's incidence of postoperative hypoalbuminemia's occurrence. Through the ROC curve, we found the diagnostic score of the scoring scale and verified the sensitivity and specificity of the scale in both the derivation group (166 patients) and the validation group (66 patients). Based on the score scale, 166 patients were divided into a low-risk group ($\leq$3 points) and a high-risk group ($\geq$4 points), we calculated the perioperative albumin changing values of the two groups and deduced both groups' recommended preoperative albumin values by combining the respective average decreased albumin value with the diagnostic threshold of hypoalbuminemia (35 g/L).

MATERIALS AND METHODS

All the participants provided their written informed consent to participate in this study before their data were stored in the hospital database and used for research purposes. The work has been reported in line with the STROCSS criteria (17).

Study Design

This study is a single-center, retrospective, and case-controlled study.

Study Period

We retrospectively reviewed the records of a total of 166 patients with STB who underwent lesion debridement in our hospital from January 2012 to May 2020 to form the derivation set.

Study Population

The included patients aged between 14 and 70 had thoracic or lumbar tuberculosis for the first time, and their involved lesions are less than 3 segments. Recorded and analyzed the clinical characteristics including age, gender, height, weight, body mass index (BMI), comorbidities (diabetes, pulmonary tuberculosis), smoking history, drinking history, operation time, operation blood loss, preoperative hemoglobin, preoperative lymphocytes, preoperative albumin, preoperative C-reactive protein (CRP), preoperative erythrocyte sedimentation rate (ESR), and course of the disease.

Patient Selection

Inclusion Criteria

Patients were included when they met the following inclusion criteria: Patients were selected if they met the following inclusion criteria: (i) complete medical records, including general information, preoperative laboratory data, imaging results (MRI and CT), and data on postoperative clinical features, (ii) surgical

treatment, (iii) involved lesions less than 3 segments, and (iv) postoperative pathological diagnosis of STB.

Exclusion Criteria

Patients were excluded if they met the following exclusion criteria: (i) STB was suspected but not confirmed with pathological examination, (ii) preliminary and pathological diagnosis of a disease like a tumor rather than STB, and (iii) a history of previous STB.

Validation of the Scoring System

From June 2020 to January 2022, we prospectively included 102 patients with STB who underwent lesion debridement in our hospital to form the validation set. The inclusion criteria and exclusion criteria of the validation set are consistent with the derivation set.

Statistical Analysis

Measurement data were listed as the mean ± standard deviation (SD) or median (minimum, maximum). Mann-Whitney rank-sum tests or *t*-tests were used to compare the measurement data between the groups. IBM SPSS (version 25.0 for Windows; SPSS, Chicago, IL, United States) was used for logistic regressions to analyze the univariate and multivariate factors for postoperative hypoalbuminemia. Univariate logistic regression analysis was conducted on clinical characteristics to obtain predict factors and then multivariate logistic regression analysis was conducted on these significant factors to confirm the final significant factors. Thresholds for continuous variables (preoperative albumin and operation time) were analyzed using ROC curves. The items of the scoring scale are determined by multivariate logistic regressions, and the weighted score of each item was based on the relative size of the OR value according to the method reported by Kharbanda et al. (18). The ROC curve analysis was used to find the diagnostic threshold of the scale of which the sensitivity and specificity were obtained to access the diagnostic accuracy.

Ethical Approval

This study was in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Institutional Ethics Board of The First Affiliated Hospital of Chongqing Medical University (No. ChiCTR1800019109).

RESULTS

Patients Population

Among the total of 166 patients, 131 patients had postoperative hypoalbuminemia and 35 patients did not suffer (**Table 1**). Seventy-six males and 55 females suffered from postoperative hypoalbuminemia, and 22 male and 13 female did not have postoperative hypoalbuminemia. The mean ages of with postoperative hypoalbuminemia group and without postoperative hypoalbuminemia group were 47.64 ± 1.42 years and 44.43 ± 2.58 years, respectively (**Table 1**). The clinical characteristics with statistical significance among the with and without postoperative hypoalbuminemia groups are listed as: operation time ($P < 0.001$), operation blood loss ($P = 0.009$), preoperative serum albumin ($P < 0.001$), preoperative CRP ($P = 0.001$), and preoperative ESR ($P = 0.027$) (**Table 1**).

TABLE 1 | Perioperative characteristics of 166 patients with thoracic and lumbar tuberculosis.

Characteristics	Postoperative hypoalbuminemia		P
	Yes (*n* = 131)	No (*n* = 35)	
Age (year)	47.64 ± 1.42	44.43 ± 2.58	0.294
BMI (kg/m^2)	20.96 ± 0.26	22.03 ± 0.67	0.080
Sex (n,%)			0.605
Female	55	13	
Male	76	22	
Diabetes mellitus			0.627
Yes	15	3	
No	116	32	
Pulmonary‘tuberculosis			0.003
Yes	58	6	
No	73	29	
Smoking history (year)	8.50 ± 1.20	6.37 ± 1.79	0.396
Drinking history (year)	5.15 ± 0.99	5.00 ± 1.60	0.945
Operative time (min)	204.17 ± 4.25	169.29 ± 9.44	<0.001
Operative blood loss (ml)	394.89 ± 27.07	246.63 ± 40.18	0.009
Preoperative hemoglobin (g/L)	121.27 ± 1.48	124.89 ± 2.74	0.258
Preoperative lymphocytes (× 10^9/L)	1.24 ± 0.04	1.41 ± 0.08	0.066
Preoperative serum albumin (g/L)	38.50 ± 0.35	41.37 ± 0.40	<0.001
Preoperative CRP (mg/L)	34.13 ± 3.20	18.16 ± 3.32	0.001
Preoperative ESR (mm/h)	56.24 ± 2.50	44.34 ± 4.41	0.027
Course of disease (month)	12.95 ± 3.05	8.09 ± 2.23	0.421

Postoperative Serum Albumin Change Trend

By consulting the clinical data, the average albumin of each day among the earliest 5 days after operation of 166 patients are calculated: the first day after the operation is 33.9 ± 3.7 g, second day after the operation is 33.7 ± 3 g, third day after the operation is 32.7 ± 3.9 g, fourth day after the operation is 33.2 ± 3.8 g, fifth day after the operation is 35.1 ± 3.6 g. Postoperative serum albumin change trend 5 days after an operation is shown in **Figure 1**.

Results of Univariate and Multivariate Analysis

Univariate logistic regression analysis found that BMI, pulmonary tuberculosis, operation time, operation blood loss, preoperative lymphocytes, preoperative serum albumin, preoperative CRP, and preoperative ESR are risk factors (**Table 2**). Multivariate logistic regression analysis was used on the above significant risk factors found that pulmonary

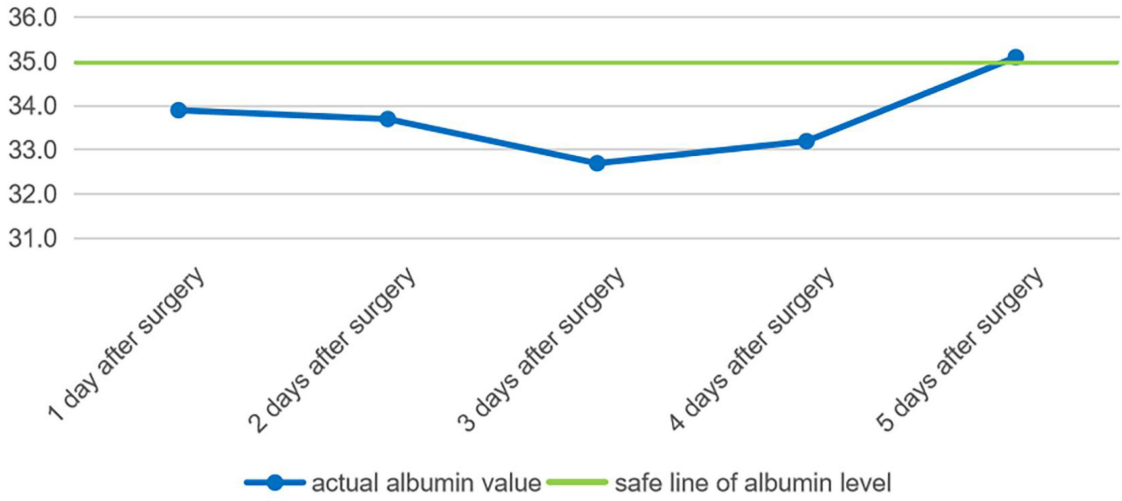

FIGURE 1 | Postoperative albumin changing trend of 166 patients.

TABLE 2 | Univariate binary logistic regression analysis of postoperative hypoalbuminemia.

Characteristics	Regression coefficient (β)	Odds ratio (OR)	95% CI	P
Age	0.013	1.013	0.989–1.036	0.293
Sex	−0.203	0.817	0.379–1.761	0.605
BMI	−0.100	0.905	0.807–1.013	0.086*
Diabetes mellitus	−0.322	0.725	0.198–2.660	0.628
Pulmonary tuberculosis	−1.346	0.260	0.101–0.669	0.005*
Smoking history	0.014	1.014	0.983–1.046	0.395
Drinking history	0.001	1.001	0.967–1.036	0.944
Course of disease	0.011	1.011	0.984–1.039	0.436
Operation time	0.017	1.017	1.007–1.026	0.001*
Operation blood loss	0.003	1.003	1.001–1.006	0.009*
Preoperative hemoglobin	−0.013	0.987	0.964–1.010	0.257
Preoperative lymphocytes	−0.692	0.500	0.237–1.057	0.070*
Preoperative serum albumin	−0.247	0.781	0.686–0.889	< 0.001*
Preoperative CRP	0.027	1.028	1.005–1.051	0.016*
Preoperative ESR	0.016	1.016	1.002–1.030	0.030*

** means statistical significance.*

TABLE 3 | Multivariate binary logistic regression analysis of postoperative hypoalbuminemia.

Characteristics	Regression coefficient (β)	Crude odds ratio (OR)	95% CI	P
BMI	−0.059	0.940	0.823–1.080	0.396
Pulmonary tuberculosis	−1.309	0.270	0.097–0.752	0.012*
Operation time	0.017	1.017	1.006–1.028	0.002*
Operation blood loss	0.001	1.001	0.999–1.004	0.295
Preoperative lymphocytes	−0.291	0.748	0.309–1.809	0.519
Preoperative serum albumin	−0.282	0.754	0.648–0.878	< 0.001*
Preoperative CRP	0.003	1.003	0.981–1.027	0.767
Preoperative ESR	< 0.001	1.000	0.981–1.019	1.000

** means statistical significance.*

tuberculosis, preoperative serum albumin, and operation time are independent risk factors of the postoperative hypoalbuminemia's occurrence (**Table 3**). ROC curve showed that the cutoff value of preoperative serum albumin was 40 g/L (sensitivity: 88.6%, specificity: 60.3%) and operation time was 181 min (sensitivity: 66.4%, specificity: 68.6%) (**Figure 2**).

Development of the Scoring Scale

Multivariate logistic regression analysis was carried out on the significant findings in univariate analysis and showed 3 clinical characteristics namely pulmonary tuberculosis, preoperative serum albumin, and operation time were significant predictors of STB postoperative hypoalbuminemia's occurrence (**Table 3**). According to the OR value, pulmonary tuberculosis (OR = 0.270) was assigned as 1-point, preoperative serum albumin (OR = 0.754) was assigned as 2 points, and operation time (OR = 1.017) was assigned as 3 points (**Table 4**). ROC curve showed that the diagnostic threshold score of the scoring scale was 4 points (sensitivity: 85.5%, specificity: 62.9%) (**Figure 3**).

Validation of the Scoring Scale

The scoring scale that was made by logistic regression was applied to the 102 cases in the validation set. A comparison of the results of the score scale on the derivation set and validation set was listed in **Table 5**. Based on the threshold score of 4 points, the sensitivity and specificity of the score for predicting STB postoperative hypoalbuminemia were 51.9 and 91.4% in the derivation set, respectively, 63.6 and 86.1% in the validation set (**Table 5**).

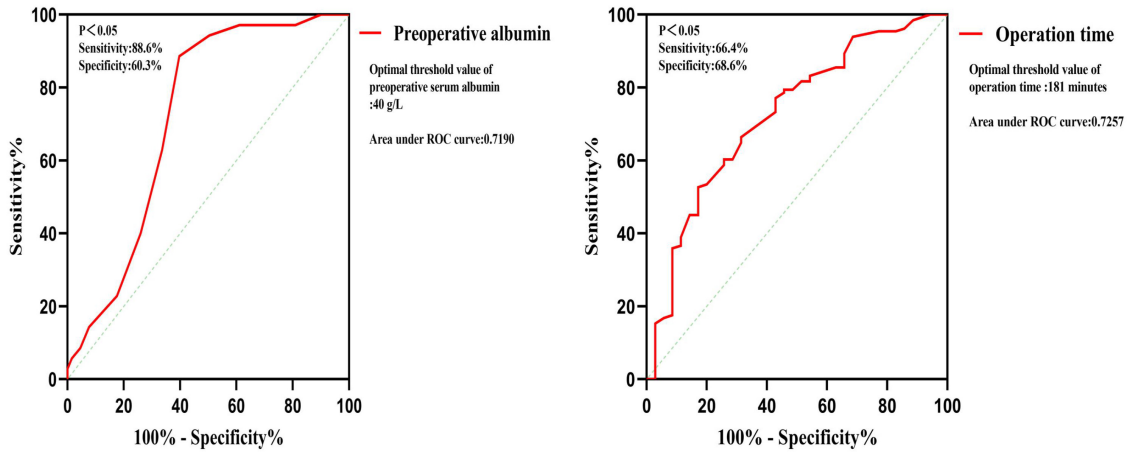

FIGURE 2 | Receiver operating characteristic (ROC) curve of preoperative albumin and operation time.

TABLE 4 | Scoring scale for occurrence of postoperative hypoalbuminemia.

Clinical characteristics	Points
Pulmonary tuberculosis (in 5 years)	
Yes	1
No	0
Preoperative serum albumin (g/L)	
≤39	2
≥40	0
Operation time (minute)	
≥181	3
≤180	0

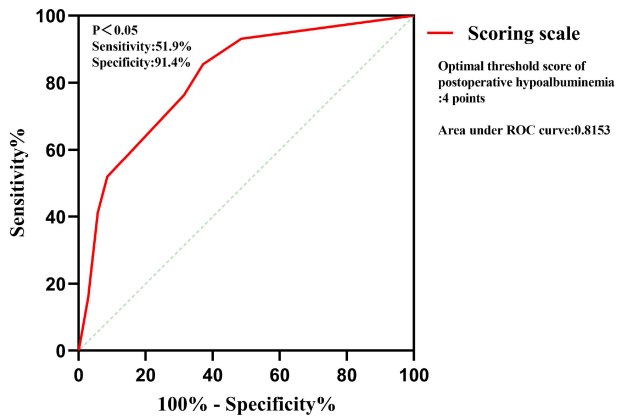

FIGURE 3 | ROC curve of the scoring scale.

TABLE 5 | Comparison of performance of the scoring scale on derivation set and validation set.

		Derivation set			Validation set		
		With POH	Without POH	Total	With POH	Without POH	Total
Clinical diagnosis	With POH	68	63	131	42	24	66
	Without POH	3	32	35	5	31	36
	Total	71	95	166	47	55	102
	Sensitivity (%)	51.9%			63.6%		
	Specificity (%)	91.4%			86.1%		

Perioperative Serum Albumin Loss Level

Applying the scoring scale on 166 patients to divide into 2 groups, respectively (score ≤3 points group and score ≥4 points group; preoperative albumin value ≤39 g/L groups and ≥40 g/L group) and get the mean value of preoperative serum albumin, postoperative lowest albumin, and postoperative albumin loss, respectively in each group (**Table 6**). The postoperative albumin changing trend in the earliest 5 days after surgery in both high-risk group and low-risk group has been shown in **Figure 4**.

Recommended Preoperative Serum Albumin Value

Combining the diagnostic threshold of hypoalbuminemia and respective average decreased albumin value in the 2 groups, we deduced the recommended preoperative serum albumin value of the patients with STB (**Table 7**).

DISCUSSION

It is reported that 4.8∼16.8% of patients who underwent spinal surgery are complicated with hypoalbuminemia before operation (19, 20). Hypoalbuminemia, as a common postoperative complication, rises frequently after the spinal operation. Zhang et al. reported that 72.8% of the 602 patients who underwent posterior lumbar fusion, developed hypoalbuminemia after the operation (21). Many studies have shown that hypoalbuminemia is an independent risk factor for wound infection after spinal surgery, which is

TABLE 6 | Comparison of perioperative albumin changing value divided by scoring scale and preoperative albumin value.

Albumin changing value	Score			Preoperative albumin		
	Score ≤3 points group	Score ≥4 points group	P	≤39 g/L group	≥40 g/L group	P
Preoperative albumin (g/L)	41.37 ± 2.39	38.50 ± 4.00	<0.001	36.06 ± 2.89	42.14 ± 1.85	<0.001
Postoperative lowest albumin (g/L)	36.74 ± 1.85	29.51 ± 3.42	<0.001	29.34 ± 3.88	32.73 ± 4.09	<0.001
Albumin loss (g/L)	4.71 ± 2.66	8.99 ± 3.37	<0.001	6.72 ± 3.90	9.41 ± 4.63	<0.001

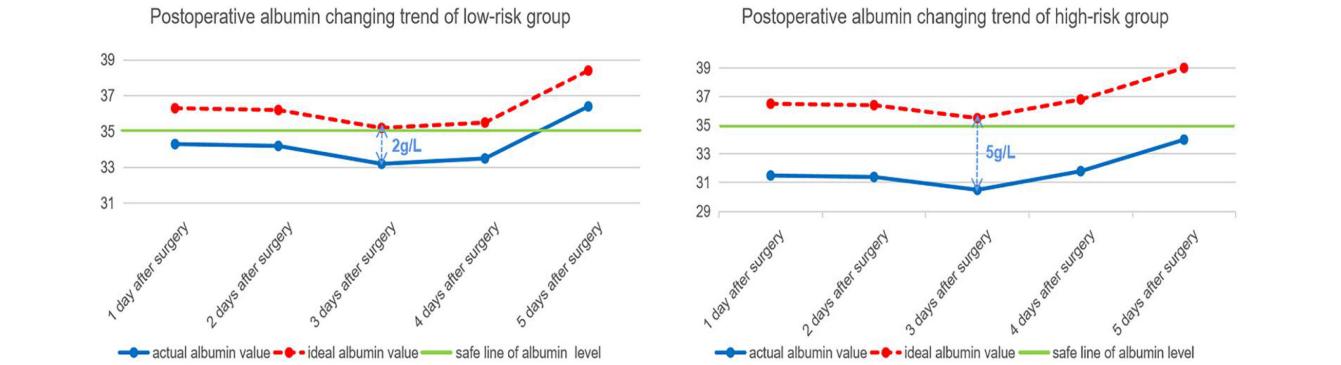

FIGURE 4 | Postoperative albumin changing trend of low-risk group and high-risk group.

TABLE 7 | Preoperative serum albumin recommended value based on the scoring scale.

Groups	Recommended value
Low risk group (score ≤3 points group)	40 g/L
High risk group (score ≥4 points group)	44 g/L

closely related to the occurrence of perioperative pneumonia, postoperative sepsis, myocardial infarction, and secondary revision operation in both spinal fusion surgery and total hip arthroplasty (22–25). Spinal tuberculosis patients suffer a long course of the disease, are prone to be complicated with malnutrition before operation. The incidence of patients with STB's postoperative hypoalbuminemia, which is closely related to many severe postoperative complications, is much higher than those with spinal degenerative diseases. It is of great clinical significance to explore the risk factors of hypoproteinemia after operation in patients with spinal tuberculosis. In our study we found that complicated pulmonary tuberculosis, preoperative serum albumin value, and operation time are independent risk factors of postoperative hypoalbuminemia. Based on the independent risk factors, we proposed a simple scoring scale to predict the occurrence of postoperative hypoalbuminemia in patients with thoracic and lumbar tuberculosis. Through the scoring scale we divided 166 patients with thoracic and lumbar tuberculosis into 2 groups, the low-risk group (≤3 points) and the high-risk group (≥4 points), respectively calculated the perioperative albumin changing the value in both groups, then, combined with a diagnostic threshold of hypoalbuminemia. At last, we proposed the recommended values of preoperative serum albumin.

Independent Risk Factors

Pulmonary Tuberculosis

The patients with STB are prone to being complicated with pulmonary tuberculosis. Another epidemiological study of STB based on the same province demographic characteristics reported that 25.7% of 284 patients with STB are complicated with pulmonary tuberculosis (26). Pulmonary tuberculosis is closely linked to malnutrition thus, patients with pulmonary tuberculosis are easier to develop hypoalbuminemia (27). In a study on the nutritional status of patients with tuberculosis, Ddungu et al. reported that 24% of 200 patients with pulmonary tuberculosis had hypoalbuminemia, of which 25% of the patients' albumin value is less than 25 g/L (28). Not only the albumin, but also these patients' concentrations of blood hemoglobin, plasma retinol, and plasma zinc are poorer than healthy people (29). The above studies can explain why patients with STB with pulmonary tuberculosis are more likely to occur hypoalbuminemia after surgery.

Preoperative Albumin Level

Some studies have shown that postoperative albumin loss is positively correlated with preoperative albumin level, which is considered to be a protective factor for postoperative hypoalbuminemia (15, 16), which is consistent with our research's results. Our study figured that preoperative albumin was an independent risk factor for postoperative hypoalbuminemia, and the diagnostic threshold value is 40 g/L. Preoperative albumin greater than or equal to 40 g/L is a protective factor for postoperative hypoalbuminemia, for patients with preoperative albumin ≥40 g/L, their postoperative protein loss is more than that of patients with preoperative albumin <40 g/L. Our group holds the view that the albumin loss

portion in patients with preoperative albumin greater than or equal to 40 g/L is more than those with preoperative albumin of less than 40 g/L, however, it is not the main cause of postoperative hypoalbuminemia. We proposed a hypothesis and for the convenience to state it, we induced the conception of albumin loss endurance, which means the difference value between the preoperative actual albumin value and the diagnostic value of hypoalbuminemia of 35 g/L. In our hypothesis, patients with preoperative albumin greater than 40 g/L had more albumin loss endurance than patients with preoperative albumin levels lower than 40 g/L, which outnumbers the postoperative excess albumin loss between the two kinds of patients. The advantage of the albumin loss endurance in our hypothesis makes the preoperative albumin value $\geq$40 g/L a protective factor for postoperative hypoalbuminemia.

The postoperative albumin changing trend in the low-risk group and the high-risk group suggested that there needs to be an improvement of 2 and 5 g/L, respectively, in the low-risk group and high-risk group patients to achieve the safe line of albumin level. The preoperative albumin recommended value proposed by our group is helpful to achieve the ideal postoperative albumin changing trend in the two groups' patients.

Operation Time

In our initial assumption, operative blood loss should be an independent risk factor for postoperative hypoalbuminemia because albumin will be lost from plasma with intraoperative bleeding, albumin was strongly correlated with reliable surrogate parameters of the extent of surgery such as blood loss (5, 30). Unexpectedly, our results suggested that operation time rather than operation blood loss is an independent risk factor for postoperative hypoalbuminemia. According to our analysis, this can be explained by another mechanism of albumin loss in surgical patients, which is called transcapillary escape of albumin. Albumin exudates from the intravascular compartment plasma by dilated capillaries to the tissue, resulting in the decrease of serum albumin after operation, especially under the condition of anesthesia and surgical stress statement (31–33). The operation time of spinal patients, on the one hand, is positively correlated with the operation blood loss, on the other hand, is consistent with patients' duration of both anesthesia and the surgical stress statement (2, 7–9, 30). Operation time can better synthesize the effects of surgical blood loss and capillaries vasodilation on postoperative albumin loss. The dynamic changes of postoperative albumin suggest that there is a significant increase in postoperative albumin (shown in **Figure 1**), which may suggest that not all of the reduced albumin is lost with intraoperative bleeding, and albumin lost in the tissue space is re-entered into the blood after the vanishing of anesthesia and the surgical stress statement, thus, resulting in the phenomenon of albumin recovery on the fourth day after operation, which is consistent with our previous explanation. The inclusion of the operation time in the scoring scale brings some difficulties to the evaluation of the patients before the operation. In practice, we should estimate the operation time of preoperative patients according to their specific conditions and combined it with their operation plan of them. This is also a limitation of this study. It also shows that this scale is more suitable for the evaluation of postoperative patients due to the explicit surgical duration.

Scoring Scale

The proposal of the simple scoring scale is helpful for clinicians to predict whether hypoalbuminemia will occur in patients with thoracic and lumbar tuberculosis after the operation and provide support for whether appropriate treatment measures can be taken to prevent the occurrence of hypoalbuminemia after the operation. Yet, there is no such predictive model of postoperative hypoalbuminemia in patients with STB. Our study is of pioneering significance. The scale is simple and easy to execute and will not add additional workload to clinicians. The specificity of the scoring scale in the derivation set and validation set was, respectively at 91.4% and 86.1%, and both data have confirmed the specificity of the scale is high, which means the patients in the high-risk group authentically have a high incidence to suffer hypoalbuminemia after the operation. The high specificity of the scoring scale brings us new questions like whether patients in the high-risk group can be treated in advance to reduce the occurrence of postoperative hypoalbuminemia, which is the further research direction of our group.

Our score scale also has some unsatisfactory aspects. The sensitivity of the scoring scale in the derivation set and validation set was, respectively 51.9 and 63.6%, which suggests that the missed diagnosis rate of the simple score scale is high. The possibility of hypoalbuminemia after operation in the low-risk group should not be ignored, thus, it is still necessary to pay close attention to the postoperative albumin value of the patients in this group. Due to the limitation of sample size and clinical feature integrity, there is still room for further improvement of our scoring scale, and there may be some independent risk factors for postoperative hypoalbuminemia that have not been revealed.

Recommended Value of Preoperative Albumin

For more than 50 years, hypoalbuminemia has been associated with increased morbidity and mortality after major abdominal surgeries. Bendersky et al. determined that a threshold preoperative serum albumin of $\geq$3.9 mg/dL is associated with improved outcomes in elective colorectal surgery patients (34, 35). Similarly, we believed that it is of great clinical significance to explore the appropriate preoperative albumin level in patients with STB, for this consideration, we divided patients with thoracic and lumbar tuberculosis into the low-risk group and high-risk group according to the scoring scale and obtained the postoperative albumin loss values of the two groups. Combined with the diagnostic threshold of hypoalbuminemia: 35 g/L, we proposed the preoperative albumin recommended values for two groups of patients: high-risk group: 44 g/L and low-risk group: 40 g/L. Our study reveals that preoperative albumin level is an independent risk factor for postoperative hypoalbuminemia, which is more likely to occur when the preoperative albumin value is less than 40 g/L. This value coincides with the preoperative albumin recommended value

of the low-risk group, which further confirms the rigor of our research results.

For high-risk patients whose preoperative albumin value did not reach the recommended value, the probability of postoperative hypoalbuminemia is very high, so we strongly recommend active treatment. Preoperative nutritional maneuvers play an important role in reducing postoperative impaired healing, morbidities, and mortalities in patients with STB (1, 21). We recommend taking active treatment for this kind of patient, such as supplementing albumin in advance, enhancing the preoperative nutrition, choosing the minimally invasive and short-duration surgical approach. However, for low-risk patients whose preoperative albumin value reached the recommended value, we hold the opinion that it is still necessary to pay close attention to the postoperative albumin change, but there is no need for active preoperative intervention. For some patients whose operation time is hard to predict due to the complex clinical conditions, in consideration of the maximum weight of surgical time on the scoring scale and the safety of postoperative albumin level, we regard the operation time of this kind of patient as greater than 181 min.

CONCLUSION

Complicated with pulmonary tuberculosis, low preoperative albumin value and long operation time are three main independent risk factors that can result in postoperative hypoalbuminemia in patients with thoracic and lumbar tuberculosis. The scoring scale proposed by our group can effectively assist the physicians to evaluate whether patients with thoracic and lumbar tuberculosis develop postoperative hypoalbuminemia. The advantage of the scale are only that it is simple and reliable, but also due to its clinical guiding significance. For low-risk patients and high-risk patients, according to our research results, the preoperative albumin value should reach 40 and 44 g/L, respectively, to effectively avoid postoperative hypoalbuminemia.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The protection of patients' privacy. Requests to access these datasets should be directed to GJ, 15227175613@163.com.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

GJ and YO: conception and design. YZ: provision of study materials of patients. YZ and WL: collection and assembly of data. GJ: data analysis and interpretation. YO: administrative support. All authors contributed to the writing and final approval of the manuscript.

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A Nutritional Metabolism Related Prognostic Scoring System for Patients With Newly Diagnosed Osteosarcoma

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Osteosarcoma is a primary malignant bone tumor with high metastatic potential. To date, achieving long-term survival of osteosarcoma patients remains a difficult task. Metabolic reprogramming has emerged as a new hallmark of cancer. However, studies on the prognostic value of hematological markers related to nutritional and metabolism in cancer patients are limited and contradictory. In this retrospective study, we extensively collected 16 hematological markers related to nutritional and metabolism in 223 osteosarcoma patients. A nutritional metabolism related prognostic scoring system (NMRS) in patients with osteosarcoma was constructed by least absolute contraction and selection operator (LASSO) cox regression analysis. Compared with individual hematological indicators, NMRS has stronger predictive power (training set: 0.811 vs. 0.362–2.638; validation set: 0.767 vs. 0.333–0.595). It is an independent prognostic factor for the survival of patients with osteosarcoma [HR: 1.957 (1.375–2.786) training set; HR: 3.146 (1.574–6.266) validation set]. NMRS-based nomograms have good and stable predictive power. NMRS facilitates further risk stratification of patients with the same clinical characteristics.

Keywords: osteosarcoma, nutrition, metabolism, prognosis, hematology

INTRODUCTION

Osteosarcoma is the most common primary malignant bone tumor, accounting for 20–40% of all bone tumors (1, 2). Tumors tend to occur in children and adolescents and have a high metastatic potential. The 5-year survival rate of patients with standard treatment is about 60–70% (3). However, approximately 15–20% of patients have developed metastases at initial diagnosis, and the five-year survival rate is significantly reduced, so far there is no effective treatment regimen (4–6). In addition, insensitivity to chemotherapy and tumor recurrence are also important factors leading to significantly reduced patient survival (7, 8). These clinical features can identify high-risk patients and aid in treatment planning (9). However, the progression of the disease may be distinct in patients with similar clinical features. Therefore, more factors need to be considered to facilitate precision treatment.

Recently, researchers have made many efforts in developing biomarkers that can more accurately predict the prognosis of patients and developed many biomarkers with potential, such as non-coding RNA (NcRNA), circulating tumor cell (CTC), and circulating tumor DNA (ctDNA) (10–12). However, these markers have not been applied to clinical work due to reasons such as the cost of testing, strict technical requirements for biopsy, etc. Fortunately, studies have shown that preoperative hematological markers such as neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) or hematology-based scores such as Glasgow Prognostic Score (GPS), Controlling Nutritional Status (COUNT) show great potential in predicting the prognosis of cancer patients (13–15). Compared with NcRNA, CTC, and ctDNA, most of these hematological markers originate from routine examinations of patients on admission without the need for additional testing costs. Many recent studies have confirmed the value of these markers in predicting survival in cancer patients, including osteosarcoma (16–18).

Studies over the past decade have shown that cancer cells can promote their survival by reprogramming metabolic pathways, and therefore, metabolic reprogramming is also considered to be one of the hallmarks of cancer (19, 20). Local tumors can even impair antitumor immunity by affecting host metabolism through cachexia (21). However, studies on the ability of hematologic metabolic markers to predict the prognosis of cancer patients are contradictory and limited compared with hematologic inflammatory markers (22).

In this study, we collected the hematological markers related to metabolism and explored the significance of disturbances in these markers in patients with osteosarcoma. Through iterative least absolute contraction and selection operator (LASSO) COX proportional hazards regression analysis we constructed nutritional metabolism related prognostic scoring system (NMRS) and assessed the predictive power of the scores through multiple dimensions. In addition, we also explored the superiority and limitations of NMRS by comparing it with existing scoring systems and clinical features.

PATIENTS AND METHODS

Patients

We reviewed the clinical data of osteosarcoma patients who visited the Musculoskeletal Tumor Center of West China Hospital from January 2016 to January 2021. Patients were screened with the following inclusion and exclusion criteria. inclusion criteria: (1) patients with histopathologically confirmed high-grade osteosarcoma; (2) patients with blood routine, liver and kidney function, coagulation function tests before neoadjuvant chemotherapy; (3) patients who completed standard osteosarcoma treatment regimen at West China Hospital. Exclusion criteria: (1) patients with concomitant metabolic disease; (2) patients with concomitant hematological diseases; (3) patients with other malignancies; (4) patients who were previously misdiagnosed or mistreated. Finally, 223 patients who met the inclusion criteria and passed the exclusion criteria were included in the study. A standard follow-up principle was developed to follow each patient, with the last follow-up date being January 2022. The follow-up principle: reexamination every 3 months within 1 year after surgery; reexamination every 4 months 1–2 years after surgery; reexamination every 5 months 2–3 years after surgery; reexamination every 6 months 3–5 years after surgery; reexamination every year more than 5 years after surgery. All patients were randomly assigned to the training set (n = 156, 70%) vs. the validation set (n = 67, 30%).

Data Collection and Processing

The following data were extracted from each patient's first blood routine, liver and kidney function: Red blood cells (RBC), Red blood cell specific volume (HCT), Hemoglobin (HB), Lymphocyte count (LYMPH#), albumin (A), Globulin (G), Glucose (GLU), Cholesterol (TCH), Triglycerides (TG), High density liptein cholesterol (HDL-C), Low Density Lipoprotein (LDL), Total bilirubin (TBIL), Indirect bilirubin (IBIL), Direct bilirubin (DBIL). The calculation formulas of AGR and PNI are as follows. AGR = A/G; PNI = A + 0.005 × LYMPH#. CONUT was calculated following previous studies. **Supplementary Table 1** provides the specific calculation formula. In the overall cohort, the receiver operating curve was used to find the optimal cutoff for continuous variables and continuous variables were transformed into binary variables based on the cutoff.

Construction of Nutritional Metabolism Related Prognostic Scoring System

First, univariate Cox regression analysis was used to screen for prognostic related indicators. The LASSO regression analysis was performed 1,000 times on the training set using the screened hematological markers to build the model. Hematological markers that were retained at high frequencies in the 1,000 times LASSO regression analyses were sequentially included in the cox model. The model when AUROC peaked was considered the best model. NMRS risk scores were calculated for each patient including the validation set based on markers and coefficients in the model.

Evaluation of the Value of Nutritional Metabolism Related Prognostic Scoring System

First, we contrasted the differences in predictive ability between NMRS scores and its constituent markers using ROC analysis. Subsequently, with the "survivalROC" package, we determined the optimal cutoff value for the NMRS score. All patients were divided into two groups according to the cutoff value and differences in overall survival between the two groups of patients were assessed using Kaplan–Meier (KM) survival analysis. Given that the effects of continuous variables on risk can be non-linear, ignoring such non-linear effects can interfere with the results, risk scores were analyzed using restricted cubic splines. To further clarify the value of NMRS, we also plotted time-dependent ROC curves to explore changes in NMRS predictive ability over time and contrasted with clinical characteristics. Subsequently, through multivariate cox regression analysis, we explored whether NMRS was an independent prognostic factor

for overall survival in patients with osteosarcoma. Finally, to explore the stability of the predictive power of NMRS scores, we set up subgroups based on clinical characteristics and explored the predictive power of NMRS in each subgroup.

Construction and Evaluation of the Nomogram

To facilitate the clinical application of NMRS, we combined NMRS with clinical features to construct a NMRS-based nomogram. The discrimination ability and accuracy of nomograms were assessed by C-index and calibration curve, respectively. To clarify whether the predictive power of nomogram prediction is stable, we predicted the overall survival of patients in the validation set using nomograms and assessed it using the C-index and the calibration curve. Finally, whether the application of nomograms can bring about clinical net benefit and net reduction was evaluated by decision curve analysis.

Relationship Between Nutritional Metabolism Related Prognostic Scoring System and Clinical Characteristics

We analyzed differences in NMRS scores across clinical characteristics to assess the relationship between NMRS and clinical characteristics. In addition, we simply combined NMRS with important clinical characteristics [tumor metastasis status, pathological fracture status, Body Mass Index (BMI)] to divide patients into multiple groups, and plotted KM survival curves to assess the difference in survival between different groups of patients to explore whether NMRS can further distinguish patients with the same clinical characteristics.

Statistical Analysis

Descriptive statistics were used to assess any differences between datasets using the *t*-test or Mann–Whitney *U* test for continuous variables and the chi-square test or Kruskal–Wallis test for categorical variables. All statistical analyses were conducted using R software, version 4.1.0 (Institute for Statistics and Mathematics, Vienna, Austria). P values < 0.05 were considered to indicate statistical significance.

RESULTS

Patient Characteristics

The study included 131 male and 92 female with a total of 223 patients. Median follow-up of patients was 3.4 ± 0.4 years. As shown in **Table 1**, variables did not differ significantly between the training set and the validation set. The mean age of the patients in the training and validation sets were 21.3 and 22.5, respectively. Metastasis occurred in 17.9% (23) of patients in the training set and 16.4% (11) in the validation set. Only a very small proportion of patients had tumors located in non-extremity sites (7 patients in training set; 2 patients in validation set). Seventeen patients in the training set and 8 patients in the validation set were presenting for pathological fractures. In addition, **Table 1** shows the markers and their coefficients

TABLE 1 | Differences in the distribution of all variables between the training set and the validation set and the respective coefficients of the 9 hematological markers that make up the NMRS.

	Train (*N* = 156)	Test (*N* = 67)	*P*-value	Coefficient
OS time				Not applicable
Mean (SD)	1,030 (545)	975 (576)	0.524	
OS				Not applicable
Alive	105 (67.3%)	42 (62.7%)	0.608	
Died	51 (32.7%)	25 (37.3%)		
Gender				Not applicable
Male	93 (59.6%)	38 (56.7%)	0.799	
Female	63 (40.4%)	29 (43.3%)		
Age				Not applicable
Mean (SD)	21.3 (12.3)	22.5 (12.4)	0.483	
Metastasis status				Not applicable
No	128 (82.1%)	56 (83.6%)	0.933	
Yes	28 (17.9%)	11 (16.4%)		
Tumor site				Not applicable
Extremities	149 (95.5%)	65 (97.0%)	0.88	
Non-extremities	7 (4.5%)	2 (3.0%)		
Pathological fracture				Not applicable
No	139 (89.1%)	59 (88.1%)	1	
Yes	17 (10.9%)	8 (11.9%)		
PNI				Excluded
High	98 (62.8%)	44 (65.7%)	0.799	
Low	58 (37.2%)	23 (34.3%)		
AGR				−0.497
High	71 (45.5%)	33 (49.3%)	0.714	
Low	85 (54.5%)	34 (50.7%)		
CONUT				0.354
High	57 (36.5%)	25 (37.3%)	1	
Low	99 (63.5%)	42 (62.7%)		
RBC				Excluded
High	125 (80.1%)	53 (79.1%)	1	
Low	31 (19.9%)	14 (20.9%)		
HB				Excluded
High	101 (64.7%)	47 (70.1%)	0.53	
Low	55 (35.3%)	20 (29.9%)		
HCT				Excluded
High	106 (67.9%)	48 (71.6%)	0.697	
Low	50 (32.1%)	19 (28.4%)		
Total bilirubin				Excluded
High	15 (9.6%)	13 (19.4%)	0.0716	
Low	141 (90.4%)	54 (80.6%)		
Direct bilirubin				Excluded
High	94 (60.3%)	48 (71.6%)	0.142	
Low	62 (39.7%)	19 (28.4%)		

(Continued)

TABLE 1 | (Continued)

	Train (*N* = 156)	Test (*N* = 67)	*P*-value	Coefficient
Indirect bilirubin				Excluded
High	73 (46.8%)	41 (61.2%)	0.0679	
Low	83 (53.2%)	26 (38.8%)		
Albumin				−0.286
High	117 (75.0%)	51 (76.1%)	0.993	
Low	39 (25.0%)	16 (23.9%)		
Globulin				0.417
High	92 (59.0%)	38 (56.7%)	0.869	
Low	64 (41.0%)	29 (43.3%)		
Glucose				0.562
High	39 (25.0%)	24 (35.8%)	0.138	
Low	117 (75.0%)	43 (64.2%)		
Triglycerides				0.596
High	43 (27.6%)	20 (29.9%)	0.853	
Low	113 (72.4%)	47 (70.1%)		
Cholesterol				−1.127
High	99 (63.5%)	42 (62.7%)	1	
Low	57 (36.5%)	25 (37.3%)		
HDL				−0.188
High	96 (61.5%)	40 (59.7%)	0.914	
Low	60 (38.5%)	27 (40.3%)		
LDL				0.901
High	82 (52.6%)	36 (53.7%)	0.989	
Low	74 (47.4%)	31 (46.3%)		

that make up the NMRS. **Supplementary Table 2** provides the optimal cutoff values for each marker.

Construction of Nutritional Metabolism Related Prognostic Scoring System

As described in the "Patients and Methods" section, based on the overall cohort, by univariate cox regression analysis, we identified 10 markers with prognostic value and used them for further analysis (**Figure 1A**). Subsequently, LASSO regression analysis was performed 1,000 times in the training cohort using prognostic markers to determine 9 hematological markers with high frequency retained and constructed NMRS (**Figures 1B,C**). The coefficients for each marker in the NMRS are shown in **Table 1**, and the NMRS was calculated for each patient based on these coefficients.

Evaluation of Prognostic Value of Nutritional Metabolism Related Prognostic Scoring System

First, by ROC analysis, we contrasted NMRS with every hematological marker to explore whether there was an advantage in NMRS. As shown by **Figures 1C,D**, NMRS has the largest area under the curve (AUC) in both training and validation sets and is significantly higher than other markers (training set: 0.811 vs 0.362–2.638; validation set: 0.767 vs 0.333–0.595). The optimal cutoff value of NMRS was 0.132, and patients were divided into two groups based on the cutoff value, and the overall survival of low-risk patients was significantly longer than that of high-risk patients in both the training and validation sets (**Figures 2A,B**, $p < 0.001$). Finally, results from restricted cubic splines show that the effect of NMRS on prognosis is linear (**Figure 2C**, $p = 0.376$).

We further assessed whether the prognostic value of NMRS would be influenced by clinical characteristics. As shown in **Figures 2D–G**, multivariate cox regression analysis revealed that only NMRS score and metastatic status were independent prognostic factors in both training and validation sets [NMRS: HR: 1.957 (1.375–2.786) training set; HR: 3.146 (1.574–6.266) validation set]. As shown in **Figures 3A,B**, the time-dependent ROC curve showed that the predictive ability of NMRS did not decrease with time.

We also assessed the stability of the predictive ability of NMRS by the subgroup analysis. It can be seen that the predictive ability of NMRS has good stability, has prognostic value in most of subgroup, and is limited only in three group of patients with small sample size (**Figure 3C**).

Construction and Validation of Nutritional Metabolism Related Prognostic Scoring System-Based Nomograms

To improve the accuracy of the prediction of overall survival in osteosarcoma, we constructed a nomogram combining NMRS with clinical features in the training set. As shown in **Figure 4A**, NMRS and tumor metastasis status were the two most important components in the nomogram. NMRS has the largest scoring interval, ranging from 0 to 100. The C-index of the nomogram was 0.80, suggesting that the nomogram has a good discriminative ability. The results of the calibration curve show that the nomogram also has good accuracy (**Figure 4B**). The nomogram also has a good performance in the validation set with a C-index of 0.80, suggesting that the predictive ability of the nomogram is stable (**Figure 4C**). The results of decision curve analysis showed that the introduction of NMRS on the basis of clinical characteristics can bring about clinical net benefit vs. net reduction (**Figures 4D,E**).

Association Between Nutritional Metabolism Related Prognostic Scoring System and Clinical Features

We also explored whether there were differences in NMRS scores between different clinical subgroups. As shown in **Figure 5**, the NMRS scores were lower in the non-metastaticgroup, and there was no significant difference in the NMRS scores among the remaining subgroups. Finally, the results of two-factor survival analysis showed that NMRS can be further risk-stratified from patients with the same clinical characteristics (**Figure 6**). Based on NMRS and tumor metastasis status, patients were divided into four groups, and the overall survival of patients with high NMRS risk was significantly higher than that of patients with low NMRS risk in the non-metastatic group ($p < 0.0001$), even close to that of patients with low NMRS risk in tumor metastasis ($p > 0.05$). Even among patients in the tumor metastasis group, there were differences in the overall survival of patients with different NMRS

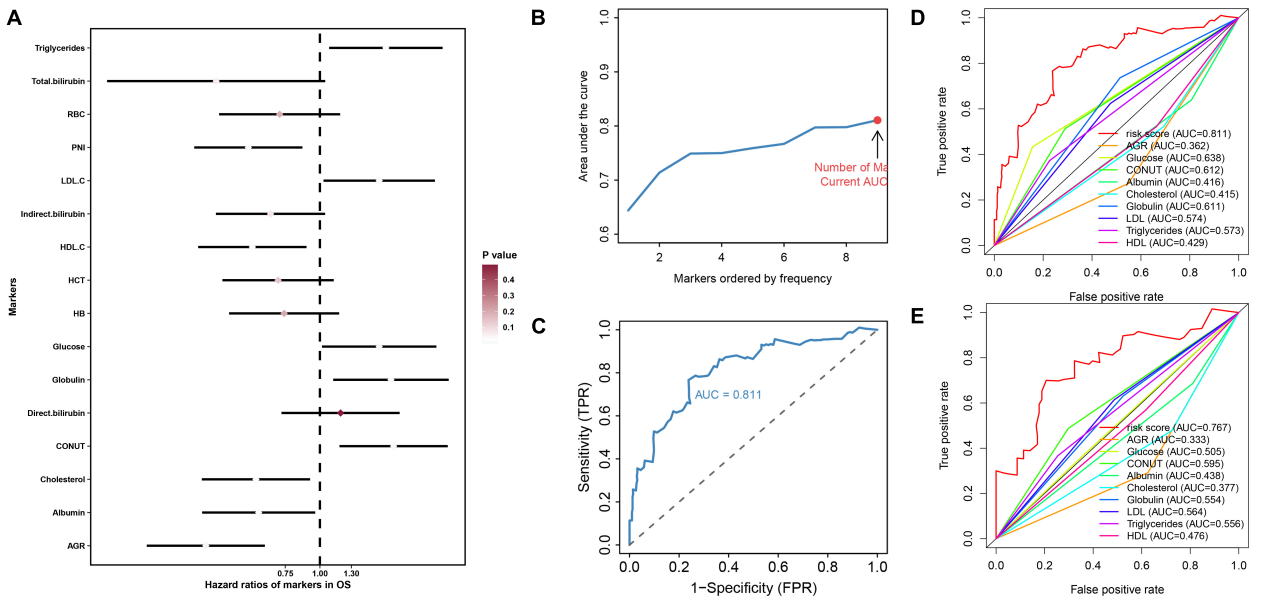

FIGURE 1 | The construction process of NMRS and the comparison between NMRS and individual hematological markers in terms of predictive ability. **(A)** Forest plot presented the results of univariate cox regression analysis of 16 hematological markers in the overall cohort. Where the smaller the *p* value, the closer the color of the diamond of the marker is to white; **(B,C)** With the continuous inclusion of the marker with high frequency retained in LASSO regression analysis into the model, the AUC value of the model continues to rise. When the 9th marker is included in the model, the AUC value of the model reaches the highest value, and this model is NMRS; **(D)** ROC curves of the predictive ability of NMRS and individual hematological markers in the training set; **(E)** ROC curves of the predictive ability of NMRS and individual hematological markers in the validation set.

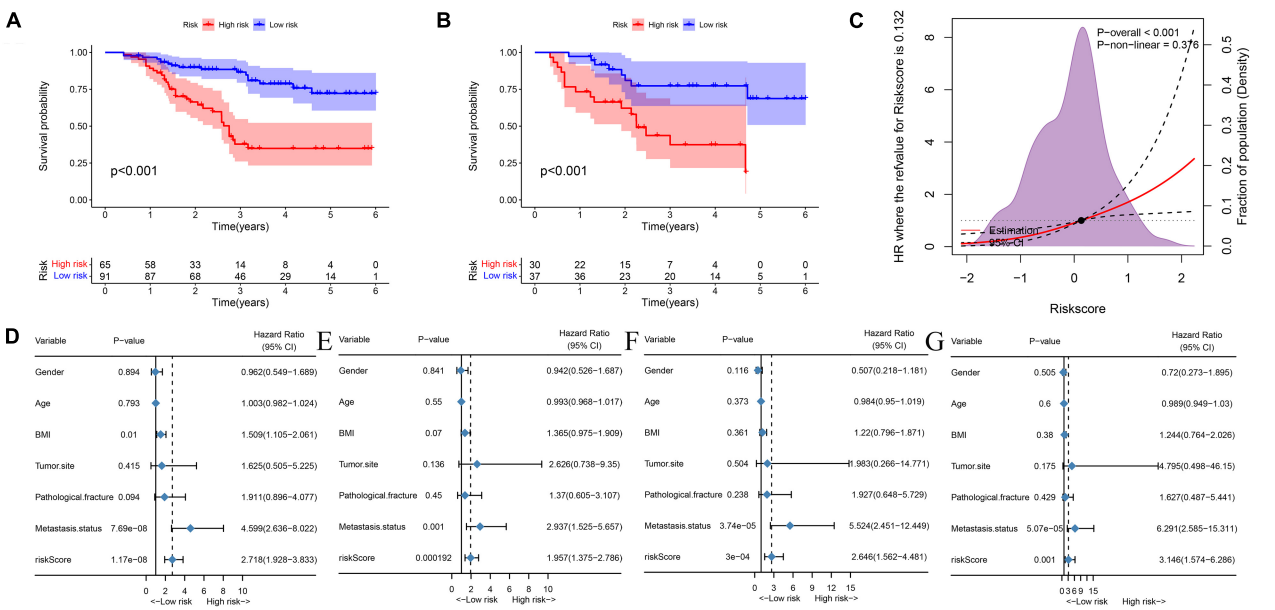

FIGURE 2 | NMRS has independent prognostic value in both the training and validation sets. **(A)** KM survival curves showing overall patient survival in the training set; **(B)** KM survival curves showing overall patient survival in the training set; **(C)** The overall cohort patient NMRS score and overall survival risk, restricted cubic splines indicated that the effect of NMRS as a continuous variable on overall survival risk was linear; **(D)** Results of univariate cox regression analysis of NMRS and clinical characteristics in the training set; **(E)** Results of multivariate cox regression analysis of NMRS and clinical characteristics in the training set; **(F)** Results of univariate cox regression analysis of NMRS and clinical characteristics in the validation set; **(G)** Results of multivariate cox regression analysis of NMRS and clinical characteristics in the validation set.

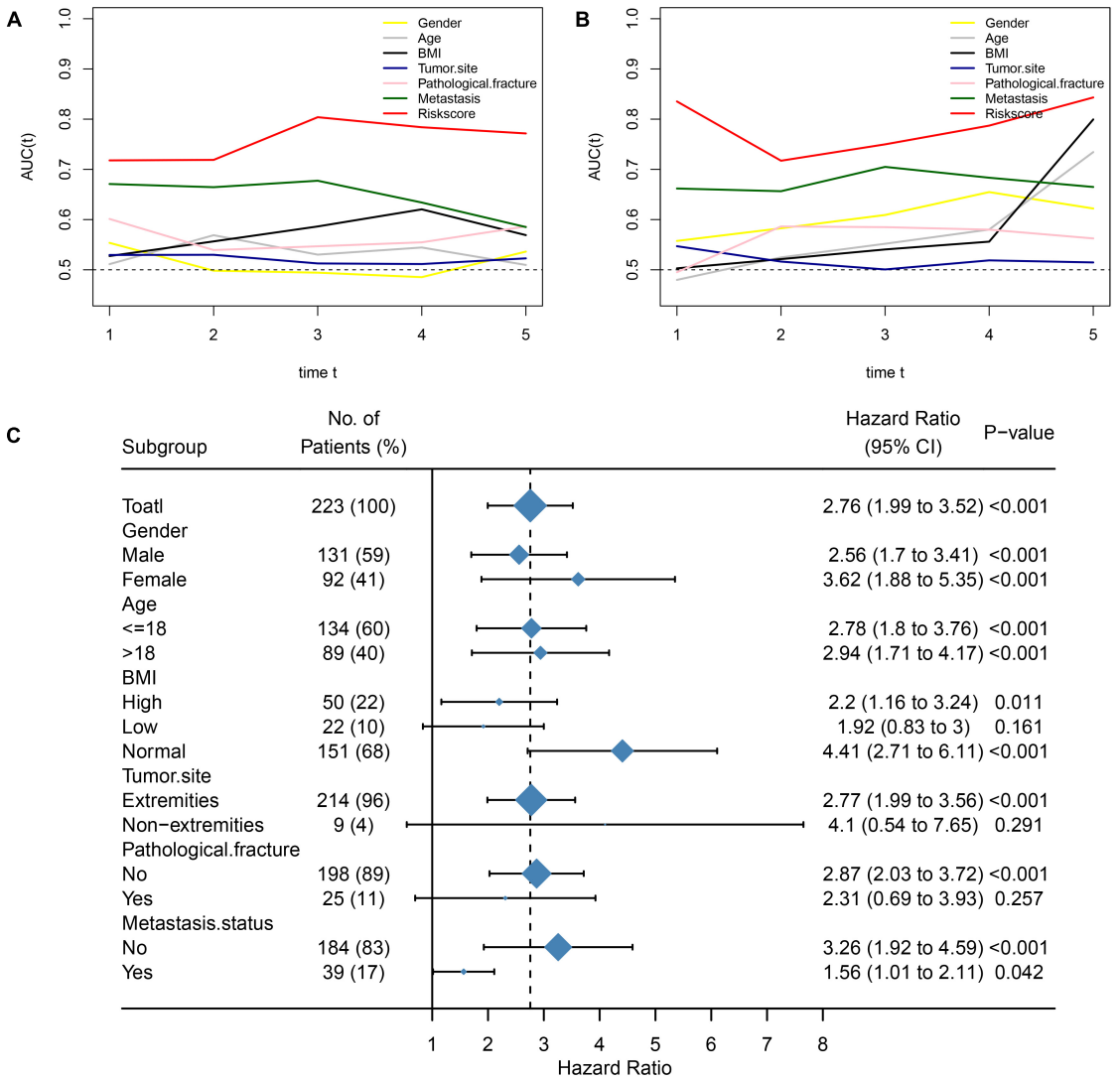

FIGURE 3 | Comparison of NMRS with clinical features and stability of NMRS prediction ability. **(A,B)** Time-dependent ROC curves for NMRS vs. clinical characteristics in terms of predictive ability (training set and validation set); **(C)** Forest plots showing the predictive power of NMRS in various subgroups.

risks ($p = 0.0471$). When patients were divided into four groups according to pathological fracture status and NMRS risk, overall survival was significantly lower in patients with high NMRS risk among patients with non-pathological fractures ($p < 0.0001$).

Prognostic Value of Individual Hematological Markers

Finally, we assessed the prognostic value of the 9 hematological markers that constitute NMRS in osteosarcoma. As shown in **Figure 7A**, the results of univariate cox regression analysis showed that AGR, TCH, COUNT, TG and LDL had significant prognostic value in the training set. However, only the prognostic value of AGR remained statistically significant in the validation set (**Figure 7B**). We further explored whether AGR is an independent prognostic factor in osteosarcoma patients in the validation set. Unfortunately, the results of multivariate cox regression analysis indicated that AGR was not an independent prognostic factor in osteosarcoma patients (**Figures 7C,D**).

DISCUSSION

Although limb salvage surgery for osteosarcoma has been continuously improved in recent years, and most patients can obtain satisfactory limb function after surgery, it is still a difficult task for osteosarcoma patients to achieve survival (1, 24, 25). Early identification of high-risk patients and development of personalized treatment options are expected to improve the prognosis of patients (26). Unfortunately, in osteosarcoma, no new prognostic marker has been truly applied to clinical work except for clinical features such as metastatic status and tumor necrosis rate (9). Although recent studies have shown

the potential of NcRNA, CTC with ctDNA as a tool for early detection, postoperative monitoring of cancer patients (23, 27–29). However, because the concentration of CTC and ctDNA collected from patients is usually very low. In addition, standard methods for isolation, enrichment, or detection of NcRNA, CTC, and ctDNA are lacking (30, 31). Therefore, there is still a certain distance between NcRNA, CTC and ctDNA for real clinical application. In contrast, because most of the hematological markers are derived from routine examinations such as blood routine and liver and kidney function, no additional testing costs are required. Moreover, the value of hematological markers in predicting the prognosis of cancer patients has been generally accepted, for example, NLR has been written into the 2020 European Association of Urology Guidelines on Upper Urinary Tract Urothelial Carcinoma (32). However, the prognostic value of indicators such as TG, HDL and LDL in cancer patients has not been determined. Therefore, we extensively collected some hematological markers that may reflect nutritional status and metabolic reprogramming in cancer patients and constructed NMRS. As expected, NMRS demonstrated greater stability and predictive power. With the exception of NMRS, none of the hematological markers had independent prognostic ability in both the training and validation sets. In addition, NMRS has better discrimination ability than clinical features such as tumor metastasis status, especially in identifying low-risk patients.

The lipid metabolism plays an important role in cancer metabolic reprogramming. Cancer cells, as well as other cell types in the tumor microenvironment, utilize various methods to obtain lipids and extensively rewrite their metabolism (33). Studies have shown that cancer stem cells maintain their stem cell characteristics through lipid metabolism (34). In addition, alterations in lipid metabolism can impair antitumor immunity and promote iron death escape (35, 36). Several large-scale lipidomics studies have provided compelling evidence for the potential of lipids as prognostic biomarkers for cancer (33, 37, 38). In this study, lipid metabolic markers were the most important cornerstone of NMRS, with TCH, TG, HDL, and LDL coefficients of −1.127, 0.596, −0.188, and 0.901, respectively, which indicated that higher TCH and HDL was associated with better prognosis, while triglyceride and LDL were the opposite.

Serum albumin and globulin reflect the nutritional and inflammatory status of individuals and have shown potential prognostic value in a variety of tumors (39–41). On the one hand, albumin reflects the nutritional status of individuals, and in general, patients with poor nutritional status have a higher risk of postoperative complications, which may greatly shorten the survival time of cancer patients (42). On the other hand, decreased serum albumin may be due to increased capillary permeability caused by cancer-related inflammation resulting in albumin escape into the interstitium and absorption by cancer cells, decomposition and utilization (43, 44). In addition, globulin is considered a pro-inflammatory protein and has been shown to be associated with poor prognosis in cancer patients (45). In this study, the coefficients of albumin, globulin and AGR were −0.286, 0.417 and −0.497, respectively, confirming the previous conclusions (46–48).

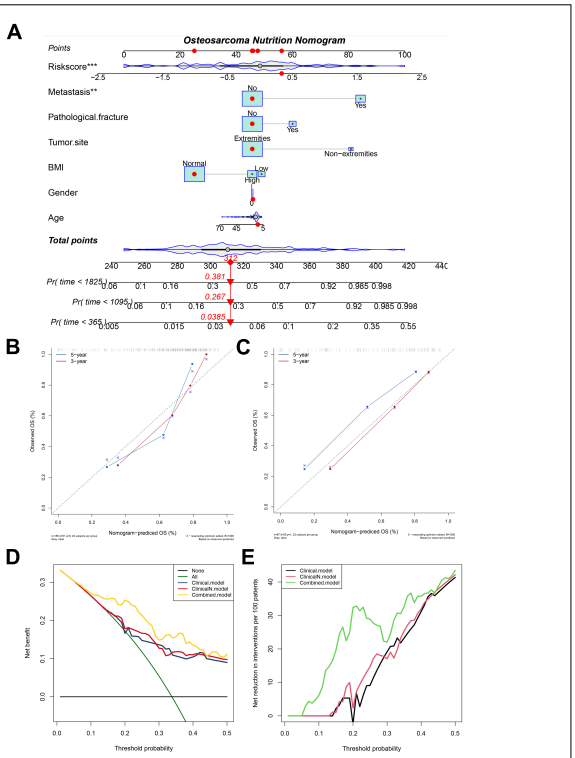

FIGURE 4 | NMRS-based nomograms have good predictive ability and predictive stability. **(A)** NMRS-based nomogram, in which NMRS with tumor metastasis status is the two most important factors; **(B,C)** Calibration curve of NMRS nomogram for predicting 3-year vs. 5-year overall survival in patients with osteosarcoma (training set and validation set); **(D)** Decision curve analysis of the NMRS nomogram, which only complements BMI to clinical features yielded little clinical net benefit (clincalN). When NMRS is introduced into clinical features it yields definite clinical net benefit (Combined); **(E)** The introduction of BMI into clinical features produced little clinical net reduction, whereas the introduction of NMRS brought clinical net reduction with certainty.

Although the mechanisms by which high glucose promotes cancer aggressiveness vary by cancer type, it is generally accepted that high glucose is associated with poor prognosis in cancer patients. Studies have shown that abnormal elevated glucose that does not reach the diagnostic level of diabetes is also associated with poor prognosis in cancer patients (49). A high glucose environment leads to the up-regulation of aerobic glycolysis (Warburg effect) -related pathways in cancer cells. In addition, glucose can also activate a variety of signaling pathways involved in cell proliferation, metastatic capacity, and chemoresistance, including ERK, STAT3, and NF-κB (50, 51). The coefficient for glucose in this study was 0.562, indicating that elevated glucose is associated with poor prognosis in osteosarcoma patients. Notably, to our knowledge, the prognostic value of glucose in osteosarcoma patients has not been previously investigated. Finally, CONUT consists of lymphocytes and albumin together with cholesterol, so it is not difficult to understand that CONUT is associated with the prognosis of cancer patients. The coefficient of CONUT in NMRS was 0.354, which is consistent with previous findings (52).

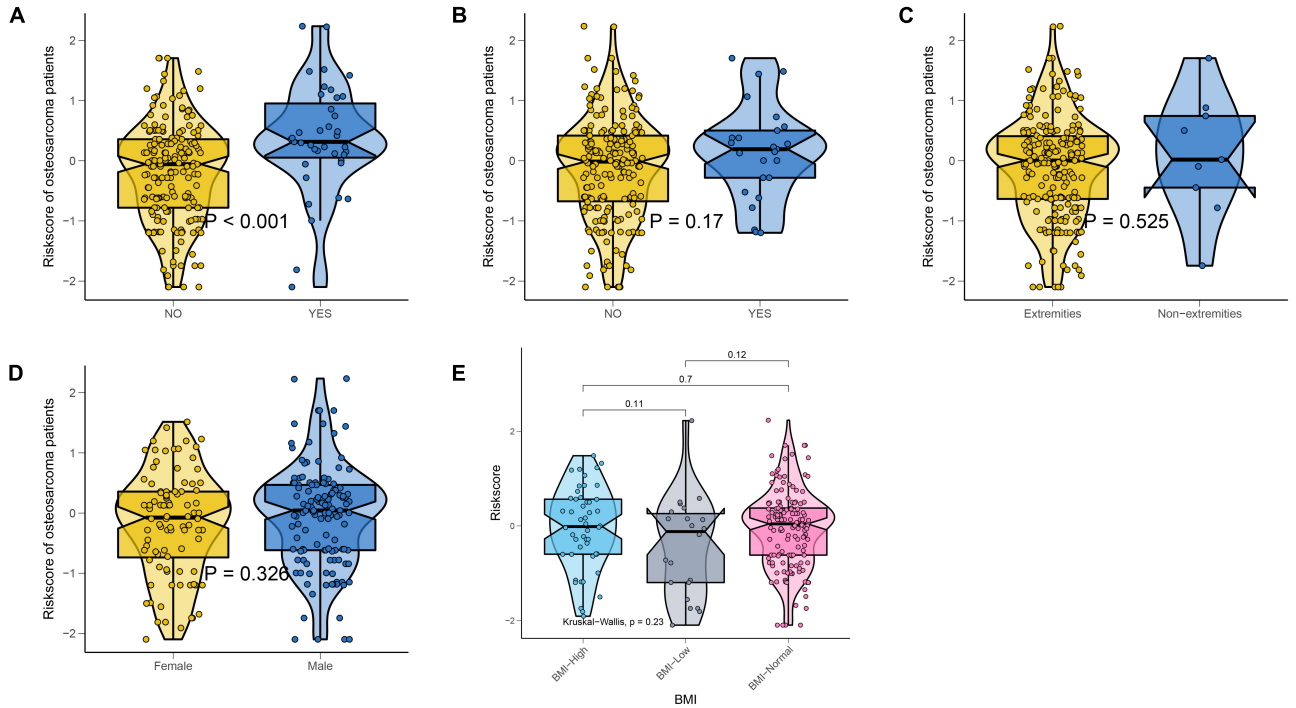

FIGURE 5 | NMRS was only associated with metastatic status. **(A)** Violin plots showing differences in the distribution of NMRS among patients with different metastatic status; **(B)** Violin plots showing differences in the distribution of NMRS among patients with different pathological fracture state; **(C)** Violin plots showing differences in the distribution of NMRS among patients with different tumor location; **(D)** Violin plots showing differences in the distribution of NMRS among patients with different gender; **(E)** Violin plots showing differences in the distribution of NMRS among patients with different BMI.

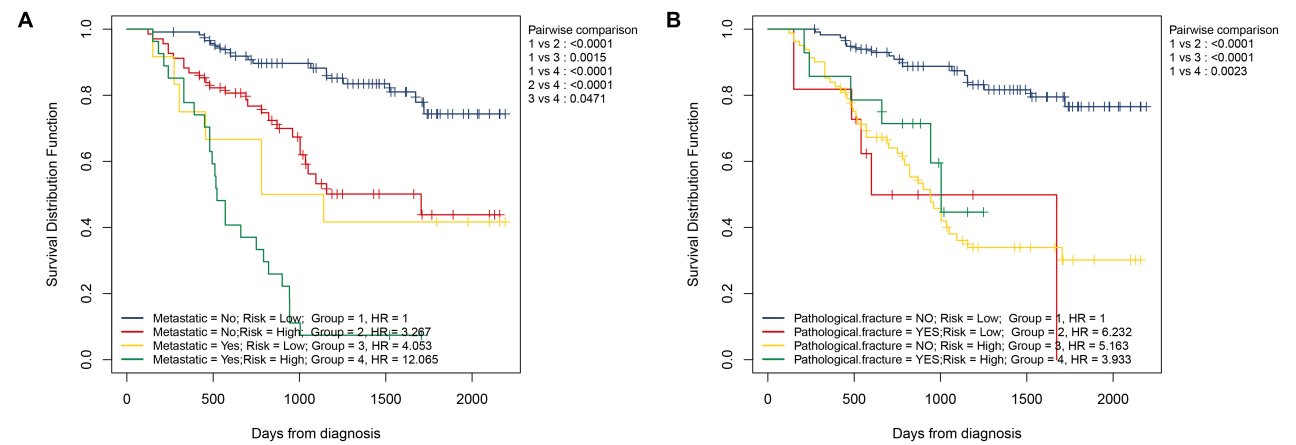

FIGURE 6 | NMRS can further identify patients with different risks on the basis of clinical characteristics. **(A)** Two-factor KM survival curves considering the risk of NMRS and the status of tumor metastasis; **(B)** Two-factor KM survival curves considering the risk of NMRS and the status of pathological fracture.

As shown by **Figure 1**, the results of our ROC analysis showed that there was no significant difference in the prognostic value of individual hematological markers between the training and validation sets. The AUC values of NMRS were higher than all the individual hematological markers. In addition, NMRS combines all of the above indicators and assigns coefficients to each indicator, which comprehensively reflects these indicators and better and more stably represents the nutritional and metabolic status of patients. In fact, we also performed further analysis of individual hematological marker. Our results showed that only AGR had some prognostic value in both the training set and the validation set. In multivariate cox regression analysis combined with clinical characteristics, none of the individual hematological parameters were independent prognostic factors for overall survival of osteosarcoma patients in both the training and validation sets. These results further demonstrate the superiority

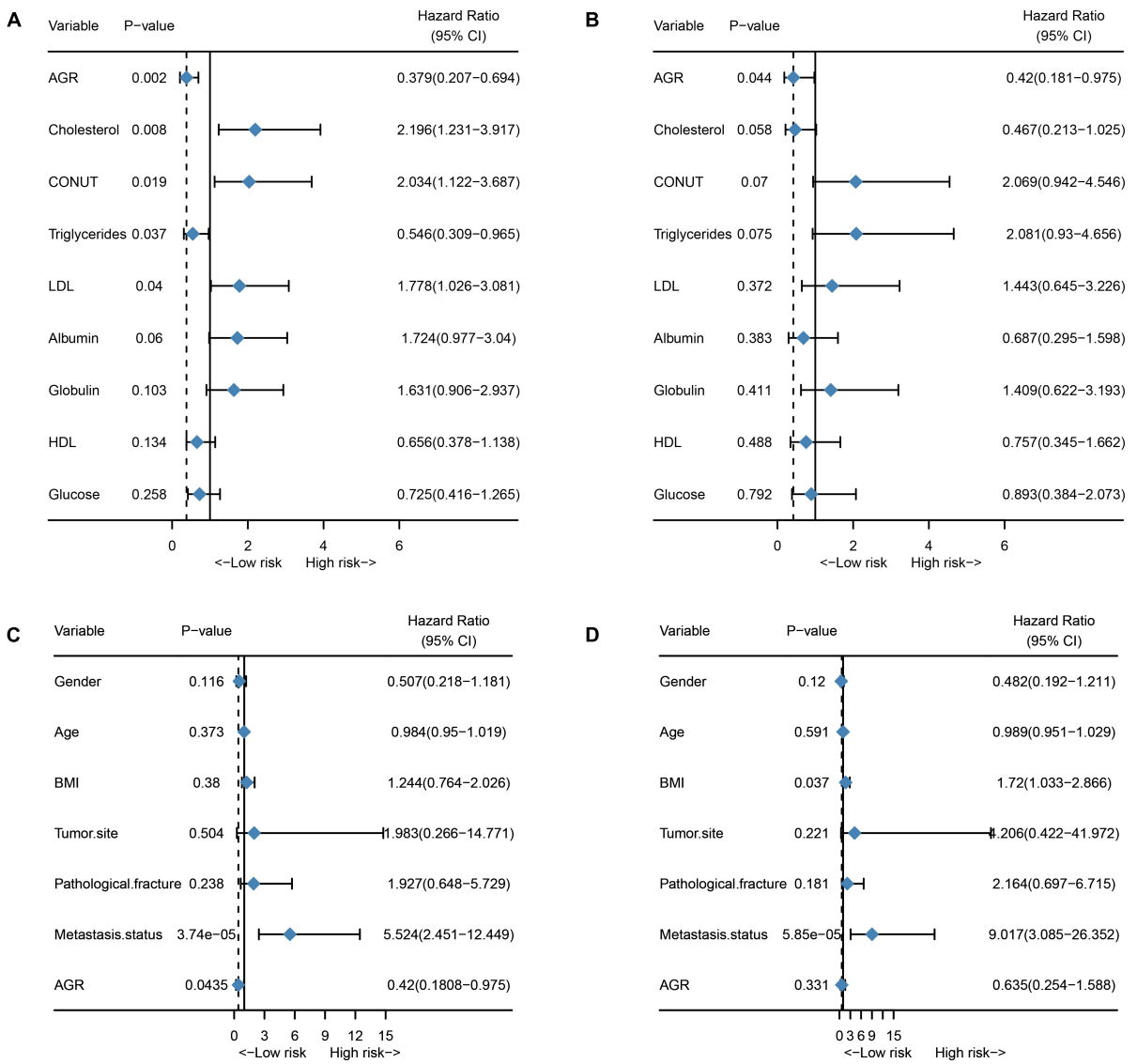

FIGURE 7 | Prognostic value of individual hematological markers and AGR in the training and validation sets. **(A)** Univariate cox regression analysis results of a single hematological marker in the training set; **(B)** Univariate cox regression analysis results of a single hematological marker in the validation set; **(C)** Univariate cox regression analysis of AGR in the validation set; **(D)** Multivariate cox regression analysis of AGR in the validation set.

of NMRS. We believe that NMRS with higher and more stable predictive ability is more likely to be applied and promoted in clinical practice.

However, it must be acknowledged that our study has certain limitations. Despite the training and validation sets of our study setup, all patients were from one clinical institution and the study was retrospective and not blinded, so there may have been a selection bias. At the same time, there were only two osteosarcoma patients over 60 years of age in the included population, so the use of NMRS in the elderly requires caution. Moreover, some patients may have problems with insufficient follow-up time. In addition, although the predictive ability of NMRS is higher than that of individual hematological markers, the computational method of NMRS is more complex. Finally, the metabolic nutrition-related indicators included in the study were derived from blood routine and liver function only. Some important indicators that reflect the patient's nutritional status, such as nutrients or dietary intake, are neglected. Therefore, well-designed prospective randomized controlled studies are needed to validate our conclusions. Finally, we believe that further studies are needed to explore the relationship between NMRS and dietary intake in patients.

CONCLUSION

Our study show the prognostic value of NMRS in osteosarcoma. Compared with individual hematological markers, NMRS

has predictive ability and predictive stability. NMRS-based nomogram also have good predictive accuracy.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of West China Hospital of Sichuan University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LL, YZ, and CT designed the study. LL, ZL, XH, YW, JL, and QC jointly collected and managed the data. LL and TG drafted the manuscript. ML, YL, and LM reviewed and corrected the manuscript. YZ and CT oversaw the entire research process. All authors contributed to the article and approved the submitted version.

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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.2022.883308/full#supplementary-material

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Associations of the Dietary Magnesium Intake and Magnesium Depletion Score With Osteoporosis Among American Adults: Data From the National Health and Nutrition Examination Survey

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Objectives: The study aimed to explore the associations between dietary magnesium (Mg) intake and magnesium depletion score (MDS) among American adults with osteoporosis.

Methods: The continuous data from the National Health and Nutrition Examination Survey 2005–2006, 2007–2008, 2009–2010, 2013–2014, and 2017–2018 were merged to ensure a large and representative sample and a total of 14,566 participants were enrolled for the analysis. The weighted multivariate linear regression model was performed to assess the linear relationship between dietary Mg intake and osteoporosis. Further, the non-linear relationship was also characterized by smooth curve fitting (SCF) and weighted generalized additive model (GAM). In addition, the odds ratios (ORs) and 95% confidence intervals (95% CIs) for associations between the MDS and osteoporosis were assessed by weighted logistic regression models.

Results: After adjusting all covariates, the weighted multivariable linear regression models demonstrated that the dietary Mg intake negatively correlated with osteoporosis, especially in participants aged 55 years or older. In addition, the non-linear relationship characterized by SCF and weighted GAM showed that the dietary Mg intake presented an L-shaped association with osteoporosis among females aged 55 years or older. Moreover, the weighted logistic regression model demonstrated that compared with MDS 0, the OR between MDS ≥3 and osteoporosis was 2.987 (95% CI 1.904, 4.686) in the male-middle intake group. Moreover, compared with MDS 0, the ORs between MDS ≥3 and osteoporosis was 5.666 (95% CI 3.188, 10.069) in the female-low intake group and 1.691 (95% CI 1.394, 2.051) in the female-middle intake group.

Conclusion: The present study indicated that in people with a daily intake of Mg level below the recommended daily intake (RDI), the dietary Mg intake and Mg bioavailability represented by MDS have a negative correlation with osteoporosis. According to the results, the combination of MDS and dietary Mg intake may be more comprehensive

and rigorous in screening the population with osteoporosis. Therefore, early monitoring and interventions for osteoporosis may be necessary for those with insufficient dietary Mg intake or high MDS scores.

Keywords: dietary magnesium intake, magnesium depletion score, bioavailability, osteoporosis, nutrition

INTRODUCTION

Osteoporosis is a disease of the skeletal system with degradation of bone tissue microstructure and low bone mineral density (BMD), which usually results in an increased risk of bone fragility and fractures (1). It is estimated that there are 1.5 million osteoporosis-related fractures per year in the US. Fractures can lead to a poor quality of life, a dependent living situation, increased fracture-related mortality, and medical care costs. Furthermore, especially in older adults, hip fractures can be devastating (2). Given the adverse consequences of osteoporosis-related diseases such as fractures, the prevention and management strategies for osteoporosis are of great significance and necessary.

The risk factors that contribute to reduced BMD and osteoporosis are multiple, including genetic, hormonal, environmental, and lifestyle-related factors (3–6). In recent years, various micronutrients, such as magnesium (Mg), have been reported to play an essential role in musculoskeletal diseases. On the one hand, Mg is an essential cofactor for enzymes related to bone matrix synthesis, which promotes bone formation by stimulating osteoblast proliferation. On the other hand, Mg deficiency affects parathyroid hormone (PTH) and Vitamin D levels while promoting inflammatory cytokine secretion and enhancing osteoclast activity (7, 8). However, the results of observational studies about the relationship between dietary Mg intake and osteoporosis were contradictory. Orchard et al. (9) reported that lower dietary Mg intake was related to lower BMD of the hip and whole body, and Ryder et al. (10) found a similar result in white women and men. Moreover, meta-analyses from Farsinejad-Marj et al. (11) and Groenendijk et al. (12) showed that dietary Mg intake was positively correlated with BMD of the femoral neck and total hip. However, no significant associations were found between dietary Mg intake and BMD at other sites. In a study with 2.8 years of follow-up, Kaptoge et al. (13) found that dietary Mg intake was not associated with hip BMD in both men and women, which was supported by Chan et al. (14) and Woo et al. (15).

In addition, previous studies have mainly focused on the effect of dietary Mg intake levels on osteoporosis but ignored the effective bioavailability of dietary Mg. The Mg depletion score (MDS) is a novel scoring tool that integrates several common factors affecting the absorption and excretion of dietary Mg in the US population (8, 16–18). The MDS has been shown to reflect the systemic utilization of the dietary Mg and can identify individuals with relatively low dietary Mg utilization. The higher score represented a lower bioavailability of dietary Mg. Moreover, Fan et al. (19) used the Mg tolerance test to validate MDS as a predictor of real body Mg deficiency in US adults. The results showed that the model containing the MDS alone had the highest area under the receiver operating characteristic curve estimator among models with single predictors, including serum and urine Mg. Thus, MDS may more accurately reflect the real Mg deficiency state of the body. To our knowledge, no previous studies are exploring the relationship between MDS and osteoporosis.

Given the above background, the purpose of the current study was to identify the relationship between dietary Mg intake and osteoporosis and further explore the association between MDS and osteoporosis in US adults.

MATERIALS AND METHODS

Study Population

Data used in this study were extracted from the National Health and Nutrition Examination Survey (NHANES). NHANES data were collected from a nationally representative sample of American civilians *via* a multistage probability design. All participants provided written informed consent, and NHANES was approved by the National Center for Health Statistics Ethics Review Board. This study merged the continuous data from NHANES 2005–2006, 2007–2008, 2009–2010, 2013–2014, and 2017–2018 to ensure a large and representative sample. The details of inclusion and exclusion process criteria are shown in **Figure 1**.

Dietary Mg Intake and Osteoporosis

Dietary Mg intake data were extracted from two NHANES 24-h recall interviews. The first interview was carried out at the Mobile Examination Center (MEC), and the second was carried out by telephone 3–10 days later. The mean value of the two 24-h recall data was determined as needed dietary Mg intake in the study.

The BMD was evaluated by dual-energy X-ray absorptiometry scans with Hologic QDR-4500A fan-beam densitometers (Hologic, Inc., Bedford, Massachusetts). The assessed femoral regions included total femur, femur neck, trochanter, and intertrochanter. According to the World Health Organization classification criteria, a BMD value in any femoral region lower than −2.5 standard deviations of the reference group can be defined as osteoporosis. The specific thresholds were 0 68, 0.59, 0.49, and 0.78 g/cm^2 for total femur, femur neck, trochanter, and intertrochanter, respectively (20).

MDS Calculation

The MDS was calculated by adding up the following 4 points:

1) Current use of diuretics was recorded as 1 point;
2) Current use of proton pump inhibitor (PPI) was recorded as 1 point;

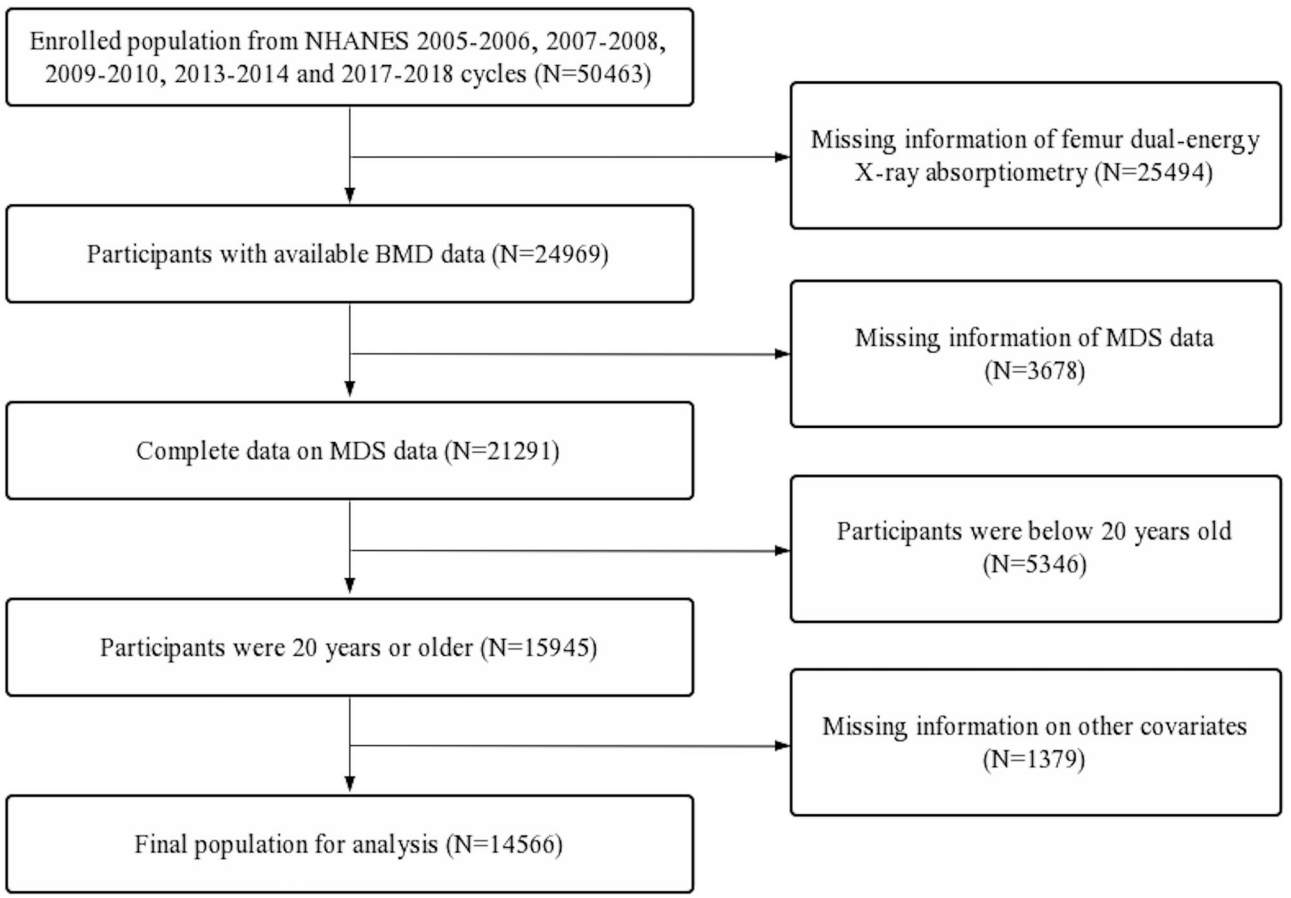

FIGURE 1 | Flow diagram of inclusion criteria and exclusion criteria. NHANES, National Health and Nutrition Examination Survey; MDS, magnesium depletion score; BMD, bone mineral density.

3) Heavy drinker was recorded as 1 point. According to 2015–2020 Dietary guidelines for Americans, the heavy drinkers were defined as >1 drink/d for women and >2 drinks/d for men (http://www.health.gov/DietaryGuidelines);
4) Mildly decreased renal function was recorded as 1 point, and chronic kidney disease (CKD) was recorded as 2 points. According to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (21, 22), the estimated glomerular filtration rate (eGFR) of participants were classified into 3 categories, eGFR ≥90 ml/(min 1.73 m^2) was defined as normal, 60 mL/(min 1.73 m^2) ≤ eGFR < 90 ml/(min 1.73 m^2) was defined as mildly decreased renal function, and eGFR <60ml/(min 1.73 m^2) was defined as CKD.

Covariates

Based on the previous literature and clinical experience, the selected covariates were obtained as follows:

1) Demographic data: age (<55 years, ≥55 years), sex (male, female), race/ethnicity (Mexican Americans, other Hispanic, non-Hispanic White, non-Hispanic Black, other race), educational level (<9th grade, 9–11th grade, high school, some college, college graduate), marital status (married, widowed, divorced, separated, never married, living with partner), and poverty income ratio (PIR) (<1, 1–3, ≥3);
2) Dietary data: dietary calcium and energy intakes (the mean value of the two 24-h recall data).
3) Examination data: body mass index (BMI) (<25, 25–30, ≥30);
4) Questionnaire data: alcohol consumption (drink/d), smoked at least 100 cigarettes (yes or no), ever use prednisone or cortisone daily (yes or no), moderate or vigorous activity (yes or no).

Comprehensive data: hypertension status (yes or no) and diabetes status (yes, no or borderline). hypertension status was defined according to the following criteria: doctor told you have hypertension, use of hypertension drugs, or mean value of 3 measured diastolic blood pressure ≥90 mmHg or the mean value of 3 measured systolic pressure ≥140 mmHg (The reading with zero is not used to calculate the diastolic average, and if only one blood pressure reading was obtained, that reading is the average). Diabetes was defined according to the following criteria: doctor told you have diabetes, self-reported diabetes for a long

time, glycated hemoglobin >6.5%, fasting glucose ≥7.0 mmol/L, random blood glucose ≥11.1 mmol/L, 2-h oral glucose tolerance test blood glucose ≥11.1 mmol/L, and use of diabetes medication or insulin (borderline diabetes = impaired fasting glycaemia or impaired glucose tolerance or prediabetes).

Statistical Analysis

According to the weight selection criteria of NHANES, sampling weights were used in all analyses. Chi-square test was used to compare the differences of categorical variables between the osteoporosis and non-osteoporosis groups, and for continuous variables, a Student's *t*-test was used. Weighted multivariate linear regression model was performed to assess the linear relationship between the dietary Mg intake and osteoporosis. Subgroup analyses based on sex and age were further performed *via* weighted stratified line regression models. Moreover, the non-linear relationship was characterized by smooth curve fitting (SCF) and weighted generalized additive model (GAM). We also used two-piecewise linear regression models and a recursive algorithm to find the inflection points. Then, the dietary Mg intake were categorized into low, middle, and high groups based on the inflection points of male and female subgroups. In addition, the odds ratios (ORs) and 95% confidence intervals (95% CIs) for associations between the MDS and osteoporosis were assessed by weighted logistic regression models. Subgroup analyses based on sex and the dietary Mg intake levels were further performed *via* weighted stratified logistic regression models. Model 1 was adjusted for no covariates. Model 2 was adjusted for age (if applicable), sex (if applicable), and race. Model 3 was adjusted for all the applicable covariates.

All analyses were performed *via* R software (4.0.3) and EmpowerStats (2.0). A two-sided $p < 0.05$ was considered to have statistical significance.

RESULTS

Baseline Characteristics of Participants

First, a total of 50,463 participants were extracted. Second, participants with missing femur BMD data ($n = 25{,}494$) and incomplete MDS data ($n = 3{,}678$) were excluded. Further, participants below 20 years old ($n = 5{,}346$) and participants with missing data on other covariates ($n = 1{,}379$) were also excluded. A total of 14,566 participants were included in the final analysis (**Figure 1**).

Baseline characteristics of selected participants were compared between osteoporosis and non-osteoporosis groups (**Table 1**). Among all participants, the prevalence of osteoporosis was 6.9% ($n = 998$). Compared with the non-osteoporosis group, participants in the osteoporosis group tended to have less dietary Mg (263.1 ± 114.3 vs. 304.5 ± 126.5, $P < 0.001$), calcium (854.3 ± 460.8 vs. 963.7 ± 499.6, $P < 0.001$), and energy (1,753.1 ± 709.1 vs. 2,125.9 ± 824.9, $P < 0.001$) intake. After grouping dietary Mg intake by recommended daily intake (RDI, 330.0 mg) and upper limit (UL, 700.0 mg), the percentage of participants whose daily dietary Mg intake below RDI was higher in the osteoporosis group. However, when daily dietary Mg intake was above RDI or UL, the result seemed to be the opposite ($P < 0.001$,

TABLE 1 | Weighted characteristics of the study population.

	Non-osteoporosis (*N* = 13,568, 93.1%)	Osteoporosis (*N* = 998, 6.9%)	*P*-value
MDS (%)			<0.001
0	40.4	19.7	
1	37.3	39.7	
2	16.5	23.8	
≥3	5.9	16.8	
Age (years, %)			<0.001
<55	61.2	13.9	
≥55	38.8	86.1	
Sex (%)			<0.001
Male	50.9	17.8	
Female	49.1	82.2	
Race (%)			<0.001
Mexican Americans	7.2	3.2	
Other Hispanic	4.4	3.3	
Non-Hispanic White	72.7	83.4	
Non-Hispanic Black	10.0	3.9	
Other race	5.7	6.2	
BMI (%)			<0.001
<25	28.8	56.3	
≥25, <30	36.1	28.5	
≥30	35.1	15.2	
PIR (%)			<0.001
<1	10.9	11.3	
≥1, <3	33.4	44.9	
≥3	55.7	43.8	
Educational level (%)			<0.001
<9th grade	4.6	6.4	
9–11th grade	10.4	12.4	
High school	23.7	29.7	
Some college	30.9	29.2	
College graduate	30.5	22.2	
Marital status (%)			<0.001
Married	61.7	47.7	
Widowed	4.8	26.1	
Divorced	11.2	16.2	
Separated	2.2	1.8	
Never married	13.6	5.4	
Living with partner	6.6	2.9	
Diabetes status (%)			<0.001
Yes	78.5	71.7	
No	13.5	17.1	
Borderline	8.0	11.2	
Hypertension status (%)			<0.001

(Continued)

TABLE 1 | Continued

	Non-osteoporosis (*N* = 13,568, 93.1%)	Osteoporosis (*N* = 998, 6.9%)	*P*-value
Yes	66.2	49.4	
No	33.8	50.6	
Ever use prednisone or cortisone daily (%)			<0.001
Yes	5.4	8.9	
No	94.6	91.1	
Smoked at least 100 cigarettes (%)			0.269
yes	46.5	48.4	
no	53.5	51.6	
Moderate or vigorous activity (%)			<0.001
Yes	41.2	56.4	
No	58.8	43.6	
Alcohol consumption (drink/d, mean ± SD)	1.4 ± 3.1	0.7 ± 1.8	<0.001
Magnesium (mg, mean ± SD)	304.5 ± 126.5	263.1 ± 114.3	<0.001
Magnesium intake level (%)			<0.001
<RDI	65	78.4	
≥RDI, < UL	33.8	20.7	
≥UL	1.2	0.9	
Calcium (mg, mean ± SD)	963.7 ± 499.6	854.3 ± 460.8	<0.001
Energy (kcal, mean ± SD)	2,125.9 ± 824.9	1,753.1 ± 709.1	<0.001
Total femur BMD (g/cm^2, mean ± SD)	1.0 ± 0.1	0.7 ± 0.1	<0.001
Femur neck BMD (g/cm^2, mean ± SD)	0.8 ± 0.1	0.6 ± 0.1	<0.001
Trochanter BMD (g/cm^2, mean ± SD)	0.7 ± 0.1	0.5 ± 0.1	<0.001
Intertrochanter BMD (g/cm^2, mean ± SD)	1.2 ± 0.2	0.8 ± 0.1	<0.001

MDS, magnesium depletion score; BMD, bone mineral density; PIR, poverty income ratio; BMI, body mass index; RDI, recommended daily intake (330.0 mg); UL, upper limit (700.0 mg); SD standard deviation; %, weighted percentage.

Table 1). In addition, the percentage of participants who had a higher MDS, hypertension, diabetes, and ever used prednisone or cortisone daily were significantly higher in the osteoporosis group. Participants in the osteoporosis group were more likely to be female, widowed, older, more emaciated, poorer, smoke more, drink more, have less activity, and have lower educational levels ($P < 0.050$, **Table 1**).

Associations of Dietary Mg Intake With Osteoporosis

Total Analyses

The levels of dietary Mg intake showed a negative association with osteoporosis in Model 1. However, after adjusting for confounding factors in Models 2 (age, sex, and race) and 3 (age, sex, race, body mass index [BMI], poverty income ratio [PIR], educational level, marital status, smoked at least 100 cigarettes, hypertension status, diabetes status, ever used prednisone or cortisone daily, moderate or vigorous activity, alcohol consumption, dietary calcium, and energy intakes), the relationship between exposed variables and outcomes remained stable (**Table 2**). Furthermore, after adjusting for all covariates, the negative associations between dietary Mg intake levels and osteoporosis were also observed in smooth curve fitting (SCF) and weighted generalized additive model (GAM) (**Figure 2A**).

Subgroup Analyses

In the age below 55 years, dietary Mg intake showed an inverse association with osteoporosis in Models 1 and 2. However, this association did not exist in Model 3 ($P = 0.080$, **Table 2**). Moreover, when the non-linear relationship was characterized by SCF and weighted GAM, the association between dietary Mg intake and osteoporosis was not significant (**Figure 2B**). In contrast, at the age of 55 years or older, the relationship between dietary Mg intake and osteoporosis was significantly negative in Models 1, 2, and 3 (**Table 2**). Further, SCF and weighted GAM presented the negative associations (**Figure 2B**).

In the male group, dietary Mg intake was negatively correlated with osteoporosis in Models 1, 2, and 3 (**Table 2**). This negative correlation was further verified by the results of SCF and weighted GAM (**Figure 2C**). Two-piecewise linear regression model and a recursive algorithm found that the inflection point was 145.5 mg (**Table 3**). The relationship between dietary Mg intake and osteoporosis in the female group was generally negative in Models 1, 2, and 3 (**Table 2**). The non-linear relationship between dietary Mg intake and osteoporosis was an L-shaped association (**Figure 2C**). Further, a two-piecewise linear regression model and a recursive algorithm found that the inflection point was 332.5 mg (**Table 3**).

When the participants were further cross-stratified by age and sex, the negative association between the dietary Mg intake and osteoporosis was mainly presented in males and females aged 55 years or older (**Table 2**). Meanwhile, SCF and weighted GAM showed that the non-linear relationship between the dietary Mg intake and osteoporosis presented a stable negative correlation in the males aged 55 years or older (**Figure 3A**). In addition, the non-linear relationship between the dietary Mg intake and osteoporosis presented an L-shape (**Figure 3B**) in females aged 55 years or older. When daily dietary Mg intake was below the inflection point of 337.5 mg, there was a clear inverse relationship between the dietary Mg intake and osteoporosis. However, when daily dietary Mg intake was more than 337.5 mg, this negative correlation did not exist (**Figure 3B**, **Table 3**). In males aged

TABLE 2 | Associations of dietary magnesium intake and osteoporosis.

	Male	Female	Total
Age < 55			
Model 1 β (95% CI) *P*-value	0.994 (0.993, 0.996)***	1.000 (0.999, 1.001)	0.998 (0.997, 0.999)***
Model 2 β (95% CI) *P*-value	0.994 (0.992, 0.996)***	0.999 (0.998, 1.000)	0.998 (0.997, 0.999)***
Model 3 β (95% CI) *P*-value	1.000 (0.997, 1.003)	1.001 (1.000, 1.002)	1.001 (1.000, 1.002)
Age ≥ 55			
Model 1 β (95% CI) *P*-value	0.998 (0.997, 0.999)***	0.999 (0.998, 0.999)***	0.999 (0.998, 0.999)***
Model 2 β (95% CI) *P*-value	0.998 (0.997, 0.999)***	0.998 (0.998, 0.999)***	0.998 (0.998, 0.999)***
Model 3 β (95% CI) *P*-value	0.998 (0.997, 0.999)**	0.997 (0.997, 0.998)***	0.998 (0.997, 0.998)***
Total			
Model 1 β (95% CI) *P*-value	0.997 (0.997, 0.998)***	0.999 (0.999, 0.999)***	0.999 (0.998, 0.999)***
Model 2 β (95% CI) *P*-value	0.997 (0.996, 0.998)***	0.999 (0.998, 0.999)***	0.998 (0.998, 0.999)***
Model 3 β (95% CI) *P*-value	0.999 (0.998, 1.000)*	0.998 (0.998, 0.999)***	0.998 (0.998, 0.999)***

Model 1: no covariates were adjusted.
Model 2: age (if applicable), sex (if applicable), and race were adjusted.
Model 3: age (if applicable), sex (if applicable), race, BMI, PIR, educational level, marital status, smoked at least 100 cigarettes, hypertension status, diabetes status, ever use prednisone or cortisone daily, moderate or vigorous activity, alcohol consumption, dietary calcium and energy intakes were adjusted.
*PIR, poverty income ratio; BMI, body mass index; *, ** and ***, for P-values <0.05, <0.01 and <0.001, respectively.*

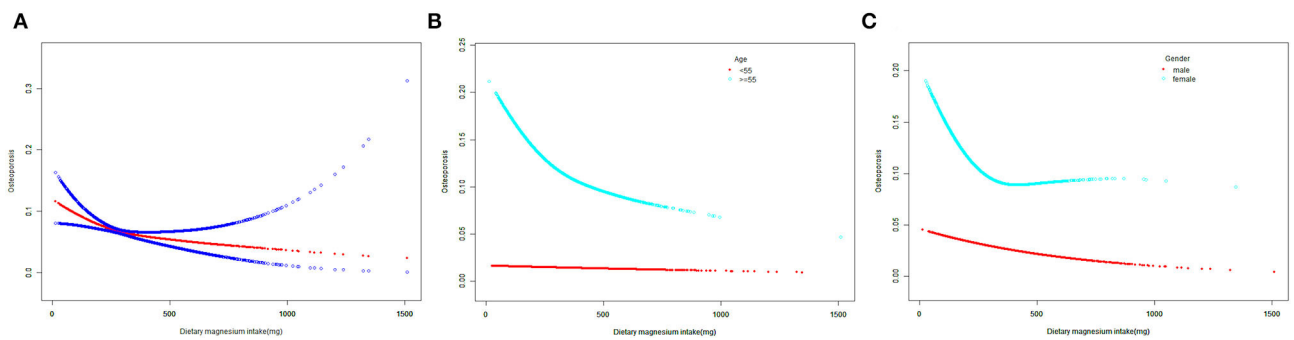

FIGURE 2 | The SCF for associations of MDS with osteoporosis. **(A)** Represents the overall trend. **(B,C)** Represent the substratum trends grouped by age and gender, respectively. Age (if applicable), gender (if applicable), race, BMI, PIR, educational level, marital status, smoked at least 100 cigarettes, hypertension status, diabetes status, ever use prednisone or cortisone daily, moderate or vigorous activity, alcohol consumption, dietary calcium and energy intakes were adjusted. SCF, smooth curve fit; MDS, magnesium depletion score; PIR, poverty income ratio; BMI, body mass index.

below 55 years, the inverse correlation was only found in Models 1 and 2. However, in females aged below 55 years, subgroup analyses did not show any significant associations between the dietary Mg intake and osteoporosis in Models 1, 2, and 3 (**Table 2**).

Associations of MDS With Osteoporosis

Total Analyses

The relationship between MDS and osteoporosis generally showed a positive correlation trend. For Model 1, the odds ratios (ORs) between MDS and osteoporosis across scores 1, 2, and ≥3 compared with score 0 were 2.403 (95% confidence interval [CI] 2.194, 2.632), 3.145 (95% CI 2.841, 3.481), and 5.642 (95% CI 5.032, 6.325), respectively. After adjusting for covariates in Model 2, the ORs across scores 1, 2, and ≥3 compared with score 0 were 1.205 (95% CI 1.092, 1.330), 1.075 (95% CI 0.961, 1.202), and 1.623 (95% CI 1.432, 1.839), respectively. Further adjusting for covariates in Model 3, the ORs across scores 1, 2, and ≥3 compared with score 0 were 1.240 (95% CI 1.116, 1.377), 1.161 (95% CI 1.025, 1.316), and 1.785 (95% CI 1.544, 2.064), respectively (**Table 4**).

TABLE 3 | Two-piecewise linear regression models of dietary magnesium intake on osteoporosis.

	Age < 55	Age ≥ 55	Total
Male			
Inflection point	222.5	138.0	145.5
< Inflection point	0.982 (0.975 0.988)***	0.973 (0.966, 0.981)***	0.973 (0.967, 0.979)***
> Inflection point	1.003 (1.001, 1.005)*	0.999 (0.997, 1.000)*	0.999 (0.998, 1.000)
Log likelihood ratio	<0.001	<0.001	<0.001
Female			
Inflection point	119.0	337.5	332.5
< Inflection point	0.979 (0.969, 0.990)***	0.995 (0.994, 0.996)***	0.996 (0.995, 0.997)***
> Inflection point	1.001 (1.000, 1.003)*	1.002 (1.000, 1.003)**	1.001 (1.000, 1.002)*
Log likelihood ratio	<0.001	<0.001	<0.001

*, ** and ***, for P-values <0.05, <0.01 and <0.001, respectively.*

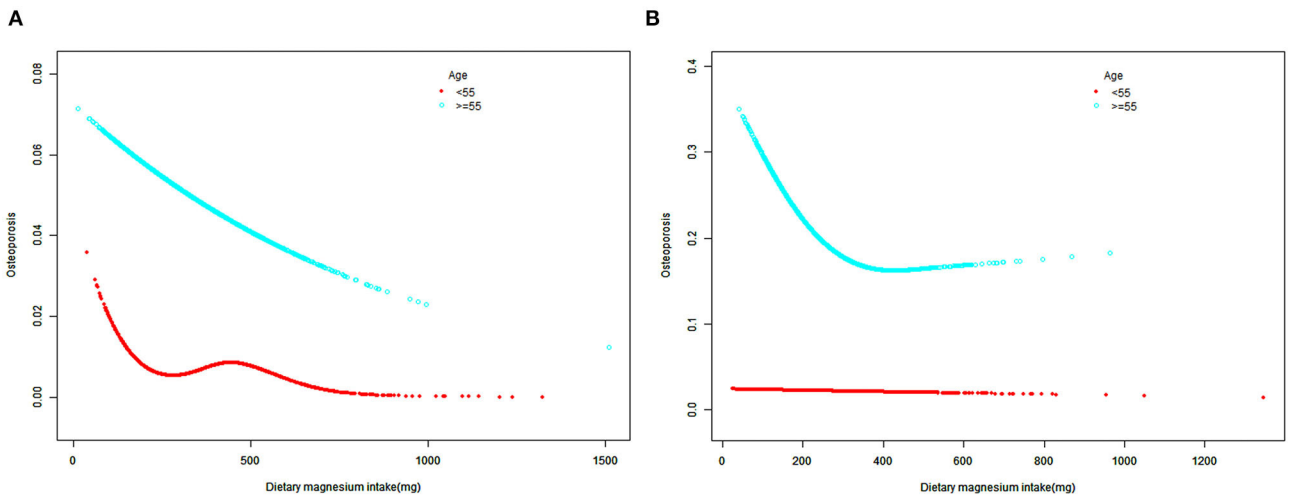

FIGURE 3 | The SCF for associations of MDS with osteoporosis after cross-stratifying by age and sex. **(A)** male; **(B)** female; Race, BMI, PIR, educational level, marital status, smoked at least 100 cigarettes, hypertension status, diabetes status, ever use prednisone or cortisone daily, moderate or vigorous activity, alcohol consumption, dietary calcium and energy intakes were adjusted. SCF, smooth curve fit; MDS, magnesium depletion score; PIR, poverty income ratio; BMI, body mass index.

Subgroup Analyses

After stratifying the participants by age, the subgroup analyses presented a similar trend to the above. Whether the participants were male or female, the MDS generally showed a positive association with osteoporosis in Model 1. When adjusting for covariates in Models 2 and 3, this trend was partially diminished but still significant. In Model 2 for males, compared with MDS 0, the OR between MDS ≥3 and osteoporosis was 1.463 (95% CI 1.083, 1.976), and in Model 2 for females, the OR was 1.677 (95% CI 1.460, 1.927). In Model 3 for males, compared with MDS 0, the OR between MDS ≥3 and osteoporosis was 2.149 (95% CI 1.521, 3.035), and in Model 3 for females, the OR was 1.761 (95% CI 1.497, 2.071) (**Table 4**).

Based on the inflection points of 145.5 mg and 332.5 mg, the dietary Mg intake levels were divided into low (<145.5 mg), middle (≥145.5 mg, <332.5 mg), and high (≥332.5 mg) groups. The significant associations between MDS and osteoporosis were mainly found in the low and middle dietary Mg intake groups. In Model 1 of the low intake group, the ORs between MDS and osteoporosis across scores 1, 2, and ≥3 compared with score 0 were 2.731 (95% CI 2.073, 3.598), 3.198 (95% CI 2.317, 4.414), and 7.857 (95% CI 5.773, 10.694), respectively. In Model 1 of the middle intake group, the ORs between MDS and osteoporosis across scores 1, 2, and ≥3 compared with score 0 were 2.351 (95% CI 2.096, 2.636), 3.675 (95% CI 3.250, 4.155), and 6.4697 (95% CI 5.643, 7.418), respectively. In Model 2 of the low intake group, compared with MDS 0, the OR between MDS ≥3 and osteoporosis was 2.492 (95% CI 1.741, 3.566), and in Model 2 of the middle intake group, the OR was 1.703 (95% CI 1.464, 1.980). In Model 3 of the low intake group, compared with MDS 0, the OR between MDS ≥3 and osteoporosis was 3.607 (95% CI 2.235, 5.823), and in Model 3 of the middle intake group, the OR was 1.809 (95% CI 1.518, 2.155) (**Table 4**).

TABLE 4 | Associations of MDS with osteoporosis.

	Dietary magnesium intake (mg) <145.5	Dietary magnesium intake (mg) ≥145.5, <332.5	Dietary magnesium intake (mg) ≥332.5	Total
Male				
Model 1				
β (95% CI) *P*-value				
MDS				
0	1	1	1	1
1	2.094 (1.206, 3.636)**	2.412 (1.796, 3.239)***	2.399 (1.689, 3.406)***	2.342 (1.902, 2.882)***
2	0.511 (0.184, 1.421)	4.455 (3.282, 6.048)***	1.486 (0.928, 2.380)	2.777 (2.194, 3.514)***
≥3	0.188 (0.026, 1.371)	7.139 (5.045, 10.103)***	3.170 (1.789, 5.616)***	4.450 (3.369, 5.877)***
Model 2				
β (95% CI) *P*-value				
MDS				
0	1	1	1	1
1	2.215 (1.200, 4.091)*	1.002 (0.729, 1.377)	1.414 (0.979, 2.042)	1.206 (0.965, 1.506)
2	0.471 (0.151, 1.466)	1.397 (0.999, 1.955)	0.699 (0.427, 1.142)	1.104 (0.855, 1.425)
≥3	0.161 (0.021, 1.246)	1.822 (1.246, 2.665) **	1.144 (0.629, 2.079)	1.463 (1.083, 1.976)*
Model 3				
β (95% CI) *P*-value				
MDS				
0	1	1	1	1
1	6.095 (1.594, 23.305)**	1.517 (1.064, 2.161)*	1.471 (0.979, 2.210)	1.480 (1.160, 1.889)**
2	3.682 (0.507, 26.764)	2.936 (1.984, 4.343)***	0.693 (0.398, 1.206)	1.694 (1.269, 2.262)***
≥3	0.329 (0.020, 5.476)	2.987 (1.904, 4.686)***	1.206 (0.610, 2.387)	2.149 (1.521, 3.035)***
Female				
Model 1				
β (95% CI) *P*-value				
MDS				
0	1	1	1	1
1	2.785 (2.031, 3.820)***	2.345 (2.070, 2.656)***	2.188 (1.778, 2.692)***	2.399 (2.168, 2.654)***
2	4.356 (3.066, 6.188)***	3.532 (3.087, 4.040)***	1.852 (1.430, 2.398)***	3.218 (2.875, 3.601)***
≥3	11.898 (8.472, 16.711)***	6.357 (5.477, 7.377)***	1.571 (1.024, 2.409)*	5.909 (5.211, 6.701)***
Model 2				
β (95% CI) *P*-value				
MDS				
0	1	1	1	1
1	1.493 (1.041, 2.140)*	1.098 (0.958, 1.259)	1.424 (1.138, 1.781)**	1.214 (1.087, 1.356)***
2	1.356 (0.907, 2.028)	1.069 (0.920, 1.242)	0.923 (0.697, 1.222)	1.075 (0.949, 1.217)
≥3	3.276 (2.195, 4.888)***	1.692 (1.435, 1.996)***	0.577 (0.370, 0.899)*	1.677 (1.460, 1.927)***
Model 3				
β (95% CI) *P*-value				
MDS				
0	1	1	1	1
1	1.683 (1.056, 2.683)*	1.1353 (0.982, 1.313)	1.209 (0.941, 1.554)	1.205 (1.071, 1.356)**
2	1.097 (0.640, 1.882)	1.073 (0.907, 1.268)	1.166 (0.840, 1.620)	1.096 (0.953, 1.261)
≥3	5.666 (3.188, 10.069)***	1.691 (1.394, 2.051)***	0.673 (0.408, 1.109)	1.761 (1.497, 2.071)***
Total				
Model 1				
β (95% CI) *P*-value				
MDS				
0	1	1	1	1
1	2.731 (2.073, 3.598)***	2.351 (2.096, 2.636)***	2.242 (1.876, 2.680)***	2.403 (2.194, 2.632)***
2	3.198 (2.317, 4.414)***	3.675 (3.250, 4.155)***	1.756 (1.401, 2.201)***	3.145 (2.841, 3.481)***

(Continued)

TABLE 4 | Continued

	Dietary magnesium intake (mg) <145.5	Dietary magnesium intake (mg) ≥145.5, <332.5	Dietary magnesium intake (mg) ≥332.5	Total
≥3	7.857 (5.773, 10.694)***	6.470 (5.643, 7.418)***	1.967 (1.397, 2.770)***	5.642 (5.032, 6.325)***
Model 2				
β (95% CI) *P*-value				
MDS				
0	1	1	1	1
1	1.551 (1.144, 2.103)**	1.072 (0.946, 1.215)	1.397 (1.155, 1.691)***	1.205 (1.092, 1.330)***
2	1.115 (0.775, 1.603)	1.119 (0.976, 1.283)	0.841 (0.660, 1.070)	1.075 (0.961, 1.202)
≥3	2.492 (1.741, 3.566)***	1.703 (1.464, 1.980)***	0.711 (0.499, 1.014)	1.623 (1.432, 1.839)***
Model 3				
β (95% CI) *P*-value				
MDS				
0	1	1	1	1
1	1.768 (1.224, 2.555)**	1.150 (1.007, 1.314)*	1.327 (1.077, 1.634)**	1.240 (1.116, 1.377)***
2	1.263 (0.794, 2.009)	1.222 (1.049, 1.423)**	1.038 (0.791, 1.362)	1.161 (1.025, 1.316)*
≥3	3.607 (2.235, 5.823)***	1.809 (1.518, 2.155)***	0.919 (0.619, 1.365)	1.785 (1.544, 2.064)***

Model 1: no covariates were adjusted.
Model 2: age, sex (if applicable), and race were adjusted.
Model 3: age, sex (if applicable), race, BMI, PIR, educational level, marital status, smoked at least 100 cigarettes, hypertension status, diabetes status, ever use prednisone or cortisone daily, moderate or vigorous activity, alcohol consumption, dietary calcium and energy intakes were adjusted.
*MDS, magnesium depletion score; PIR, poverty income ratio; BMI, body mass index; *, ** and ***, for P-values <0.05, <0.01 and <0.001, respectively.*

When the participants were further cross-stratified by sex and dietary Mg intake levels, the male group mainly presented a significantly positive association between MDS and osteoporosis in the middle intake level. On the other hand, the female group mainly presented significantly positive associations between MDS and osteoporosis in both low and middle intake levels. In the male-middle intake group, Models 2 and 3 showed that compared with MDS 0, the ORs between MDS ≥3 and osteoporosis were 1.822 (95% CI 1.246, 2.665) and 2.987 (95% CI 1.904, 4.686), respectively. In the female-low intake group, Models 2 and 3 showed that compared with MDS 0, the ORs between MDS ≥3 and osteoporosis were 3.276 (95% CI 2.195, 4.888) and 5.666 (95% CI 3.188, 10.069), respectively. Moreover, in the female-middle intake group, Models 2 and 3 showed that compared with MDS 0, the ORs between MDS ≥3 and osteoporosis were 1.692 (95% CI 1.435, 1.996) and 1.691 (95% CI 1.394, 2.051), respectively (**Table 4**).

DISCUSSION

According to the representative sample of U.S. adults in the National Health and Nutrition Examination Survey (NHANES), we demonstrated that dietary Mg intake levels and osteoporosis were negatively correlated, especially in participants aged 55 years or older. This result suggests that adequate dietary Mg intake may be a factor that prevents osteoporosis in older adults. In addition, we proved that MDS generally presented a significantly positive relationship with osteoporosis. The results of subgroup analyses showed that for males, the positive association mainly presented in the middle Mg intake group. For females, the positive associations mainly presented in both low and middle Mg intake groups. To our knowledge, this is the first study to combine the dietary Mg intake with the bioavailability of Mg and comprehensively explore the association between MDS and osteoporosis.

As we all know, Mg is an essential mineral involved in bone metabolism (7, 8). However, more than half of US adults do not meet the estimated average requirement (EAR) or RDI of daily Mg intake (23, 24). In the present study, the mean daily dietary Mg intake levels were 304.5164 mg ± 126.4613 mg in males and 263.1411 mg ± 114.2658 mg in females (**Table 1**) and were also far below EAR and RDI. Dietary Mg mainly comes from green vegetables, unpolished grains, nuts, and shellfish, and Mg content in food is easily lost during cooking and refining processes. Moreover, western diets are often rich in refined foods while deficient in green vegetables, and this may help explain the widespread dietary Mg deficiency in the US (25). Given the prevalence of dietary Mg deficiency in the US and the essential role of dietary Mg plays in the bone, comprehensively evaluating the relationship between dietary Mg intake levels and osteoporosis in the US is necessary. The present study showed a negative effect of dietary Mg intake deficiency on the prevention of osteoporosis, which was supported by the results of several previous literature (9–12). To better characterize the relationship in detail, we also performed a two-piecewise linear regression model and a recursive algorithm to find the inflection points of dietary Mg intake in the subgroups with a significant relationship.

In the participants aged 55 years or older, the inflection points were 138.0 mg for males and 337.5 mg for females (**Table 3**). From the perspective of preventing osteoporosis, we recommend that the subpopulation whose daily dietary Mg intake was below the inflection point should be more alert to osteoporosis.

When exploring the effect of dietary Mg on osteoporosis, the bioavailability of dietary Mg should also not be ignored. Clinically, serum Mg is routinely used to diagnose systemic Mg deficiency. However, Mg in the human body is mainly stored in bones and soft tissue (26). Serum Mg only accounts for 0.3% of the whole body Mg content (27). Previous studies have shown that serum Mg was not sensitive to a decline in the actual Mg stores of the body. In addition, individuals with normal serum Mg may have Mg deficiency and respond to Mg supplementation (28, 29). Compared with other methods, the Mg tolerance test (MTT) is more accurate in evaluating the systemic Mg status (30). The test requires measuring the Mg level in 24 h urine and then performing an intravenous drip of Mg for 4 h and collecting the second 24 h urine. However, the relatively complex process limits its widespread application (19, 30, 31). Therefore, an accurate, simple, and convenient tool that can be widely used to evaluate the bioavailability of dietary Mg is urgently needed. The absorption and excretion of dietary Mg can be affected by several factors. For instance, alcohol abuse can lead to a rapidly increased excretion of urinary Mg (18, 32). Proton pump inhibitors (PPIs) can reduce intestinal Mg absorption by interfering with the activity of epithelial Mg^{2+} transient receptor potential channel subfamily M, member 6, and the use of thiazide and loop diuretics were also proved to result in a Mg deficiency (16). In addition, plasma Mg homeostasis is primarily regulated by the kidneys, which are responsible for over 80% of the ultrafiltration and reabsorption of plasma Mg (33). Thus, renal insufficiency of various causes can also affect Mg reabsorption (8). Fan et al. (19) found that Mg levels as determined by MTT had a significant correlation with estimated glomerular filtration rate (eGFR), and Mg deficiency is positively correlated with the severity of renal insufficiency. As a reflection of the Mg bioavailability, MDS combined all the above factors. Meanwhile, the higher the MDS, the poorer the bioavailability of dietary Mg. The accuracy of MDS as a predictive tool for systemic Mg deficiency has been validated by MTT (19).

This study comprehensively assessed the relationship between MDS and osteoporosis. In subgroup analyses based on dietary Mg intake levels, this study found that MDS positively correlated with osteoporosis in the low and middle dietary Mg intake levels. Furthermore, when adding sex to the stratification factor, the positive associations remain stable in the male-middle intake, female-low, and female-middle intake groups. However, this relationship was not significant in the male and female-high intake groups. Similarly, the percentage of participants whose daily dietary Mg intake below RDI was higher in the osteoporosis group, but when daily dietary Mg intake was above RDI or UL, the result seemed to be the opposite. Given that this study used a daily dietary Mg intake of 337.5 mg as the cut-off point for the middle and high groups, which were close to the daily RDI of a US adult (23, 34), these findings may indicate the following three points: (1) in the case of inadequate dietary Mg intake, insufficient Mg bioavailability by the body may further increase osteoporosis, especially in people whose daily dietary Mg intake is below the RDI; (2) the adverse effect of insufficient Mg bioavailability on bone appeared to be partially eliminated by the adequate intake of dietary Mg; and (3) from the perspective of preventing osteoporosis, when dietary Mg intake is below the RDI, increasing dietary Mg intake is beneficial. However, when the dietary Mg intake exceeds the RDI or even reaches the UL, the effect on preventing osteoporosis may deserve further exploration.

There are several strengths in our study. First, we used a large nationally representative database collected *via* standardized protocols to minimize possible bias. Secondly, we adequately controlled for confounders and performed subgroup analyses according to different stratification variables to make the study results more rigorous. In addition, our study has some potential limitations. First, since the present study was an across-sectional analysis, the evidence for a causal relationship may not be sufficient. In the future, more prospective studies need to be performed to confirm the results in the present study. Second, the data collected from the questionnaires and interviews may result in recall bias. Third, although we have adjusted some covariates, other unmeasured confounding factors may also lead to potential bias. Lastly, vitamin D metabolism is dependent on Mg as a cofactor (35), and metabolic balance between PTH and vitamin D has been shown to be closely related to osteoporosis (36). Therefore, dietary Mg deficiency may affect osteoporosis (37) by altering the balance between vitamin D metabolite and PTH. The present study was unable to verify this mechanism due to lack of information on PTH and vitamin D.

CONCLUSION

This study indicated that in people with a daily intake of Mg level below the recommended daily intake (RDI), the dietary Mg intake and Mg bioavailability represented by MDS have a negative correlation with osteoporosis. According to the results, the combination of MDS and dietary Mg intake may be more comprehensive and rigorous in screening the osteoporosis population. Therefore, early monitoring and interventions for osteoporosis may be necessary for those with insufficient dietary Mg intake or high MDS scores.

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

JW, FX, and NS: conceptualization and investigation. JW: methodology, analysis, and writing—original

draft. JW, FX, NS, and ZX: writing—review and editing. All authors contributed to the development of this manuscript and read and approved the final version.

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Effects of Dental Implants and Nutrition on Elderly Edentulous Subjects: Protocol for a Factorial Randomized Clinical Trial

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Background: Loss of masticatory function consequent to tooth loss has been associated with changes in food choices and insufficient nutritional intake. To date, interventions based on dental prostheses alone did not significantly improve nutrient intake. Pilot studies have shown positive impacts of interventions combining implant-supported fixed dental prosthesis with brief dietary advice. The relative contribution and the potential synergy of the components of such interventions need to be determined as it has major public health implications for the community-dwelling aging population that continues to disproportionately suffer from tooth loss and its consequences.

Objective: To assess the effect of rehabilitation of masticatory function with fixed implant supported dentures and nutrition education in older subjects with terminal dentition (stage IV periodontitis) or full edentulism.

Methods: A 2 × 2 factorial randomized controlled trial with 16-month follow-up of eligible adults (≥60 years) with loss of masticatory function consequent to full arch edentulism or terminal dentition (n = 120) will be conducted to test whether the rehabilitation of masticatory function with fixed implant supported dentures, nutrition education and/or their combination improves intake of fresh fruits and vegetables for aging subjects. The study has been designed to detect changes in fresh fruits and fresh vegetables intake at 4 months using the 24-h dietary recall method. Changes in protein as percentage of total energy, nutritional biomarkers, plasma metabolomics, oral and gut microbiome, quality of life and masticatory function will also be assessed.

Discussion: We hypothesize that receiving rehabilitation of masticatory function with fixed implant dentures together with nutrition education is the most effective intervention for improving nutrient intake in aging community-dwelling subjects with extensive tooth loss. The results of this study will assist in designing better treatment regimens, guide medical care for individual subjects, and inform public health and policy.

Clinical Trials Registration: NCT05334407.

Keywords: tooth loss, masticatory function and nutrition, diet, nutrition—clinical, healthy aging, randomized control trial (RCT)

INTRODUCTION

Over the course of life unmanaged caries and periodontitis, the most common diseases of mankind, lead to tooth loss and associated loss of quality of life and eventually compromised masticatory function. Older adults and aging subjects may be disproportionately affected (1). At the end of the disease spectrum, subjects with complete tooth loss (edentulism) or presence of only few remaining teeth that do not enable adequate chewing function show changes in their food choices and seem to prefer softer diets with higher carbohydrates and fat and less fresh fruits and vegetables (2, 3).

Accumulating evidence points to the presence of an association between changes in dietary behavior consequent to tooth loss and insufficient nutrition intake (4–6). A recent systematic review indicated that subjects lacking a functional dentition had a 21% increased likelihood of being at risk of malnutrition or being malnourished (7). Such impaired nutrition may have long term effects on muscle strength and physical decline and be detrimental to general health (8, 9). Indeed, the recent Global Burden of Disease study of dietary risk factors identifies 15 important disease associated exposures. Their analysis shows that 5 of the health associated exposures: consumption of fruit, vegetables, whole grains, nuts, and fiber require a good level of mastication (10).

A recent systematic review has addressed the efficacy of tooth replacement with dental prostheses and identified clear benefits in terms of restoration of masticatory function (11). Among fully edentulous subjects, greater benefits have been observed with dental implant retained prostheses with respect to conventional dentures.

While the physiology of mastication is an essential component of alimentation and contributes to the broader process of nutrition, recent research has focused on the nutritional benefits of tooth replacement to better focus the relevance of oral health on general health. Several studies have tried to improve the nutrient intake among edentulous individuals with various types of dentures. However, this goal has been elusive for interventions based on either complete dentures or implant-retained overdentures, given the functional limitation on these prostheses and perhaps the lack of concomitant dietary intervention (12–15). A small-scale case series has shown that implant-supported fixed prosthesis resulted in more efficient mastication and improved nutrient intake compared with conventional and implant-based removable dentures in partial edentulism (16).

Within dentistry, the long-held assumption that restoration of masticatory function alone—i.e., without dietary re-education intervention—brings nutritional benefits is being questioned. Sparse evidence points to the positive impact of nutrition counseling on the dietary intake of edentulous subjects receiving dental prostheses: brief dietary advice has been advocated to help patients take full advantage of the enhanced masticatory function to improve their diet (17, 18). Ellis et al. further showed that the impact of dietary advice on patient's satisfaction with dentures and oral health-related quality of life depends on the nature of the prosthesis (19). A recent systematic review on the impact of oral rehabilitation coupled with dietary advice on nutritional status has indicated that in most studies the dietary interventions were not theory based and poorly described (20). Not unexpectedly, the meta-analysis found only a trend toward significant changes in fruit and vegetable consumption and marked heterogeneity among the included pilot case series. No trial has been performed to assess the benefit of dietary advice alone or the combined effect of re-establishment of masticatory function with an implant-supported fixed prosthesis and dietary advice in edentulous elderly subjects. Understanding the relative contribution of restoration of masticatory function and nutrition education is critical to design effective interventions and improve public policy related to nutrition and prevention of physical decline in aging populations.

Based on the current equipoise about the relative contribution of dental and dietary interventions and the clinical and public health relevance of defining appropriate interventions to improve nutrition of older adults with extensive tooth loss, this protocol describes a 2 × 2 factorial clinical trial to assess the effect of rehabilitation of masticatory function with fixed implant supported dentures and/or brief nutrition education on the dietary intake and nutrition in older subjects with terminal dentition (stage IV periodontitis) or full edentulism. The clinical trial is being implemented. The effectiveness of the intervention will be validated during the trial. Results will identify the relative importance and optimal sequence of dental and dietary interventions, providing critical information with major implications for caring of individual subjects and for public health and policy. The results of the clinical trial will be available in 2 years.

METHODS AND MATERIALS

Study Design

This protocol has been prepared according to the SPIRIT guideline for clinical trial protocols (21).

Study Overview

The study is designed as a factorial randomized controlled clinical trial testing the benefits of dental and/or dietary interventions on changes in fresh fruits and fresh vegetables intake at 4 months (**Table 1**). Group A (DE+/DI+) will receive the full treatment regimen. Group B (DE+/DI–) will receive implant-supported fixed prosthesis at first and nutrition education after a 4-month waiting period. Group C (DE–/DI+) will receive the nutrition education at first and implant treatment after a 4-month waiting period. Group D (DE–/DI–) will receive the same treatment as Group A after a 4-month waiting period (**Figure 1**). The waiting period is equal to the current waiting list in the department. Subjects will be followed for an additional 12-month period. All procedures will follow the principles of

Abbreviations: DE, dental intervention; DI, dietary intervention; HbA1c, glycated hemoglobin; CRF, case report form; CBCT, Cone beam computed tomography; hs-CRP, high-sensitivity-C-reactive protein; TNF-α, tumor necrosis factor-α; IL-1β, Interleukin-1β; IL-6, Interleukin-6; Co Q10, coenzyme Q10; LS-MS, liquid chromatography-mass spectrometry; HDL, high density lipoprotein; LDL, low density lipoprotein; HBM, health belief model.

TABLE 1 | Overview of the schedule of enrolment, interventions, and assessments.

	Study period						
	Enrolment	Allocation	Post-allocation				Close-out
Timepoint	**−7 ± 7 d**	**0**	**−0 ± 7 d**	**4 m ± 14 d**	**8 m ± 14 d**	**12 m ± 14 d**	**16 m ± 14 d**	
Intervention*			**DE**	**DI**	**DE**	**DI**		
Enrolment								
Eligibility screen	X							
Informed consent	X							
Demographics	X							
Medical history	X							
Concomitant medications	X							
Allocation		X						
Interventions								
[Group A:DE+/DI+]			X	X				
[Group B:DE+/DI–]			X			X		
[Group C:DE–/DI+]				X	X			
[Group D:DE–/DI–]					X	X		
Assessments								
24-h dietary recall	X			X				
food-frequency questionnaire	X			X	X	X	X	
Anthropometric measurement	X			X	X	X	X	
Masticatory function	X			X	X	X	X	
Peri-implant soft tissue condition				X	X	X	X	
OHIP-14	X			X	X	X	X	
Blood sample	X			X	X	X	X	
Saliva sample	X			X	X	X	X	
Subgingival plaque sample	X			X	X	X	X	
Stool sample	X			X				
Nutritional status	X			X	X	X	X	
Muscle strength	X			X	X	X	X	
Cognitive function	X			X	X	X	X	
Depressive symptoms	X			X	X	X	X	

**Interventions included DE (dental intervention) and DI (Dietary Intervention).*

the Declaration of Helsinki on experimentation involving human subjects, all subjects will provide written informed consent. The trial has been approved by the Institutional Review Board of the Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (Approval No. SH9H-2021-T321-3) and is registered in ClinicalTrials.gov (NCT05334407). The trial will be independently monitored by the office of clinical research of the Shanghai Ninth People's Hospital. Any modifications to the protocol with impact on the conduct of the study will require a formal amendment to the protocol. The SPIRIT summary of the trial procedures is illustrated in **Supplementary Table 1**.

Recruitment

Older subjects (≥60 years of age) with full arch edentulism or terminal dentition seeking care at the Dept. of Oral and Maxillofacial Implantology of Shanghai Ninth People's Hospital will be screened and invited to participate while attending new patient clinics.

Eligibility Criteria

All potential participants will be assessed for eligibility based on the inclusion and exclusion criteria.

Inclusion Criteria

- Being edentulous or having a terminal dentition (22) and accepted treatment plan for fixed implant-supported prosthesis restoring at least 10 pairs of occluding teeth.
- Self-reported inadequate fresh vegetables or fresh fruits or protein foods intakes (daily intake thresholds based on the Chinese Dietary Guidelines for the Elderly recommendations).
- Understanding written and spoken Chinese and ability to respond to Chinese questionnaires.
- Able and willing to give informed consent for participation in the study.
- Able and willing to comply with 12-month follow-up.

Exclusion Criteria

- General and local contraindications to implant-supported immediate-loading fixed prosthesis.

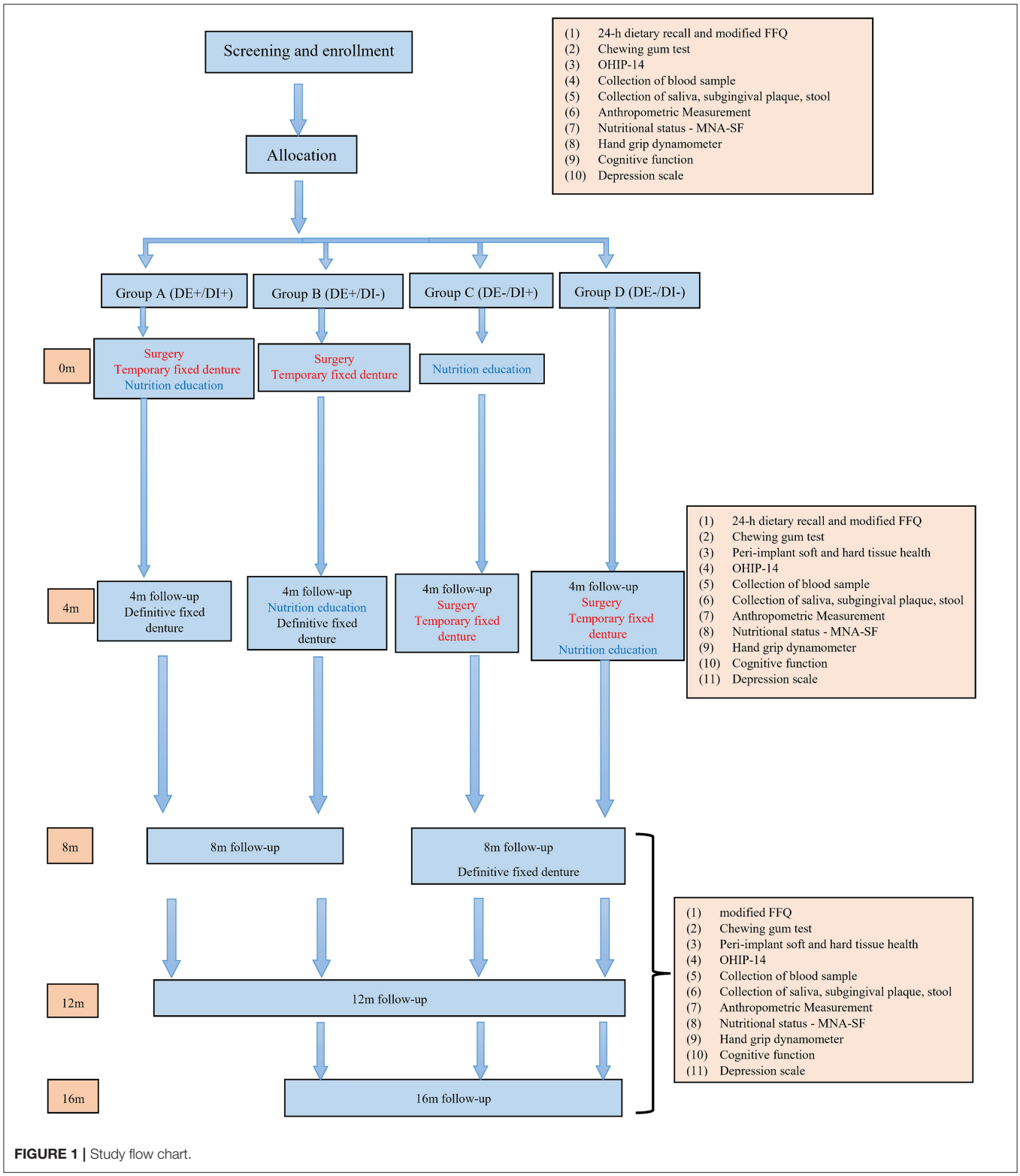

FIGURE 1 | Study flow chart.

- Looking for replacement of existing implant-retained overdenture with implant-supported fixed denture treatment.
- Presence of infectious disease, acute or chronic symptoms of TMJ disorder.
- Psychiatric disorder, dementia.
- Any dietary restriction, currently taking nutrient supplements or inability to choose his/her diet.

- Uncontrolled diabetes (HbA1c $\geq$ 7.0%).
- Self-reported heavy smokers (>10 cigarette/day).

Screening

Screening evaluations for this study will be performed in the context of routine patient evaluation in the clinic. The investigators will approach consecutive patients with the condition for possible inclusion in the study. During screening the investigator will also verify eligibility criteria. Additionally, social media (WeChat & Weibo) advertisement will be utilized to help recruit study participants. For some of these subjects, initial screening will be performed by phone. Following the telephone screening, the potential subjects will be invited for clinical screening evaluation.

Enrollment

Subjects fulfilling the inclusion and exclusion criteria will be invited to participate in the study and receive an explanation of the study, its objectives, benefits and risks by the investigator in the context of informed consent. The following information will be collected and recorded after the inclusion of the participants.

(1) Demographics: date of birth, gender, education, ethnicity, family income and anthropometric measures.
(2) Lifestyle factors: smoking (including tobacco consumption and smoking history), drinking habits and oral hygiene habits using the items from the Fourth National Oral Health Survey Questionnaire in Mainland of China (23) will be recorded.
(3) Health literacy: oral health literacy will be recorded using the Chinese version of the Short-Form Health Literacy in Dentistry (HeLD) scale (24) and nutrition literacy using the Nutrition Literacy Questionnaire for the Chinese Elderly (25).
(4) Medical History: Details of medical, including diabetes mellitus, cardiovascular diseases and other systemic diseases will be recorded. For subjects with diabetes, levels of glycated hemoglobin (HbA1c) will be obtained from the patients' medical records.
(5) Concomitant Medications: All over the counter or prescription medication, vitamins, and/or herbal supplements will be recorded on CRFs.

Randomization

Subjects will be randomly assigned to one of four groups with a 1:1:1:1 ratio by stratified block randomization. The block size will be 8 and stratifying factors will be diabetes status and smoking. Subjects will be registered into the study by a study registrar who will assign the treatment number and organize the sequence of the bookings of the patient according to the random allocation. The registrar will not be involved in any other study procedures.

Blinding and Allocation Concealment

Timing of treatment will be concealed to the therapists and to the examiners. Two separate masked therapists will perform the dental or the nutritional interventions. All laboratory assessments will be performed blindly.

Removal and Withdrawal Criteria

Those who have been selected for this trial and fall into one of the following circumstances are regarded as removed cases.

(1) Violation of important entry criteria;
(2) Receiving no study interventions;

Each participant has the right to withdraw from the study at any time. In addition, the investigators may discontinue a participant from the study at any time if the investigators consider it necessary for any reason including:

(1) Best interest of the patient.
(2) Ineligibility (either arising during the study or retrospective having been overlooked at screening).
(3) Significant protocol deviation.
(4) Significant non-compliance with treatment regimen or study requirements.
(5) An adverse event which requires discontinuation of the study or results in inability to continue to comply with study procedures.
(6) Inability to continue to comply with study procedures (e.g., moving to another city).
(7) Consent withdrawal.
(8) Loss to follow up.

If any subjects withdrawn from the study, no particular observation or treatment would need to continue. Subjects would be replaced if anyone withdrawn after the study started.

Regardless of the reason, complete clinical data should be retained for subjects who withdraw from the trial. The reason for withdrawal or early termination will be recorded in the CRF (case report form). If the participant is withdrawn due to an adverse event, if any, the investigator will arrange for follow-up visits until the adverse event has resolved or stabilized.

Study Intervention

Treatment of Trial Participants

The treatment process will include provision of implant-supported full-arch fixed prostheses (dental intervention, DE) and dietary intervention tailored to the dental status (dietary intervention consisting of nutrition education, DI). Subjects will be randomized to either a waiting period or treatment regarding dental intervention and dietary intervention. Ethical justification for the waiting period comes from the current waiting list in the regular care at the hospital. In order to improve the compliance with the protocol, all subjects participating in the study will have an alert in their patient record identifying them as participants to this protocol to alert administrative staff on the need to follow a stringent timing of follow-up appointments.

Dental Intervention

All participants will receive implant-supported full-arch fixed prostheses in at least one jaw (26, 27) and appropriate treatment in the opposing jaw regarding periodontal disease, caries, replacement of missing teeth and soft tissue disorders to get at least 10 pairs of occluding teeth (22).

Before surgery, a treatment plan will be made according to the clinical examination, study model and the CBCT data. Pre-surgery mock-up will be produced to guide implant placement and to facilitate the fabrication of the immediate prosthesis. A surgical guide with tooth set-up will be used for implant placement and bite registration, as needed.

After administrating local anesthesia any remaining tooth will be exacted atraumatically and the sockets will be carefully curetted. A crestal incision will be made and a full thickness mucoperiosteal flap will be reflected. For the preparation of the osteotomy site for implant placement, a modification of the drilling protocol according to the manufacture's recommendation will be followed as needed for immediate placement in case of the presence of residual teeth/roots. Tapping may not be used depending on the bone density to ensure primary stability of the implant. After the site preparation, 4–8 Nobel Active® implants (Nobel Biocare, Goteborg, Sweden) will be placed. Multi-Unit abutments (Nobel Biocare, Goteborg, Sweden) will be placed onto the implants. The abutment will be tightened with a torque of 35Ncm for straight multi-unit abutments and 15 Ncm for angulated multi-unit abutments. Healing caps will be placed on the abutments to support the peri-implant mucosa. The flap will be closed with a 5-0 resorbable suture (Vicryl, Johnson & Johnson Medical, Pomezia, Italy). Then, splinted impression will be taken at abutment level using an individual open tray. Pre-surgery mock-up or surgery guide with tooth set-up will be used to register the occlusal relationship. Patients will receive amoxicillin (Xinya Co, 500 mg, 3 times/day for 7 days). Decongesting nasal drops (phenylephedrin, 0.1 ml, 3 times/day for 3 days) will be prescribed if sinus elevation will be performed. Mouth rinsing with chlorhexidine 0.12% three times per day and modified oral hygiene procedures will be prescribed for the first 2 weeks of healing (sutures still in place).

A screw-retained, metal-reinforced, acrylic resin interim restoration will be delivered within 24 h of surgery. All centric and lateral contacts will be assessed and modified, until occlusal contacts are uniformly distributed on the entire prosthetic arch. Sutures will be removed at 2 weeks. After a healing period of 4 month, a definitive screw-retained, full-arch prosthesis will be delivered.

Brief Nutrition Education

The nutrition education will be conducted based on the health Belief Model (HBM) (28) addressing perceived susceptibility and severity of lacking the targeted behavior, perceived benefits and barriers of carrying out the targeted behavior, cues to action, and self-efficacy. With behavioral goals being increasing an individual's likelihood of food intake regarding fresh vegetables, fresh fruits, and high-quality protein foods (i.e., poultry, meat and aquatic product), the nutrition education session has been designed to be culturally tailored.

Participants will receive a 20-min coordinated nutrition education in the form of a slideshow presentation by a nutritionist in the clinical setting. On completion they will receive a copy of a pamphlet prepared in three parts (overall dietary goal, recipe examples mainly composed of softer and easy-to-chew food, and recipe examples composed of various food without restriction on the texture). The advice has been compiled with reference to the 4th edition of Dietary Guidelines for Chinese Elderly Residents (2016) by the Chinese Nutrition Society (29) that will be given to the participant separately. If a participant does not prepare his or her own meals, the person who does the cooking receives the dietary advice as well. A dietary checklist aiming to evaluate the compliance will be delivered with the pamphlet and patients will send it back after 1-week's recording.

Measurements and Outcomes

Timing of Assessment

Study assessments will be performed at baseline, 4, 8, and 12 months unless otherwise stated below. For Group B, C and D, an additional assessment will be performed at 16-month follow-up.

Food and Nutrient Intake

The primary outcome measure will be changes in intake of fresh fruits and fresh vegetables measured at 4 months using the 24-h dietary recall method. Protein% of total energy will also be calculated. Three 24-h dietary recall will be conducted through face-to-face interview, twice on weekdays and once on weekend. The data on food consumption will be converted into the corresponding nutrient contents based on the 6th version of China Food Composition Tables Standard Edition. Moreover, a modified simplified food-frequency questionnaire (FFQ) of 33 food group items (30) will be conducted at baseline, 4, 8, 12, and 16 months.

Masticatory Function

Masticatory function will be assessed at baseline (before treatment) and 4, 8, 12 and 16 months after insertion of a fixed implant retained prosthesis using the quantitative method described by Schimmel et al. (31) as previously described (32). In brief, subjects will be asked to mix a two-color chewing gum with 20 masticatory cycles. The obtained bolus will be pressed to a standardized height and a color image will be acquired. Quantitative data will be obtained by digital analysis of the image using variance of hue as the outcome.

Peri-Implant Soft and Hard Tissue Health

Peri-implant soft tissue condition will be measured by periodontal probing (UNC/CP-11.5B Screening Color-Coded Probe, Hu-Friedy, Chicago, IL, USA). Modified plaque index (mPI), probing depth (PD), and modified bleeding index (mBI) will be evaluated (33). Standardized panoramic radiographic imaging will be conducted to assess the peri-implant bone level. The assessment will be performed at 4-, 8-, and 12-month post-surgery.

Oral Health Impact Profile (OHIP)-14

The oral health impact profile (OHIP)-14 (34) will be administrated to assess the impact of oral health on the quality of life of participants using a validated Chinese translation of the instrument (35).

Biological Samples

Biological samples will be collected and processed in a standard way by dedicated study personnel blind with respect to treatment

status. The blood sample collection will be scheduled at 8 a.m.-9 a.m. Patients will fast overnight (12–14 h) prior to blood collection. Subjects will be advised to avoid strenuous exercise 1 h prior to collection. Samples will be processed at the clinical research center laboratory to meet the preservation standards for the assay of each marker and will be either assayed immediately or stored at −80°C in the Shanghai Ninth People's Hospital Biobank facility for later analysis.

Metabolic and Inflammatory Biomarkers

The following biomarkers will be assessed by a specialized GCP approved clinical pathology laboratory.

a) Blood serum concentration of homocysteine.
b) Plasma hs-CRP, TNF-α, IL-1βand IL-6.
c) Co Q10, Uric acid and superoxide dismutase.
d) Blood lipids (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and Lpa).

Plasma Nutrient Biomarkers

Nutrient biomarkers will be assessed at baseline, 4, 8, 12, and 16-month follow-up by an accredited laboratory according to international standards.

a) Plasma vitamins A, B2, B12, folate, C and E will be measured by liquid chromatography-mass spectrometry (LS-MS).
b) Plasma carotenoids (α- and β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin) and tocopherols (α- and γ) will be measured by LS-MS.

Plasma Metabolomics

a) Metabolites will be profiled with untargeted metabolomics using liquid chromatography coupled with mass spectrometry (36).
b) Oxylipin changes will be assessed by oxidative lipidomics (37).

Oral and Fecal Microbiome

Oral rinse, subgingival plaque and fresh stool samples will be collected for 16S rRNA gene sequencing at baseline, 4, 8, 12, and 16 months.

Oral microbiome samples will be obtained by oral rinsing for 1 min with 5 mL of buffer solution (38, 39). Additionally, in dentate patients a subgingival plaque sample will be taken from the deepest periodontal pocket/lesion with a sterile paper point inserted to the depth of the pocket. The subgingival plaque sample will be collected after isolating the sampling area with cotton rolls gentle air drying, and supragingival plaque removal. Samples will be immediately stored at −80°C.

Sterile stool tube with a spatula inside will be given to the participants with detailed instructions on how to collect the specimen. Fresh stool samples will be collected by the participants at home the night before the visit day or the morning of the visit day (40). Samples will be stored in the patient's refrigerator at 4°C until submission. During transportation, samples will be kept on ice in a cooling bag.

Nutritional Status

Mini-nutrient status form (MNA-SF) (41) will be used to screen patients for risk of malnutrition at baseline, 4, 8, 12, and 16 months.

Muscle Strength

A hand grip dynamometer will be used to assess muscle strength at baseline, 4, 8, 12 and 16 months essentially as described (32).

Cognitive Function

Cognitive function will be assessed with the Mini-Mental State Examination (MMSE) (42) and the Ascertain Dementia 8 (AD8) questionnaire (43) at baseline, 4, 8, 12, and 16 months.

Depression Symptoms

Depressive symptoms will be assessed with the shortened Center for Epidemiologic Studies Depression Scale (CES-D10) (44).

Safety Evaluation

Adverse Events

The collective evidence from numerous clinical trials reveals consistent findings that the implant supported fixed full-arch prosthesis is a safe and effective treatment approach for terminal dentition or full edentulism. Dietary advice is also a safe intervention for edentulous elderly. No significant adverse events have been reported. Occasionally the patient may experience early implant failure and/or mechanical complications of the prosthesis. These will be recorded in the case report forms and will be managed according to standard of care with additional implant placement or refabrication/modification of the existing prosthesis.

Follow-Up for Adverse Events

Adverse events (AE), if any, will be managed according to current standard of care for the specific condition and will be reported to the ethics committee. The principal investigators will assess and manage the condition to the best of his knowledge and refer to specialist care if appropriate and in the best interest of the participant. AE will be considered resolved once the principal investigators concur that to be the case.

DATA ANALYSIS

Sample Size

The sample size has been determined based on the primary outcome: changes in the intake of fresh fruits and fresh vegetables. Based on the relevant studies where the average fruit and vegetable intake was about 255 ± 200 g per day in edentulous elderly (17), we assume that the average fruit and vegetable intake will increase 50 g, 70 g and 245 g in patients receiving dental prostheses alone, brief dietary advice alone, or the combination dental treatment with brief dietary advice, respectively. With an anticipated 20% loss to follow-up, 30 patients are needed in each group to test the significance of dental treatment, brief dietary advice, alone or in combination with alpha set at 0.05 and with 80% power. Due to limitations in the baseline knowledge for precise sample size calculations and the efforts required for an adequate pilot study, adaptive adjustment

of sample size will be performed. Sample size calculation will be re-estimated after obtaining the primary outcome of 10 patients in each group. Based on the interim analysis for adaptive design, several scenarios have been identified: (i) the original sample size estimation is appropriate and the study will be completed accordingly; (ii) the original sample size will be insufficient but will still be within the capability of recruitment of the study center, in such case the sample size will be expanded according to the adaptive design principles; (iii) the original sample size will be insufficient but too large for successful completion of the study at the study center alone, in such scenario the study will be completed as a pilot study with the original sample size and a multicenter trial will be designed and implemented based on the results.

Statistical Software and General Requirements

Data analysis will be performed in SPSS software, version 26.0 (IBM Corp., Armonk, NY, USA). The level of statistical significance will be set at 0.05 for all tests.

Statistical Analysis Plan

(1) Descriptive statistics will report the demographic, clinical and biological characteristics of the study population. Means with standard deviations (SD) or medians with interquartile range (IQR) will be used to describe continuous variables. Frequencies will be used to describe categorical variables.
(2) The normality of clinical and biological parameters at baseline and each re-evaluation visit will be tested for normality using the Kolmogorov-Smirnov test. The homogeneity of the clinical and biological parameters at baseline and each re-evaluation visit will be tested using Levene's test.
(3) ANOVA analysis will be used to test the effect of dental treatment, brief dietary advice, both dental treatment and brief dietary advice, sequence of two interventions for continuous variables with a normal distribution. The Kruskal–Wallis test will be used for non-continuous variables or continuous variables not normally distributed.

Data Quality and Assurance

All investigators involved in this study will be trained appropriately for the standard operating procedures including the questionnaire conduction, blood sampling and preservation, presurgical examinations and treatment (regarding periodontal diseases, caries, missing teeth and soft tissue disorders) and delivery of interventions.

(1) The 24-h dietary recall will be conducted by clinical nutrition specialists trained appropriately for the standardized interviewing procedures and assessment of dietary intake. The inter- and intra-examiner reliability with respect to the measurement of fresh foods and fresh vegetables intake will be assessed by the intraclass correlation coefficient (ICC). In order to ensure optimal inter- and intra-examiner reliability, ICCs needs to be more than 0.75.
(2) The therapists, who will deliver the dental intervention, will be experienced specialists in implant dentistry fulfilling the Shanghai requirements. Treatment will be provided to the satisfaction of the clinician and patient. For logistic reasons, five therapists will be included in this study.
(3) The investigator who performs dietary interventions will be trained in delivering a standardized dietary instruction session including the verbal instruction and demonstration of the pamphlet. In addition, the investigator will be trained in the use of questionnaires.

Regular monitoring for assuring protocol compliance, and data quality at the clinical site, including review of source documents and records, consent forms, etc will be performed by an investigator trained in both GCP and the specific procedures. Furthermore, the clinical research coordinator will audit the case report forms for the first few patients to ensure correct filling of forms. The study will also be monitored by the compliance office of the National Clinical Research Center of Oral Diseases and Clinical Research Center of the 9th People's Hospital.

Confidentiality

All trial-related data will be stored securely at Shanghai PerioImplant Innovation Center. The participant information will be stored in locked cabinets with limited access. All data will be anonymized by assigning a Research ID used for data collection and processing to maintain participant confidentiality. All records containing personal identifiers will be stored separately from study records identified by the research ID number.

DISCUSSION

Many older adults with severe tooth loss and masticatory dysfunction change their food choices and incorporate softer food with more carbohydrates and fats and depleted of essential micronutrients and fibers. They also progressively lose weight, become frail and dependent on others for their daily necessities. Replacement of missing teeth alone restores masticatory function but does not positively influence diet. Great attention is currently being paid to the combination of dental and dietary interventions. Their relative importance and optimal sequence, however, remain unknown. This lack of knowledge has far-reaching consequences in the design of optimal treatment regimens and testing their health benefits in definitive studies. The present study will provide critical information with major implications for caring of individual subjects and for public health and policy (45).

The design of this trial has posed significant challenges in terms of experimental design, choice of the population/condition, definition of the dental and the dietary interventions as well as the choice of outcomes. These will be briefly discussed following the PICOT format.

The selected 2 × 2 factorial randomized clinical trial design provides greater efficiency in terms of sample size while allowing testing of multiple clinically relevant questions on the relative effect size of dental and/or dietary interventions. The

incorporation of an adaptive design that will recalculate sample size after data will be available from a third of the planned subjects provides robustness to the approach even considering the possible imprecision of the preliminary data used for sample size calculation. While sample size assumptions have been piloted and confirmed in the specific patient population the approach offers added robustness against type II errors. This is particularly important given the high costs of rendering the treatment to this population and the consequent difficulty in properly funding a pilot trial. Specific a priori scenarios have been identified with regards to completion of the trial.

Tooth loss is frequently incremental over the course of life and subjects in the population present with a spectrum of severity of loss of masticatory function. This study will focus on the more severe end of the spectrum as these subjects are both likely to suffer from greater changes in diet and more likely to show improvements in masticatory function because of tooth replacement. It will also recruit aging subjects who represent most edentulous subjects. Additional studies expanding the observations to milder forms of edentulism will be needed. To ensure that subjects will suffer from both masticatory dysfunction and a degree of malnutrition, an inclusion criterion has been added in terms of verification of poor fruit, vegetable, or protein intake. Pilot nutritional analysis of edentulous subjects reporting for treatment in the specific setting has verified that most of them reported at least one aspect of impairment and fit the inclusion criteria. These aspects are important for the external applicability of the results of the trial and ongoing epidemiologic research will provide additional information.

The definition of both the dental intervention and the nutrition education are also notable. To address masticatory dysfunction this study will employ fixed dentures supported by dental implants—a well-defined intervention routinely performed in the specific setting—as these have been shown to provide better objective and subjective chewing benefits (11). The masticatory function will be restored to provide at least 10 occluding pairs of teeth, a number generally considered compatible with adequate function (22, 46).

HBM has been used in aiding behavior change intervention for decades, and it has been applied to Asian populations. With the HBM-based nutrition education, the objective is to motivate participants from the pre-contemplative stage to the contemplate stage, and even to preparatory stage with the materials provided. Combining with the dental intervention which will solve the physical barrier, the hypothesis is that participants will progress to the executive stage at home. During the follow-up period, the importance of dietary intake will be reinforced to help them to stay in the maintenance stage. The intervention, its instruments and their delivery have been tailored to local circumstances, evaluated, and revised in our pilot study before implemented for the trial. Details are presented in the online appendices as a potential resource for additional trials.

While the equipoise to justify randomization is strong, recruiting patients with edentulism/terminal dentition for a trial is challenging due to the severity of the condition and the impact on quality of life. The opportunity arises in the specific setting due to the waiting list for treatment that justifies the delay in the delivery of the care initially sought by the patient.

Lastly the choice of the primary outcome has been complex due to the limited previous information on clinically relevant outcomes and the need to maintain the size of this trial within the recruitment possibilities of the single center. The choice to focus on a proxy outcome—changes in fresh fruits and fresh vegetables consumption—as the primary outcome, rather than a health gain measure, is based on the need to establish the effectiveness of the treatment regimen and logistic considerations. The limitation of 24-h recall method in providing an accurate estimate of long-term energy intake has been realized. Thus, the study plans to combine food frequency questionnaires which replied on generic rather than specific memory to offer detailed assessment of the study period. Furthermore, the study plans to assess a wide palette of secondary outcome that will provide insight into mechanisms of a potential benefit by exploring both biochemical markers, metabolomics and changes in the oral-gut microbiome axis and functional quality of life instruments.

The relatively short follow-up time for the factorial design component of the study is adequate to assess the efficacy of the interventions. The 12-month extension is relevant as it will supply critical information about retention of subjects in the trial and medium-term compliance with the dietary intervention and effectiveness of the dental intervention. It will also provide the basis for future longer-term trials focusing on health outcomes.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (Approval No. SH9H-2021-T321-3). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MT conceived and designed the study. CY, H-CL, S-JQ, and J-YS contributed to the study design and protocol development. H-CL, BL, JS, S-CQ, YT, and KD assisted with preliminary analyses on the patient population, piloting of material, and preparation of study launch. XZ provided the sample size calculations and the statistical plan. S-JQ, JS, and BL drafted the manuscript based on the original protocol. All authors revised and approved the final version of the manuscript.

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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.2022.930023/full#supplementary-material

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OPINION
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Phosphate Burden and Organ Dysfunction

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INTRODUCTION

Phosphorus is a ubiquitous mineral in nature and one of the most abundant minerals in the human body, representing about 1% of the total body weight (Calvo and Lamberg-Allardt, 2015). The body utilizes phosphorus in the form of phosphate (PO_4). Phosphate maintains cellular membrane integrity, nucleic acid structure, generation of ATP, and key regulation of virtually every molecular pathway through phosphorylation or dephosphorylation of numerous enzymes and other proteins important for cell function and homeostasis. With so much utility, the body needs to maintain blood phosphate concentration at 2.5–4.5 mg/dl. The body maintains phosphate homeostasis via crosstalk among bone, kidney, and intestine. Phosphate enters the extracellular fluid pool and constantly moves in and out of bone to meet the body's needs (Razzaque and Lanske, 2007; Razzaque, 2009a; Penido and Alon, 2012). Bones are a major phosphate reservoir, releasing it *via* the enzymatic activities of alkaline phosphatase. Alkaline phosphatase is found on the outer portion of the cell membrane and is responsible for catalyzing hydrolysis of organic phosphate esters present in extracellular space, allowing for intracellular movement of phosphate (Penido and Alon, 2012). The kidneys also regulate phosphate homeostasis, with most reabsorption occurring at the proximal tubule. The rate-limiting step of this reabsorption is mediated by two type II transporters: sodium-dependent phosphate cotransporter (NaPiIIa and NaPiIIc), located on apical membranes of proximal tubule cells where these cells reabsorb a total of 80% of filtered phosphate (Penido and Alon, 2012). NaPiIIa reabsorbs about 50% of filtered phosphate load, and its expression is partly regulated by parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and dietary phosphate levels (Penido and Alon, 2012). NaPiIIc reabsorbs about 30% of proximal tubule phosphate and is regulated by FGF23, metabolic acidosis, dietary magnesium, and phosphate (Penido and Alon, 2012). Intestines absorb phosphate through various cellular and paracellular pathways, including passive diffusion, load-dependent processes, and active transport. Intestines regulate how much phosphate is absorbed into the bloodstream, and this process is controlled partly by vitamin D (Penido and Alon, 2012). Vitamin D-regulated expression of NaPiIIb brings phosphate into enterocytes via secondary active transport (Penido and Alon, 2012). PTH, by influencing the synthesis of vitamin D, indirectly regulates phosphate absorption in the duodenum and jejunum (Penido and Alon, 2012).

Numerous hormones are also involved in maintaining systemic phosphate homeostasis (Razzaque, 2022a). PTH acts on the kidneys to decrease phosphate reabsorption and increases the production of 1α-hydroxylase, which catalyzes the hydroxylation of calcifediol into calcitriol (the bioactive form of vitamin D). Increased production of $1,25(OH)_2D_3$ (active vitamin D) enhances both calcium and phosphate absorption in the gut and also determines the extent of phosphate reabsorbed in the proximal tubule of the kidney via suppressing PTH activity (Penido and Alon, 2012). FGF23 increases renal excretion of phosphate and inhibits the synthesis of $1,25(OH)_2D_3$ in attempts to lower serum phosphate concentrations (Prié and Friedlander, 2010; Agoro et al., 2020; Akimbekov et al., 2022; Nakatani et al., 2022). Renal excretion is accomplished by decreasing NaPiIIa

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and NaPiIIc protein expression levels (Prié and Friedlander, 2010). For FGF23 to be functional and lower serum phosphate levels, it needs Klotho, which increases FGF23's affinity to its receptor at the target organs (Urakawa et al., 2006). Klotho is a single-pass transmembrane protein in the renal tubules, parathyroid gland, brain, and skeletal muscle (Prié and Friedlander, 2010). Klotho acts as an obligate cofactor for FGF23 binding and activation of cognate FGF receptors (Urakawa et al., 2006). The absence of FGF23 or Klotho leads to hyperphosphatemia and resulting premature aging features in mice (Razzaque et al., 2006; Nakatani et al., 2009a; Nakatani et al., 2009b). These features include but are not limited to loss of body weight, kyphosis, hypogonadism, infertility, generalized tissue atrophy, and reduced life span (Ohnishi and Razzaque, 2010), many of these alteration parallel potential aging sequelae.

Phosphate Burden and Inflammation

Recent studies have found that phosphate burden can lead to the activation of inflammatory responses to propagate gingival inflammation and dental decay among children (Goodson et al., 2017; Goodson et al., 2019; Erem et al., 2022; Michigami et al., 2022). An increased salivary phosphate concentration has been associated with higher inflammatory markers and could predict childhood obesity (Hartman et al., 2013; Razzaque, 2022b). Hyperphosphatemia has associations with immune dysfunction. According to Plantinga et al., high phosphate levels early during dialysis were associated with an increased risk of infection when adjusting for secondary hyperparathyroidism, uremia, or poor dialysis (Plantinga et al., 2008). Patients with end-stage renal disease (ESRD) poorly respond to immunizations against pathogens, have impaired cell-mediated immunity, and reduced $CD4^+/CD8^+$ T lymphocyte ratio (Plantinga et al., 2008). Decreases in T lymphocyte numbers are likely due to increased oxidative stress and accumulation of uremic toxicity, both are features of ESRD (Plantinga et al., 2008). Investigators also discovered a negative correlation between hyperphosphatemia severity and a number of naive subsets of T lymphocytes, raising the possibility that hyperphosphatemia plays a role in reduced numbers of T cells seen in ESRD (Plantinga et al., 2008).

One of the many parameters categorizing aging is the functional decline of the healthy immune system, leaving the older population more susceptible to pathogens causing bacterial pneumonia and influenza (Sosa et al., 2020). Sosa et al. found pro-inflammatory cytokine expression higher in aged mice, with 40% serum phosphate levels beyond those of their counterparts. These cytokine values were decreased when they were fed a low phosphate diet (Sosa et al., 2020). They also found a positive correlation between Interleukin-1β (IL-1β) expression and serum phosphate levels, effectively showing hyperphosphatemia increases inflammation *in vivo* (Sosa et al., 2020). IL-1β is an essential factor for acute host responses and resistance to pathogens, while exacerbating damage during chronic disease and acute injury (Lopez-Castejon and Brough, 2011). Dietary phosphate loading promotes systemic inflammation and oxidative stress measured by serum Tumor necrosis factor alpha (TNF-α) and urinary 8-hydroxy-2′-deoxyguanine to creatinine (8-OHdG/Cr) levels; 8-OHdG is a metabolite of DNA repair and is measurable in the urine (Yamada et al., 2014). TNF-α is released by macrophages or monocytes and has many functions including necrosis or inflammation (Besse et al., 2022). Dietary phosphate loading increases TNF-α in a dose-dependent fashion; serum TNF-α levels were significantly correlated with urinary 8-OHdG/Cr levels (a measure of oxidative stress) (Yamada et al., 2014). The investigators also observed a decrease in TNF-α and OHdG/Cr when lowering dietary phosphate intake or reducing serum phosphate levels (Yamada et al., 2014). This may also be one way to explain the chronic low grade of inflammation found in the elderly with altered phosphate balance. Although intermittent inflammation is needed for survival during infections and physical injury, chronic systemic inflammation is detrimental to human health (Pinti et al., 2014). Chronic inflammation increases the incidence of many diseases in the elderly, such as cardiovascular disease (CVD), cancer, diabetes mellitus, chronic kidney disease (CKD), autoimmune, and neurodegenerative disorders (Furman et al., 2019). High phosphate burden also increases inflammatory responses in experimental studies, as demonstrated by Yamada et al., who found that increasing phosphate loads in the body led to increased mRNA levels of TNF-α in the aorta, heart, and kidney in rats (Yamada et al., 2014). Cancer patients are also known to experience increased phosphate burden compared to non-cancer patients, most likely due to increased metabolic activity of cancer cells. Elevated phosphate burdens have been shown to positively correlate with the risk of lung, pancreas, thyroid, and bone cancers in men, and cancers of the esophagus, lung, and nonmelanoma skin cancer in women (Brown and Razzaque, 2018). Studies have shown that elevated phosphate levels can induce epithelial to mesenchymal transition (EMT), a major cellular event related to tumor invasion and metastasis (He et al., 2021; Alexander et al., 2022; Lewis et al., 2022).

Phosphate Burden and Aging

Aging is a complex biological process where progressive accumulation of age-associated changes with time are associated with or directly responsible for the increased susceptibility to disease and death, which accompanies advancing age (Boss and Seegmiller, 1981; Ohnishi and Razzaque, 2010). It decreases cardiac output by 1% a year after 30, mostly due to reduced response of catecholamines and cardiac glycosides by cardiac muscle cells (Boss and Seegmiller, 1981). Blood pressure increases as there is progressive stiffening of arteries with age, particularly in the aorta, increasing afterload or the load against which the heart has to contract to eject blood (Boss and Seegmiller, 1981). Natural lipid deposits in vessels increase the risk for arteriosclerosis and coronary artery disease. Decreases in lung volume and elastic recoil leads to an increase in residual volume, which is the volume of air that cannot be exhaled from the lungs (Boss and Seegmiller, 1981). Decreasing elastic recoil causes a greater tendency for airways to collapse, resulting in ventilation-perfusion mismatches (Boss and Seegmiller, 1981). Kidney size and glomeruli number decrease by about 30% by age 65 (Boss and Seegmiller, 1981).

Many of these age-associated changes are not pathologic but stack the odds towards pathology.

Hyperphosphatemia is most often caused by renal failure, as the kidneys excrete up to 90% of daily phosphate, leaving the other 10% to the gut (Goyal and Jialal, 2022). High phosphate levels are caused by its high intake, vitamin D intoxication, and several genetic diseases. Potential symptoms associated with hyperphosphatemia are hypocalcemia due to calcium-phosphate precipitation in the skin and soft tissues, vascular calcifications, and arteriosclerosis. High phosphate levels manifest with central nervous system disturbances such as coma, seizures, delirium, neuromuscular excitability, muscle cramping, tetany, and eventual cognitive decline (Acquaviva et al., 2022; Goyal and Jialal, 2022). It leads to cataracts and conjunctivitis in the eye from induction of symptomatic hypocalcemia due to calcium-phosphate precipitation (Goyal and Jialal, 2022). Renal failure results in reduced synthesis of calcitriol and secondary hyperparathyroidism, causing increased osteoclastic bone reabsorption and release of calcium and phosphate into the circulation and this lengthened bone demineralization leads to increased occurrences of fractures (Goyal and Jialal, 2022). Hyperphosphatemia induces changes in endothelial cells, such as declines in nitric oxide (NO) production due to oxidative stress, thereby leading to reduced cell viability and increased apoptosis (Peng et al., 2011). High phosphate levels lead to endothelial cell senescence via cell cycle arrest, thereby leading to senescence rather than death via apoptosis (Terzi et al., 2016; Olmos et al., 2017; Maique et al., 2020; Hu and Moe, 2022).

Aging is a process that is characterized by increased susceptibility of individuals, as they age, to factors that eventually lead to their morbidity and mortality (Weinert and Timiras, 2003; Jayanthi et al., 2010). As individuals age, they have progressive loss of tissue and organ functions, leading to the development of the oxidative stress theory of aging (OSTA) hypothesis. OSTA suggests the aging rate is directly related to the accumulation of oxidative damage (Salmon et al., 2010). It is based on structural damage resulting from the accumulation of oxidative damage to macromolecules (DNA, protein) via reactive oxygen (ROS) and nitrogen (RONS) species. High ROS levels over a long period activate signaling pathways, which accelerate proteolysis and eventual cell death (Barreiro, 2016). In a study performed by Nagai et al. using Klotho deficient mice, the investigators demonstrated that hyperphosphatemia resulted in cognition impairment due to increased oxidative damage and apoptosis in hippocampus neurons, which could be rescued by administering an antioxidant (Nagai et al., 2003).

Hyperphosphatemia leads to extensive oxidative stress in the mitochondria, although it is unclear how phosphate increases ROS generation and mitochondrial permeability transition (MPT). The most conceivable hypothesis is that phosphate catalyzes reactions that favor ROS formation (Kowaltowski et al., 2001). MPT is one of the ways mitochondria release apoptotic signal molecules into the cytosol. MPT causes non-selective increased permeability of the inner mitochondrial membrane resulting in loss of matrix components, swelling, and eventual rupture and cytochrome C release (Zoratti and Szabò, 1995). Zhao et al. have found that hyperphosphatemia induced calcification with oxidative stress of mitochondria (Zhao et al., 2011). A decline in mitochondrial function has long-held associations with an increase in features of aging (McGuire, 2019). Such changes lead to programmed cell death or apoptosis. Long-term exposure to high phosphate levels potentiates aging and age-related disorders (Ohnishi and Razzaque, 2010; Jacob et al., 2013; He et al., 2021; Hetz et al., 2021).

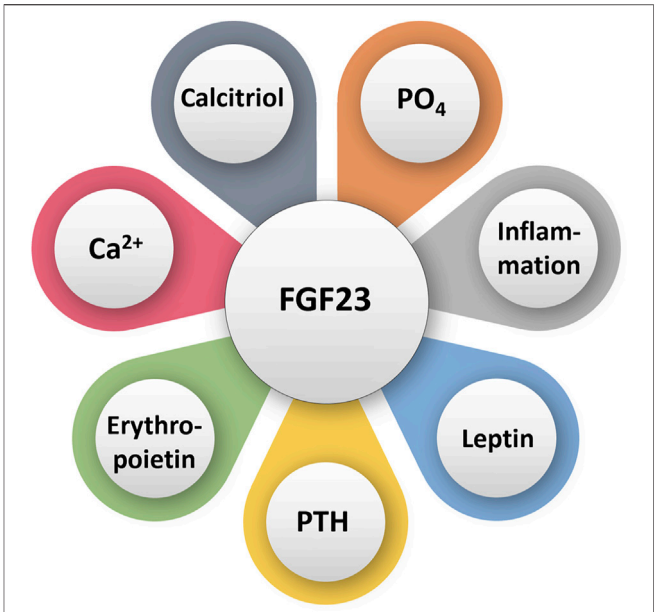

FIGURE 1 | Simplified diagram illustrating various factors that can directly or indirectly influence FGF23 activities. PO_4: phosphate; Ca^{2+}: calcium.

Phosphate Burden and Cardiovascular Pathology

Hyperphosphatemia impairs endothelial cell function through endothelin-1 and NO imbalances leading to dysfunction of the endothelium, an important step in the pathogenesis of atherosclerosis which can impair functionality of all the organs, including renal and cardiac functions (Olmos et al., 2017). High phosphate levels caused a decline in NO production *via* bradykinin and increased ROS, thereby leading to endothelial dysfunction (Peng et al., 2011). Hyperphosphatemia also reduced intracellular calcium levels, increased protein kinase C-B_2, increased apoptosis, and reduced cell viability (Peng et al., 2011). Hyperphosphatemia accelerated vascular aging by collagenization of the tunica media in the walls of arteries, with phosphate and calcium crystals accumulating in the elastic fibers of the vessel (Boss and Seegmiller, 1981).

FGF23 is a hormone that lowers blood phosphate levels (Razzaque and Lanske, 2007; Razzaque, 2009a; Razzaque, 2009b). When phosphate levels are high, bone secretes FGF23,

which acts on the kidney to increase the excretion of phosphate (Pinti et al., 2014) (**Figure 1**). FGF23 also suppresses vitamin D synthesis by inhibiting cytochrome P27B1 and stimulating cytochrome P24 to reduce the levels of $1,25(OH)_2D_3$ (Pinti et al., 2014). Vitamin D increases the absorption of calcium and phosphate in the intestines and mobilizes bone tissue via enhancing osteoclastic activities to increase blood levels of phosphate and calcium (Furman et al., 2019). Severe hyperphosphatemia is induced in human diseases where FGF23 is mutated (Benet-Pagès et al., 2005; Onishi et al., 2008; Chakhtoura et al., 2018). FGF23 gene deletion from mice resulted in hyperphosphatemia (Sitara et al., 2004), thus solidifying the role of FGF23 in reducing serum phosphate levels. The mouse with nonfunctioning FGF23 had lower lifespan than the wild-type counterparts. This decrease in lifespan was partially due to generalized tissue and organ atrophy and vascular calcifications. Hyperphosphatemic mice also had lower adipose and skeletal muscle mass than mice with normal phosphate levels, further attesting to the accelerated aging in these mice (Sitara et al., 2004). Finally, FGF23 deficient mice were also infertile, hypoglycemic, and had increased total serum cholesterol (Shimada et al., 2004; Sitara et al., 2004).

Another gene that regulates phosphate homeostasis and is critical for FGF23 function is Klotho. Both Klotho and FGF23 deficient mice consistently display signs of premature aging and CKD-associated with mineral and bone disorders. Klotho deficient mice are completely resistant to FGF23 and thus develop hyperphosphatemia (Nakatani et al., 2009a). Some of the symptoms seen in these mice have shorter lifespan, infertility, arteriosclerosis, skin atrophy, and emphysema (Nakatani et al., 2009b; Ohnishi and Razzaque, 2010). It is important to note that the Klotho deficient mice could be rescued from all symptoms by reducing phosphate levels towards the normal ranges (Ohnishi and Razzaque, 2010). CKD and its complications, such as vascular calcification, CKD-mineral and bone disease (MBD), all result from a Klotho deficiency, which manifests as accelerated aging due to phosphate burden.

Hyperphosphatemia leads to vascular dysfunction through endothelin 1 and NO imbalances. A study by Foley et al. have found evidence for hyperphosphatemia increasing incidence of cardiac calcification, left ventricular hypertrophy, and cardiovascular events, including deaths, were all accelerated with hyperphosphatemia (Foley, 2009; Foley et al., 2009). Although the reason is not entirely mapped out yet, one thought could be via a mechanism other than vascular calcification. It could be Klotho or FGF23 dysfunctions, which, as previously discussed, have shown to lead to CVD in mice. Hyperphosphatemia changes the amount of Klotho, FGF23, PTH, and calcitriol in the body, therefore increasing CVD incidence (Foley, 2009). Hyperphosphatemia increases CVD risk in individuals who do not have CKD and CVD (Dhingra et al., 2007). Phosphate burden (higher than 3.5 mg/dl) was associated with 55% increased CVD risk. This could be because high phosphate levels inhibit vitamin D synthesis; low levels of vitamin D have been hypothesized to decrease cardiac contractility and vascular dysfunction (Dhingra et al., 2007). High phosphate levels have also been found to induce endothelial cell dysfunction via lowering NO levels and intracellular calcium levels, and attendant apoptosis and reduced cell viability (Olmos et al., 2017). It is important to note that sevelamer carbonate, a phosphate scavenger, improved endothelial function and reduced mortality in patients with type 2 diabetes mellitus and inflammation in patients on peritoneal dialysis for kidney failure (Chennasamudram et al., 2013). Sevelamer, which binds phosphate in gut and prevents absorption, improved endothelial function and decreased plasminogen activator inhibitor 1, C-reactive protein, and IL-6 (Chennasamudram et al., 2013). Dysfunction of endothelial cells has been associated with the development of cardiovascular and renal damage in diabetes, hypertension, or atherosclerosis (Peng et al., 2011; Chennasamudram et al., 2013). A high phosphate burden increases oxidative stress in endothelial cells leading to cellular dysfunction. When dysfunctional, endothelial cells are unable to synthesize nitric oxide, aggravating atherosclerotic plaque formation occurs in Apo-E deficient mice (Shiota et al., 2011). A high-phosphate diet accelerated atherogenesis in Apo-E deficient mice (Ellam et al., 2011).

Phosphate Burden and Muscular Pathology

A high phosphate burden accelerates skeletal muscle atrophy through mechanisms not fully understood yet. Chung et al. demonstrated increased phosphate levels leading to increased muscle wasting owing to reduced myotubule size, increased ROS generation, decreased protein synthesis, and accelerated protein degradation (Chung et al., 2020). This is especially important with aged individuals, as their musculoskeletal system tends to breakdown with increase in age. Aging is associated with progressive and involuntary loss of muscle mass and strength, a condition known as sarcopenia. Sosa et al. have found hyperphosphatemia induces cellular senescence in murine myoblasts, leading to sarcopenia as one potential consequence (Sosa et al., 2018; Sosa et al., 2021). Of relevance, cellular senescence is the inability to progress through the cell cycle. This occurred to myoblasts due to increased mTOR activation and reduced autophagy under hyperphosphatemia conditions via Integrin-linked kinase (ILK) activation (Sosa et al., 2018), which is essential to myoblast senescence; suppressing ILK expression resulted in increased autophagy and protected myoblasts from senescence triggered by hyperphosphatemia (Sosa et al., 2018). With myoblast losing their proliferative abilities, sarcopenia may develop (Sosa et al., 2018). This was identified through hyperphosphatemia inducing senescence in cultured myoblasts through ILK overexpression via gene transfer using adenoviral expression vectors encoding ILK gene, lowers cell replication capacity since older mice have a considerable loss of muscle strength, which correlates with hyperphosphatemia and increased ILK and p53 (Sosa et al., 2018). Overexpression of ILK upregulates p53, which is a cell cycle inhibitor (Sosa et al., 2018). It is also important to discuss CKD and its role in accelerating muscle loss. Muscle atrophy is a major clinical issue in CKD patients, and muscle preservation has an integral part in the patient treatment and outcomes (Chung et al., 2020). A high phosphate burden has been suggested to suppress myogenic differentiation *in vitro* and promote skeletal muscle atrophy *in*

vivo in diseases such as CKD. This is mainly accomplished through enhanced nuclear factor erythroid 2-related factor 2 (Nrf2) transcriptional activity via increased ROS generation and p62 expression (Chung et al., 2020). Nrf2 is a sensor of oxidative stress and is prevented from binding to DNA by Kelch-like ECH-associated protein 1 (Keap1). Keap1 is inactivated during oxidative stress which allows Nrf2 to influence multiple mechanisms including drug metabolism, oxidant signaling, and antioxidant defense (Chung et al., 2020). P62 is a stress induced protein which leads to inclusion body formations and can also target ubiquitinated proteins for digestion (Chung et al., 2020). Experimental animal studies have shown that hyperphosphatemia increases inflammation to intensify anemia and skeletal muscle wasting (Czaya et al., 2022); phosphate burden induces hepatic levels of IL-6 and IL-1β to enhance the expression of hepcidin, a potential causative link between hyperphosphatemia, anemia, and skeletal muscle dysfunction (Czaya et al., 2022). Hepcidin regulates systemic iron homeostasis by blocking intestinal iron absorption and macrophage iron recycling at high levels (Czaya et al., 2022).

Phosphate Burden and Renal Pathology

CKD is associated with hyperphosphatemia, which increases the odds of developing various diseases, such as coronary artery disease (John et al., 2011). As mentioned above, kidneys are responsible for phosphate excretion to keep levels in an optimal range. CKD leads to increased numbers of nonfunctioning nephrons as well as increased amounts of phosphate in the body (Foley, 2009). CKD does not allow for successful aging, which is desirable by most. Successful aging is defined as aging while remaining free of CVD, cancer, chronic obstructive pulmonary disease (COPD), and personal/cognitive disability or impairment (Sarnak et al., 2008). Sarnak et al. have found that impaired renal function, such as CKD, promotes unsuccessful aging (Sarnak et al., 2008). Although not completely understood why CKD promotes unsuccessful aging, Sarnak et al. proposed three potential mechanisms. First, kidney dysfunction may be the secondary symptom due to vascular disease or hypertension. Second, kidney function may mediate an increase in several other risk factors for aging, like anemia, insulin resistance, and inflammation. Third, kidney dysfunction may be linked to unsuccessful aging related to insufficient glomerular filtration rate (GFR) (Sarnak et al., 2008). Even early stages of CKD can drop a minimum of 5 years to the normal life span (Sarnak et al., 2008). CKD culminates in systemic mineral metabolism and bone composition along with a decrease in GFR (Hou et al., 2018). This creates a scenario known as CKD-MBD. With falling GFR levels, serum calcium and phosphate levels rise (Hou et al., 2018). Disruption in mineral homeostasis increases secretion of PTH, FGF23, and decreases calcitriol. These effects combined lead to increased bone turnover and extra-skeletal calcifications (Sprague et al., 2021). Hyperphosphatemia, vascular calcification, and elevated FGF23 concentrations are the components of CKD-MBD, which exacerbate cardiovascular disease, accounting for around 60% of deaths among patients with CKD on dialysis (Sprague et al., 2021).

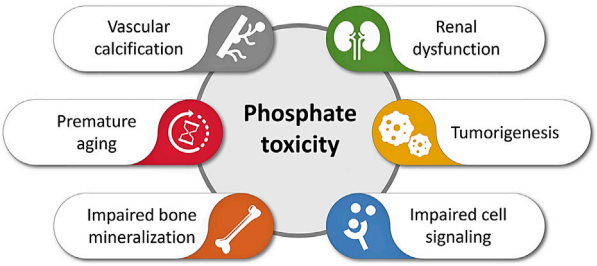

FIGURE 2 | Simplified diagram illustrating various adverse events of phosphate toxicity, ranging from impaired bone mineralization to vascular calcification to accelerated aging (inspired based on earlier publications) (Akimbekov et al., 2022; Razzaque, 2022a; Erem et al., 2022; Michigami et al., 2022; Nakatani et al., 2022).

CONCLUSION

Phosphate is an important nutrient that has various roles in the human body. It is imperative to keep its concentration in normal homeostatic ranges to avoid increasing chances of developing numerous systemic pathologies, as discussed earlier. Hyperphosphatemia has a role in many aspects of accelerated aging, prominent among them sarcopenia, decreased immune function, skin atrophy, development of arteriosclerosis, tumorigenesis, or the progression of various neurodegenerative disorders (**Figure 2**). Potential interventions to delay phosphate-associated aging-like features could be through decreasing phosphate burden with phosphate scavengers. Reducing dietary phosphate intake is another intervention, which could be achieved through avoiding artificially added phosphate-rich processed foods (Miyamoto et al., 2022). As phosphate is commonly found in additives and preservatives, the FDA does not require food industries to list amounts of phosphate on labels, thus making the task of controlling the amount of consumed much more challenging. In closing, it is becoming increasingly clear that hyperphosphatemia represents a major driver of accelerated aging, emphasizing the unmet needs for further interventional studies with the potential to yield therapeutic breakthroughs.

AUTHOR CONTRIBUTIONS

NM: collected information and drafted the manuscript. AA: reviewed and edited the manuscript. MR: conceptualized and edited the manuscript.

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N-linoleyltyrosine ameliorates high-fat diet-induced obesity in C57BL/6 mice *via* cannabinoid receptor regulation

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Objectives: N-linoleyltyrosine (NITyr) showed mild effects in preclinical studies. The research discussed the effect of NITyr on a high-fat diet (HFD) induced obese (DIO) mice, and preliminarily explored its mechanism.

Methods: The DIO mice were established by feeding an HFD for 12 weeks and subsequently administrated orally with NITyr (30, 60 and 100 mg/kg) for four weeks. The indexes of serum and liver samples were determined by ELISA kit. The pathological status of adipose and liver were detected by HE staining. The factors related to energy and lipid metabolism were measured *via* western blot.

Results: NITyr at 60 and 100 mg/kg/day suppressed the weight gain without affecting water and food intake. Accordingly, NITyr reduced adipose weight and the area of individual adipocytes and increased the number of adipocytes. Moreover, NITyr didn't affect the appetite-related indexes such as ghrelin, peptide YY and brain-derived neurotrophic factor. Besides, NITyr didn't affect other organ coefficients except for the liver. Correspondingly, NITyr reduced alanine aminotransferase and aspartate aminotransferase levels, yet didn't influence IL-1β and TNF-α levels, and the liver injury. The levels of triacylglycerol (TG), total cholesterol (TC), glucose, insulin, adiponectin and leptin in serum were assessed to evaluate the effect of NITyr on glucose and lipid metabolism. NITyr decreased the levels of TG, TC and glucose, and didn't affect insulin, adiponectin and leptin levels. Meanwhile, NITyr up-regulated p-AMPK and the cannabinoid receptor 2 (CB_2) expressions, and down-regulated PPAR, FAS and cannabinoid receptor 1 (CB_1) expressions.Overall, NITyr suppressed lipid accumulation *via* improving lipid and glucose metabolism involving CB_1 and CB_2 receptors.

KEYWORDS

N-linoleyltyrosine, endocannabinoid, diet-induced obesity, cannabinoid receptor, glucose and lipid metabolism

Introduction

Obesity is defined as an imbalance between caloric intake and energy consumption (1). Obese people are often accompanied by abnormal blood sugar, blood lipids, blood pressure, and insulin levels, and with prone to diabetes, hypertension, cardiovascular and cerebrovascular diseases (2).

The World Health Organization estimated that two out of five adults worldwide will be obese by 2030. Within several decades, obesity has become a global problem (3). Except for obesity caused by genetic and pathological factors, most obesity is diet-induced obesity (DIO) (4). The effect of exercise and diet intervention in losing weight is not satisfactory enough, and long-term intervention with drugs is required (5, 6). Medications used for obesity treatment such as cannabinoid receptor 1 (CB_1) antagonist Rimonabant with side effects of either depression or gastrointestinal reactions, respectively (7), resulting in low patient compliance. Since obesity is closely associated with the disorder of glucose and lipid metabolism, hormone disturbance and low-grade inflammation (8, 9), therefore, a compound comprehensively intervening in the above pathological pathways, while not affecting drinking and appetite, has advantages over traditional combinations in promoting weight loss and safety concerns.

N-linoleoyltyrosine (NITyr), an endocannabinoid analog, exerts neuroprotective effects in APP/PS1 transgenic mice, protects against transient cerebral ischemia in gerbils, and protects PC12 cells against oxidative damage *via* mediating cannabinoid receptors (CB_1 and CB_2) as a neuroprotective agent *in vitro* (10–12). CB_1 and CB_2 are potential therapeutic targets for obesity (13–15). CB_1 is highly expressed in the central nervous system, as well as adipose, muscle, adrenal gland, liver, gastrointestinal tract and other tissues (16). CB_1 activation improves glucose uptake and increases peroxisome proliferator-activated receptor gamma (PPAR-γ) and lipoprotein lipase expressions, which promote adipocyte proliferation and increase the size and quantity of triglyceride in adipocytes of diet-induce obese mice (17). Additionally, CB_1 activation decreases adiponectin expression and increases leptin expression in mouse white adipose tissue (18). Moreover, CB_1 activation causes an expansion of the adipose tissue in the liver (19). CB_2 is mainly distributed in brain regions related to appetite, and peripheral regions, metabolically active, such as liver, adipose, skeletal muscle, islets, etc. (20). Meanwhile, CB_2 activation improves insulin sensitivity, energy homeostasis and inflammation (21). And 60 mg/kg/day NITyr promotes weight loss (data unpublished). Importantly, NITyr improves the learning and memory ability of mice through CB_1 and CB_2 receptors, but not anxiety and depression in Alzheimer's disease. It should be noted that NITyr produces positive effects on metabolic pathologies. Therefore, all these characters mentioned above highlight the need for further research for NITyr on obesity.

In the present study, the anti-obese effect and possible mechanisms of NITyr were confirmed. Firstly, a DIO model was established, and the basic information of mice such as drinking, appetite and body weight were recorded. Next, the glucose and lipid metabolism related factors were measured. Furthermore, whether the effect of NITyr was associated with CB_1 and CB_2 was discussed.

Materials and methods

Materials

NITyr was independently synthesized in our laboratory according to the literature (12), Orlistat (MACKLIN, purity: 98%, CAS: 96829-58-2), Poloxamer 188 (Solarbio, CAS: 9003-11-6), 45% kcal high-fat diet (MD12032, Medicine, Jiangsu, China; protein 24%, fat 24% and carbohydrate 41%), RIPA lysis Buffer (Strong) (Cwbio, CW2333), SDS-PAGE Loading Buffer (Cwcio, CW0027S, 5 ×), Protease inhibitor cocktail (Cwbio, CW22005, 100 ×), Phosphatase inhibitor cocktail (Cwbio, CW2383S, 100 ×), CNR1 Ab - DF4918 (Source: Rabbit, Cat. #: DF4918, Affinity Biosciences), CNR2 Ab - DF8646 (Source: Rabbit, Cat. #: DF8646, Affinity Biosciences), GAPDH (Source: Rabbit, Cat. #: AF7021, Affinity Biosciences), FAS Ab - AF5342 (Source: Rabbit, Cat. #: AF492, Affinity Biosciences), PPAR gamma Ab - AF6284 (Source: Rabbit, Cat. #: AF6284, Affinity Biosciences), Phospho-AMPK alpha (Thr172) Antibody (Source: Rabbit, Cat. #: CY6027, Abways Technology), Goat Anti-Rabbit lgG (H + L) HRP - S0001 (Source: Goat, Cat.#: S0001, Affinity Biosciences), mouse insulin enzyme-linked reaction kit (MM-0579M, Meimian, Jiangsu), mouse adiponectin enzyme-linked reaction kit (MM-0547M, Meimian, Jiangsu), mouse leptin enzyme-linked reaction kit (MM-0622M, Meimian, Jiangsu), mouse ghrelin enzyme-linked reaction kit (MM-0621M, Meimian, Jiangsu), mouse peptide YY (PYY) enzyme-linked reaction kit (MM-0649M, Meimian, Jiangsu), mouse brain-derived neurotrophic factor (BDNF) enzyme-linked reaction kit (MM-0204M, Meimian, Jiangsu), mouse alanine aminotransferase (ALT) enzyme-linked reaction kit (MM-44625M, Meimian, Jiangsu), mouse aspartate aminotransferase (AST) enzyme-linked reaction kit (MM-4415M, Meimian, Jiangsu), mouse tumor necrosis factor α (TNF-α) enzyme-linked reaction kit (MM-0132M, Meimian, Jiangsu), mouse interleukin-1β (IL-1β) enzyme-linked reaction kit (MM-0040M, Meimian, Jiangsu), mouse triacylglycerol (TG) enzyme-linked reaction kit (A110-1-1, Jiancheng, Nanjing), mouse total cholesterol (TC) enzyme-linked reaction kit (A111-1-1, Jiancheng, Nanjing), mouse glucose enzyme-linked reaction kit (A154-1-1, Jiancheng, Nanjing).

Animals and diets

The operations were approved by the Animal Experiment Ethics Committee of Chengdu Medical College. Seventy-two male 3-week-old C57BL/6 mice (Chengdu DaShuo, Sichuan,

China) weighing ~11.8 g were fed (6 mice per cage) in an environment with 23 ± 1°C and 60 ± 5% humidity. The mice were allowed free access to food and water. After being adapted to a 12% kcal fat standard diet (MD12031, Medicine, Jiangsu, China; protein 19.2%, fat 4.3% and carbohydrate 67.3%) for one week, the mice were treated with NITyr and Orlistat. The mice were randomly divided into the control group (12 mice, fed 12% kcal fat standard diet) and the high-fat group (60 mice, fed 45% kcal high-fat diet). The body weights, food and water intake (Formula (1) (2),) were recorded weekly for 11 weeks. Then the mice with no significance in body weight between the high-fat group and the control group were removed ($P > 0.05$).

Daily food intake = $(W_i - W_f) \div N \div D$, g/mouse/day (1)

Daily water intake = $(V_i - V_f) \div N \div D$, mL/mouse/day (2)

In the formula, W_i: initial mass of feed; W_f: final mass of feed; V_i: initial water supply; V_f: final water; N: number of mice per cage; D: days.

Drugs treatment and tissue collection

Drug treatment began at the 13th week and lasted for four weeks, and the feeding of each group followed the above method. The body weights, food and water intake (Formula (1) (2),) were recorded weekly. NITyr and Orlistat were suspended in 0.05 g/mL Poloxamer 188 aqueous solution, and then administered orally at 0.1 mL/10 g per day. The control group (mice served a standard diet) and the DIO1-DIO5 group (mice served a fat diet). The experiment group was as follows: the control group (the normal mice treated with Poloxamer 188 aqueous solution), The DIO group (the obesity mice treated with Poloxamer 188 aqueous solution), the 30 NITyr group (the obesity mice treated with 30 mg/kg NITyr), the 60 NITyr group (the obesity mice treated with 60 mg/kg NITyr), the 100 NITyr group (the obesity mice treated with 100 mg/kg NITyr), the Orlistat group (the obesity mice treated with 100 mg/kg Orlistat).

After drug intervention, mice were weighed and euthanized by cervical dislocation. Serum was prepared by keeping the blood at room temperature for 20 min until coagulation, and then centrifuged (4°C, 3,000 g for 10 min) and stored at - 80°C. The heart, spleen, liver, lung, kidney, brain and adipose tissues were removed, rinsed with phosphate-buffered saline (PBS: 135 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8 mM K_2HPO_4, pH 7.2) at 4°C and weighted, then stored in - 80°C. The organ coefficient was calculated as Formula (3).

Organ coefficient = (organ weight/body weight) × 100, g/100 g (3).

Biochemical analysis of serum and liver

TG, TC, glucose, insulin, leptin, ghrelin, PYY and BDNF levels in serum and ALT, AST, TNF-α and IL-1β levels in the liver were determined respectively using corresponding enzyme kits according to the manufacturer's instructions.

Histological analysis of liver and adipose tissue

The liver and adipose tissue were fixed in 4% paraformaldehyde solution (BL539A, BioSharp, Shanghai, China) for 48h, and subsequently embedded in paraffin. The embedded tissue was cut into slices with five μm-thick sections, subsequently stained with hematoxylin and eosin (H&E), and finally, the cellular structure and lipid accumulation of liver and adipocytes were observed under observed using a light microscope (BA210Digital.) under magnification 400 ×.

Western blot

For protein extraction, brain tissues were chopped and weighed, and then its homogenate was lysed with RIPA solution for 30 min (tissue: RIPA = 0.1 g: 500 μL). Meanwhile, the protease inhibitors and phosphatase inhibitors were added to the above solutions. The operations were performed on ice. The lysates were centrifuged (4°C, 3,000 g, 10 min) and their supernatants were collected. The western blot method was consistent with the literature (16) and the primary antibody was changed (CNR1 Ab - DF4918, CNR2 Ab - DF8646).

Statistical analysis

The data were represented by mean ± standard deviation (SD) and were analyzed by SPSS software. The statistical method applied in the study was one-way ANOVA, followed by the turkey test. A significant difference was obtained when $P < 0.05$.

Results

Establishment of the diet-induced obesity

The weight of mice was measured at the end of DIO establishment (Figure 1). Compared to the control group, the body weights of DIO mice was significantly increased (17-24%, $P < 0.001$) and no significance in body weights was observed among the DIO1-DIO5 group, indicating that the high-fat food (HFD) did induce obesity.

Body weight during 4-week drug administration

When the DIO mice were given Orlistat for two weeks, a significant decrease ($P < 0.01$) was observed in the Orlistat group as compared with the DIO group (Figure 2), until the DIO mice

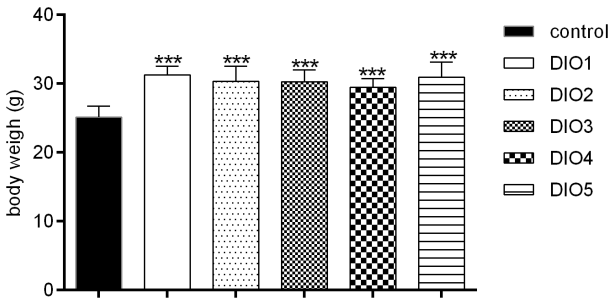

FIGURE 1
Body weight under 12-week HFD dietary intervention. Control was given a 12% kcal normal fat diet. DIO1-5 were given a 45% kcal high-fat diet. All values were expressed as means ± SD (n = 8). ***$P < 0.001$, as compared with the control group.

were treated with NITyr for four weeks, the body weights in the NITyr group decreased compared with the DIO group (all $P < 0.01$).

Effect of NITyr on appetite

As shown in Figure 3, compared with the control group, the food intake and water intake in the DIO group were reduced ($P < 0.001$), and the treatment group didn't attenuate the above phenomena. Meanwhile, the factors related to appetite were detected (Table 1). The levels of peptide YY (PYY), a feeding inhibitor, and ghrelin, a feeding stimulator, were measured. The levels of ghrelin and PYY increased in the DIO group compared with that of the control group, NITyr didn't affect the above factors. Besides, compared with the control group, the levels of brain-derived neurotrophic factor (BDNF) were decreased in the DIO group, and no significance in the BDNF level was investigated after NITyr intervention.

Effect of NITyr on organ coefficient in mice

A significant difference ($P < 0.05$, $P < 0.001$) in the organ coefficient of liver and adipose was observed in the DIO group compared to the control group (Table 2), and NITyr decreased the above factors. Besides, no significance was observed in the organ coefficient conclude heart, spleen, lung, kidney and brain among all groups.

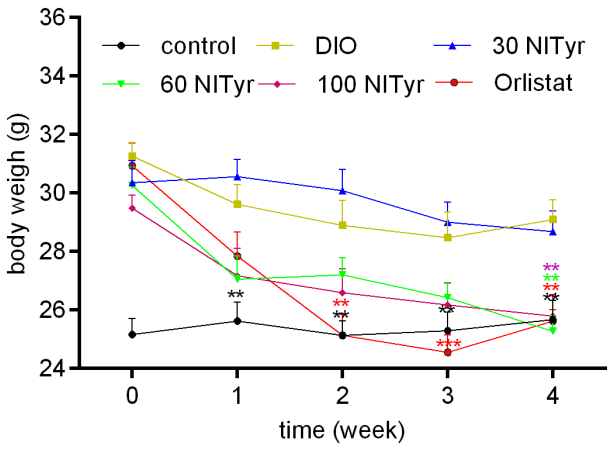

FIGURE 2
Figure 2 Body weight during 4-week drug intervention period. NITyr and Orlistat promoted weight loss of mice. Control or DIO was treated with Poloxamer 188 aqueous solution. 30 NITyr, 60 NITyr, 100 NITyr and Orlistat were treated with 30 mg/kg NITyr, 60 mg/kg NITyr, 100 mg/kg NITyr, 100 mg/kg Orlistat, respectively. All values were expressed as means ± SD (n = 8). **$P < 0.01$, ***$P < 0.001$, as compared with the DIO group.

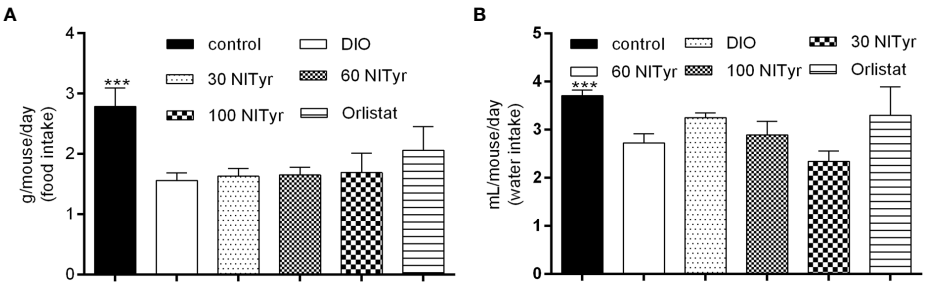

FIGURE 3
(A) Average food intake during 4-week drug intervention. **(B)** Average water intake during 4-week drug intervention. Control or DIO was treated with Poloxamer 188 aqueous solution. 30 NITyr, 60 NITyr, 100 NITyr and Orlistat were intervened with 30 mg/kg NITyr, 60 mg/kg NITyr, 100 mg/kg NITyr and 100 mg/kg Orlistat, respectively. All values were expressed as means ± SD (n = 4). ***$P < 0.001$, as compared with the DIO group.

Effect of NITyr on adipocyte

As shown in Figure 4, compared with the control group, the adipocyte area in the DIO group increased and the number of adipocytes decreased in each field ($P < 0.001$, $P < 0.001$). The image of adipocytes showed an expansion in the DIO group compared with the control group, suggesting HFD-induced adipose tissue hypertrophy. NITyr attenuated the above phenomena, indicating that NITyr inhibited adipocyte hypertrophy.

Effect of NITyr on liver

The levels of ALT, AST, IL-1β and TNF-α correlated with liver injury and inflammation were tested (Table 3). All the above indicators increased significantly ($P < 0.01$, $P < 0.05$, $P < 0.05$, $P < 0.05$, respectively) in DIO group compared with the control group. NITyr treatment (100 mg/kg) weakened ALT and AST levels, while didn't affect IL-1β and TNF-α levels. The H&E staining of the liver showed that the hepatic injury didn't exist among all groups (Figure 5).

Effect of NITyr on glucose and lipid metabolism

TG, TC, glucose, insulin, adiponectin and leptin were detected to investigate the effects of NITyr on lipid and carbohydrate metabolism (Table 4). Compared with the control group, the levels of TG, TC, glucose and insulin in the DIO group were significantly increased ($P < 0.001$, $P < 0.01$, $P < 0.001$, and $P < 0.01$, respectively). In contrast, the levels of adiponectin and leptin were descended ($P < 0.05$, $P < 0.05$, respectively), indicating that HFD led to an imbalance of lipid and carbohydrate metabolism. NITyr decreased TG, TC and glucose levels in DIO mice ($P < 0.05$, $P < 0.05$, $P < 0.01$, respectively), yet didn't affect the level of insulin, adiponectin and leptin.

Effect of NITyr on the expression of key factors in adipogenesis

As shown in Figure 6, NITyr treatment noticeably suppressed the elevated expression of PPAR ($P < 0.05$) and FAS ($P < 0.05$, $P < 0.05$, and $P < 0.01$, respectively) in DIO group. The p-AMPK expression was significantly decreased in the DIO group compared with the control group but was increased by NITyr treatment ($P < 0.05$, and $P < 0.01$, respectively).

Effect of NITyr on CB_1 and CB_2 protein levels in DIO mice

Compared with the control group, the CB_1 expressions were upregulated in the DIO group ($P < 0.01$) and no significance was

TABLE 1 Analysis of several appetite indexes in serum of mice after drug administration for 4 weeks.

Value	control	DIO	30NITyr	60 NITyr	100 NITyr	Orlistat
Ghrelin (ng/L)	133.58 ± 34.36*	234.65 ± 55.62	201.46 ± 28.44	224.56 ± 54.96	262.62 ± 35.65	191.20 ± 47.82
PYY (pg/mL)	62.69 ± 10.21*	106.26 ± 29.57	71.19 ± 15.13	95.51 ± 20.05	70.82 ± 13.92	76.76 ± 13.85
BDNF (ng/L)	644.05 ± 127.62*	408.97 ± 135.62	296.22 ± 132.35	336.76 ± 60.88	321.91 ± 105.15	304.89 ± 129.81

All values were expressed as means ± SD (n = 3). *P < 0.05, as compared with the DIO group.

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TABLE 2 Effect of NITyr on organ coefficient in mice.

Value	control	DIO	30 NITyr	60 NITyr	100 NITyr	Orlistat
Heart	0.46 ± 0.04	0.50 ± 0.10	0.38 ± 0.06	0.45 ± 0.73	0.46 ± 0.09	0.60 ± 0.13
Liver	3.87 ± 0.14*	3.06 ± 0.45	2.76 ± 0.74	4.04 ± 0.41**	3.91± 0.27**	4.43 ± 0.28***
Spleen	0.29 ± 0.08	0.35 ± 0.12	0.29 ± 0.04	0.35 ± 0.05	0.34 ± 0.09	0.44 ± 0.13
Lung	0.64 ± 0.14	0.58 ± 0.11	0.60 ± 0.08	0.57 ± 0.04	0.60 ± 0.11	0.66 ± 0.07
Kidney	1.10 ± 0.04	1.37 ± 0.25	1.14 ± 0.24	1.35 ± 0.06	1.30 ± 0.12	1.10 ± 0.26
Brain	1.38 ± 0.24	1.33 ± 0.08	1.04 ± 0.17	1.26 ± 0.16	1.36 ± 0.24	1.34 ± 0.20
Adipose	1.11 ± 0.32***	5.07 ± 1.52	3.57 ± 1.03	3.28 ± 0.89*	3.15 ± 1.42*	2.30 ± 0.89**

All values were expressed as means ± SD (n = 8). *P < 0.05, **P < 0.01 and ***P < 0.001, as compared with the DIO group.

TABLE 3 Analysis of liver injury and inflammation indexes in serum in mice after 4-week administration of drugs.

Value	control	DIO	30 NITyr	60 NITyr	100 NITyr	Orlistat
ALT (ng/L)	28.92 ± 5.97**	53.81 ± 15.40	38.06 ± 9.36	41.11 ± 7.45	35.21 ± 9.38*	36.92 ± 9.73
AST (ng/L)	31.84 ± 11.79*	55.50 ± 14.46	50.49 ± 16.09	34.30 ± 11.67	33.70 ± 14.00*	38.45 ± 13.04
IL-1β (ng/L)	199.84 ± 11.79*	372.33 ± 57.54	251.87 ± 43.85	350.29 ± 106.20	387.87 ± 115.97	446.74 ± 108.79
TNF-α (ng/L)	18.71 ± 6.42*	33.78 ± 8.24	29.45 ± 6.92	37.63 ± 6.58	34.68 ± 9.87	41.81 ± 5.85

All values were expressed as means ± SD (n = 4). *P < 0.05, **P < 0.01, as compared with the DIO group.

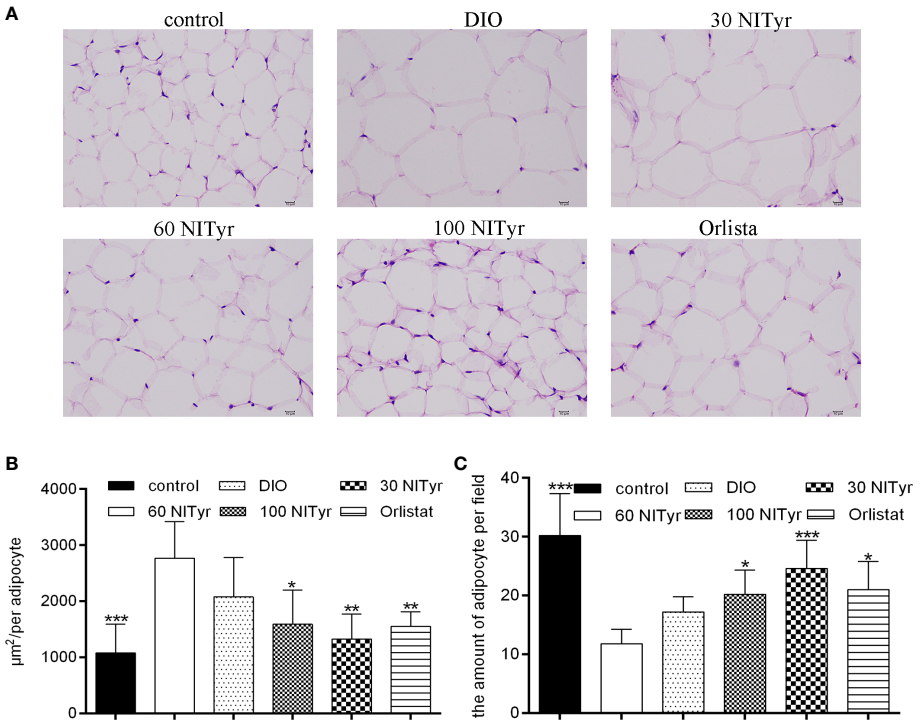

FIGURE 4
(A) The adipose tissue of mice stained by hematoxylin and eosin (H&E). scar bar = 10 μm. **(B)** The area of adipocytes in each group under the same field. **(C)** the number of adipocytes in each group of mice under the same field. The control or the DIO group was treated with Poloxamer 188 aqueous solution. 30 NITyr, 60 NITyr, 100 NITyr and Orlistat were treated with 30 mg/kg NITyr, 60 mg/kg NITyr, 100 mg/kg NITyr and 100 mg/kg Orlistat, respectively. All values were expressed as means ± SD. (n = 4). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, as compared with the DIO group.

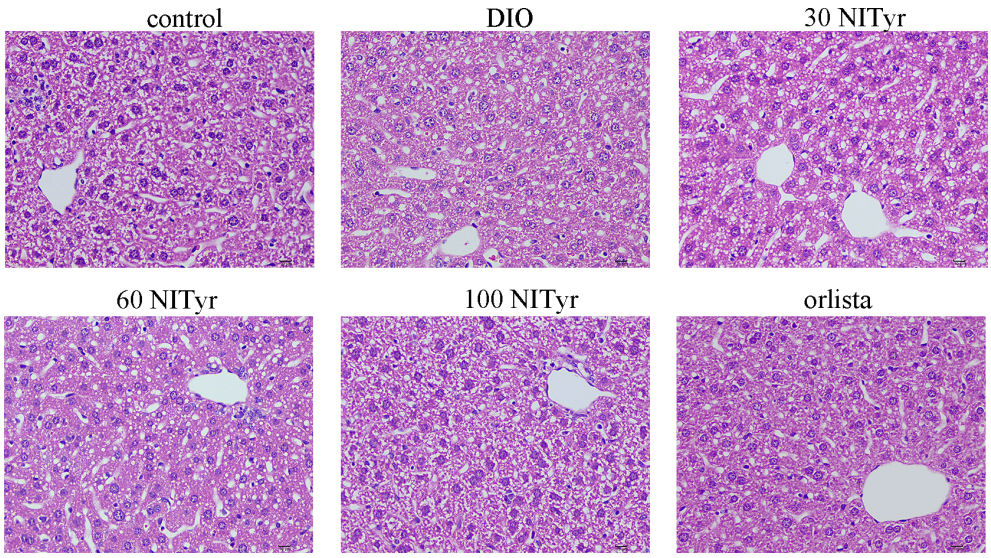

FIGURE 5
The liver tissue of mice stained by the H&E method. scar bar = 10 μm. The control or DIO group was treated with Poloxamer 188 aqueous solution. 30 NITyr, 60 NITyr, 100 NITyr and Orlistat were treated with 30 mg/kg NITyr, 60 mg/kg NITyr, 100 mg/kg NITyr, 100 mg/kg Orlistat, respectively.

observed in CB_2 expressions (Figure 7). Besides, NITyr treatment downregulated CB_1 expressions ($P < 0.05$, $P < 0.01$) while upregulated CB_2 expressions ($P < 0.01$, $P < 0.05$).

Discussion

Obesity is a chronic metabolic disorder caused by genetic or environmental factors (22). When energy intake is greater than energy consumption, the body will cause fat accumulation, resulting in obesity. Establishing an obesity model suitable for the experiment is a prerequisite for obesity study. Nutritional obesity without definite etiology was caused by energy intake exceeding consumption (23). The nutritional obesity model induced by HFD is widely used, similar to human obesity (24, 25). Therefore, we use the commercially available high-fat feed to establish the obesity model, which is stable and straightforward. As C57BL/6 mice are sensitive to an HFD, they are constantly applied to obesity (26). Orlistat, a weight-loss drug, is associated with gastrointestinal reactions, but it exerted benign efficacy and high safety compared with the other weight-loss drugs such as naltrexone and bupropion (27). Hence, it was selected as the positive drug in the experiment. The DIO mice were induced by continuous feeding with HFD for 12 weeks according to the method of Tang (28). Because some mice own obesity genes and love to grab food, free-feeding leads to individual differences in the weight of mice. Thus, in the study, mice with no significance compared with the control group were excluded. Meanwhile, mice with no difference in weight between the high-fat groups were retained as the model mice for subsequent experiments.

Body weight directly reflects the weight loss activities of drugs. In the first week of drug intervention, the weight loss of mice decreased sharply, but it became gentle in the later stage of drug intervention. We speculated that the intragastrical administration

TABLE 4 Analysis of glucose and metabolism indexes in serum in mice after 4-week administration of drugs.

Value	control	DIO	30 NITyr	60 NITyr	100 NITyr	Orlistat
TG (mM)	1.49 ± 0.06***	1.87 ± 0.14	1.71 ± 0.21	1.66 ± 0.13*	1.63 ± 0.10*	1.53 ± 0.15**
TC (mM)	2.36 ± 0.62**	3.94 ± 0.83	3.32 ± 0.96	2.66 ± 0.34*	2.75 ± 0.22*	2.47 ± 0.88*
Glucose (mM)	4.51 ± 0.71***	8.81 ± 1.29	6.55 ± 1.67	5.45 ± 1.58**	5.19 ± 0.90**	6.46 ± 1.69*
Insulin(mIU/L)	0.42 ± 0.07**	0.63 ± 0.09	0.54 ± 0.09	0.56 ± 0.13	0.51 ± 0.06	0.47 ± 0.06
Adiponectin (μg/L)	139.49 ± 13.16*	104.73 ± 16.2	131.61 ± 16.23	131.43 ± 17.99	132.36 ± 18.62	140.79 ± 19.84*
Leptin (pg/L)	556.75 ± 106.33*	410.25 ± 38.49	422.56 ± 43.73	545.68 ± 83.70	521.42 ± 73.89	556.83 ± 69.16*

All values were expressed as means ± SD (n = 8). *P < 0.05, **P < 0.01, ***P < 0.001, as compared with the DIO group.

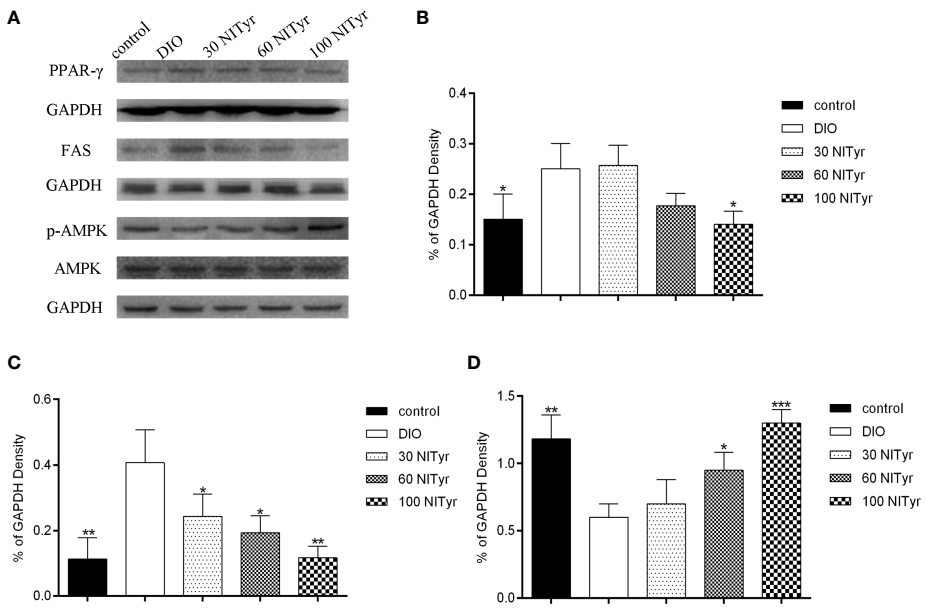

FIGURE 6
Effect of NITyr on the expression of key factors in adipogenesis. **(A)** Western blot analysis of PPAR-γ, FAS and p-AMPK. **(B)** PPAR-γ expressions were normalized that of GAPDH. **(C)** FAS expressions were normalized that of GAPDH. **(D)** p-AMPK expressions were normalized that of AMPK. The control or DIO group was treated with Poloxamer 188 aqueous solution. 30 NITyr, 60 NITyr and 100 NITyr were treated with 30 mg/kg NITyr, 60 mg/kg NITyr, 100 mg/kg NITyr, respectively. All values were expressed as means ± SD. (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, as compared with the DIO group.

reduced the food intake of mice, resulting in significant weight loss. As the mice gradually adapted to the intragastrical continuous administration, the weight loss became gentle. Meanwhile, the mice treated with Orlistat showed side effects such as loose stool and malaise, while mice treated with NITyr didn't show the above reaction. Thus, NITyr owns a good safety. On account of the efficacy of NITyr on weight loss, the effect of NITyr on organ coefficient was further investigated. The organ coefficient of adipate in DIO mice significantly increased, while the organ coefficient of liver decreased compared with the control mice. The changes in the liver may not be consistent with our expectations. As obese mice showed excessive fat deposition, the organ coefficient of adipose tissue increased. And the weight gain of the liver is less than the overall weight gain of mice, so the organ

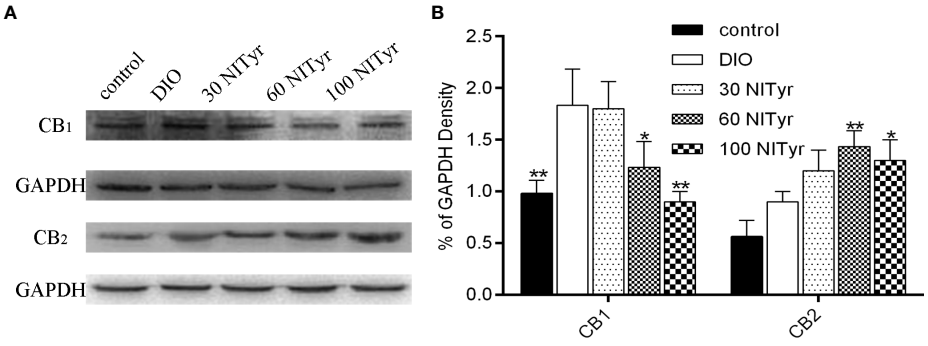

FIGURE 7
Effect of NITyr on the expression of CB_1 and CB_2 in brain. **(A)** Western blot analysis of CB_1 and CB_2. **(B)** CB_1 and CB_2 expressions were normalized that of GAPDH. The control or DIO group was treated with Poloxamer 188 aqueous solution. 30 NITyr, 60 NITyr and 100 NITyr were treated with 30 mg/kg NITyr, 60 mg/kg NITyr, 100 mg/kg NITyr, respectively. All values were expressed as means ± SD. (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, as compared with the DIO group.

coefficient of the liver descended. NITyr improved the above phenomenon. The above results indicated that NITyr did interfere with fat synthesis or decomposition, while its effect on the liver is unclear. Therefore, we further discussed the pathological sections and indexes of fat and liver.

Adipocyte enlargement and hepatic steatosis are essential features of obesity (29). NITyr reduced the area of individual adipocytes and increased the number of adipocytes, consistent with the above results. As we know that the liver is the most important metabolic organ responsible for lipid metabolism including lipogenesis, lipolysis, and lipid oxidation, which maintains lipid homeostasis (30, 31). Thus, the liver is the hub of fat transport. The liver is one of the organs that severely suffer from obesity. Obesity disrupted the overall metabolic function of the liver to promote the accumulation of fat in the liver to form "fatty liver", which further aggravated obesity, resulting in a vicious circle (32, 33). Thus, if there were methods to intervene in hepatic lipid metabolism, they would be expected to offer a potential strategy for obesity alleviation. Due to the extremely high sensitivity of ALT and AST (34), they are used as indicators to evaluate liver injury clinically. TNF-α and IL-1β reflected the inflammatory status of the liver (35). Compared with the control group, a notable increase in ALT, AST, TNF-α and IL-1β was detected in the DIO mice. NITyr reduced the ALT and AST but didn't affect the level of TNF-α and IL-1β. The liver of DIO mice didn't show steatosis or inflammatory infiltration, and no significance was observed after NITyr intervention. The above results showed that the liver injury of DIO mice established by our research group was not noticeable.

DIO is often accompanied by abnormal metabolism of glucose and lipids in the body (36), so TG, TC, glucose and insulin levels in serum were detected. NITyr reduced the high levels of TG, TC and glucose induced by DIO mice, indicating that the glucose and lipid metabolism in DIO mice was unbalanced, and NITyr intervention restored the glucose and lipid balance. Due to insulin deficiency or resistance caused by hyperglycemia (37), the insulin levels were tested. Insulin levels in the DIO group were significantly increased compared to that in the control group. In contrast, neither group under drug treatment showed significant differences, indicating the effect of NITyr on regulating blood glucose is independent of insulin.

Ghrelin produced by P/D1 cells at the bottom of the stomach promotes appetite (38). PYY, a derived intestinal hormone, makes the body feel full, and then reduces food intake (39). In the study, the PYY and ghrelin levels of DIO mice increased compared with the control group. The food intake and water consumption of DIO mice decreased compared with the control group. On the one hand, the increase of PYY levels induced by HFD is higher than that of ghrelin; on the other hand, mice fed a high-fat diet rich in energy for a long time will produce a strong sense of satiety, thus long-term consumption of high-fat diet inhibited the appetite of mice. NITyr didn't affect PYY and ghrelin levels, suggesting that the weight loss induced by NITyr was independent of food intake. In the previous studies, NITyr as a neuroprotective agent enhanced

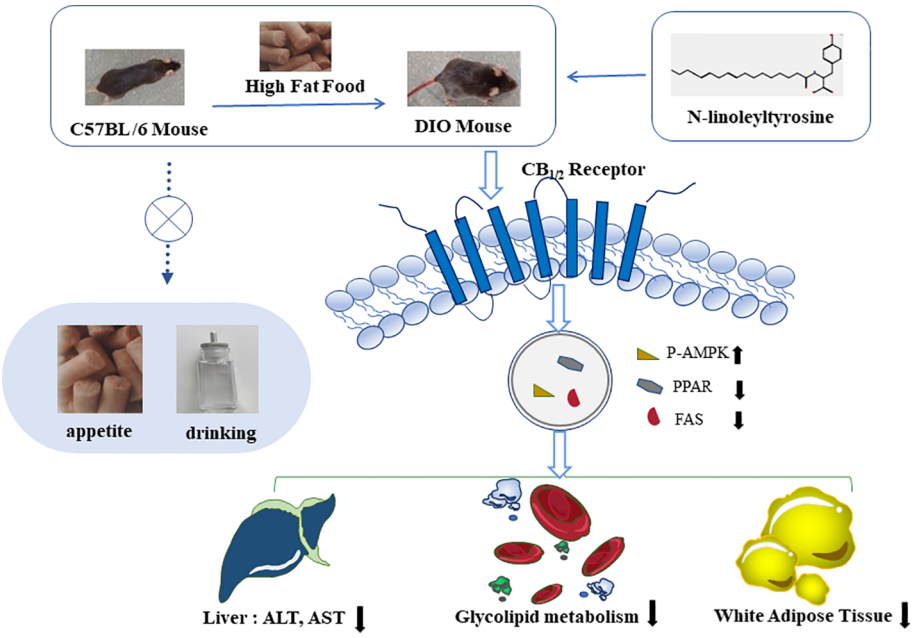

FIGURE 8
N-linoleyltyrosine ameliorates high-fat diet-induced obesity in C57BL/6 mice via cannabinoid receptor regulation, involving in regulating lipid and glucose metabolism, but not appetite.

BDNF levels in the brain of mice (data unpublished), and BDNF regulated food intake and energy metabolism (40). Thus, the levels of BDNF were investigated in our study. NITyr didn't interfere with the BDNF level in DIO mice, further indicating that the weight loss induced by NITyr was independent of appetite.

PPAR, a transcriptional regulator of adipogenesis, contributes to lipid accumulation and adipocyte differentiation. It was found to be activated in the process of adipogenesis and regulate the expression of AMPK (41). AMPK is an AMP-dependent protein kinase, which plays a role in energy homeostasis through the upregulation of catabolic processes that generate ATP. AMPK activation enhances the catabolism of the body, and reduce the expression of lipid synthesis-related factors such as FAS to regulate the synthesis and utilization of lipids (42). FAS is a key enzyme in lipogenesis, and when activated, it increases fatty acid synthesis and insulin resistance in adipose tissues. In the present study, we found that PPAR and FAS were up-regulated in HFD mice, the expression of which was down-regulated by the introduction of NITyr. P-AMPK was lowly expressed in adipose tissues in HFD mice, while highly expressed after NITyr administration.

The endocannabinoid system consists of endocannabinoid (AEA), cannabinoid receptors (CB_1 and CB_2) and hydrolase (FAAH). The inhibition of FAAH increased the AEA level, thus indirectly activating the CB_1 and CB_2 receptors (43). Activation of the CB_1 receptor promoted weight gain, while activation of the CB_2 receptor urged weight loss (13). In the study, NITyr upregulated CB_2 expressions and downregulated CB_1 expressions. We speculated that the downregulation of the CB_1 receptor was due to receptor desensitization caused by long-term drug action. Besides, CB_2 receptors were more stable; thus, they were only up-regulated even after long-term drug stimulation. In addition, OEA and PEA, as AEA analogs, activated TRPV1 and PPAR receptors to promote weight loss. Hence, NITyr as an AEA analog, may also bring a similar role to OEA and PEA (44).

Overall, NITyr fight against obesity *via* balance glycolipid metabolism involved in CB_1 and CB_2 activation (Figure 8).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Animal Experiment Ethics Committee of Chengdu Medical College.

Author contributions

Methodology, Z-YY and Y-YW. Project administration, Z-YY and J-HZ. Data curation and analysis, YZ and Y-QY. Writing-original draft manuscript, SL and TH. Writing-review, SL. Funding acquisition, SL and TH. All authors approved the published version of the manuscript.

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Conflict of interest

YZ was employed by Sichuan Yuanda Shuyang Pharmaceutical Co.

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

Publisher's note

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Effect of vitamin D supplementation on COVID-19 patients: A systematic review and meta-analysis

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Objective: To systematically evaluate the impact of vitamin D supplementation on mortality, ICU admission, and the rates of mechanical ventilation or intubation among COVID-19 patients.

Data sources and study selection: The PubMed, Embase, Cochrane Library, CBM, CNKI, VIP, and WanFang databases were searched from 1 December 2019 to 31 December 2022. The authors sought to identify randomized controlled trials and cohort studies that examined the relationship between vitamin D supplementation and mortality, ICU admission, and mechanical ventilation or intubation rates among COVID-19 patients.

Data extraction and synthesis: Two investigators independently searched the literature, extracted the data, and assessed the quality of the included studies. The Grading of Recommendation, Assessment, Development, and Evaluation approach was used to evaluate the quality of the evidence. Meta-analysis was conducted using RevMan 5.3, STATA 15.1, and R 4.1.3 software.

Results: Eight randomized controlled trials (RCTs) and eight cohort studies were included, involving 3359 COVID-19 patients. The pooled analysis of randomized controlled trials showed that vitamin D supplementation did not have a significant effect on reducing mortality (Relative Risk, RR = 0.94, 95% CI 0.69–1.29, $P = 0.7$), while the results of cohort studies indicated that vitamin D supplementation had a positive impact on reducing mortality among COVID-19 patients (RR = 0.33, 95% CI 0.23–0.47, $P < 0.001$). There was no statistically significant difference in the rates of ICU admission (RCTs: RR = 0.64, 95%CI 0.38–1.08, $P = 0.10$; cohort studies: RR = 0.32, 95% CI 0.08–1.29, $P = 0.109$) or rates of mechanical ventilation or intubation (RCTs: RR = 0.77, 95% CI 0.58–1.02, $P = 0.07$; cohort studies: RR = 0.93, 95% CI 0.55–1.58, $P = 0.789$).

Conclusion: The results of this systematic review and meta-analysis suggest that vitamin D supplementation does not have a significant impact on reducing mortality, ICU admission, and the rates of mechanical ventilation or intubation among COVID-19 patients. However, due to the limited number and quality of the studies included, further high-quality studies are needed to confirm these findings.

Systematic review registration: www.crd.york.ac.uk, identifier CRD42021299521.

KEYWORDS

vitamin D, meta-analysis, COVID-19, mortality, ICU admission, mechanical ventilation, intubation

Introduction

The global outbreak of coronavirus disease 2019 (COVID-19) has caused a major health crisis with 655,689,115 confirmed cases and 6,671,624 confirmed deaths as of 3 January 2023 (1). The infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) leads to a wide range of symptoms, and patients with comorbidities such as diabetes, cardiovascular disease, and hypertension may face adverse outcomes (2), including ICU admission, mechanical ventilation or intubation, and death.

While vaccines and antiviral drugs have demonstrated efficacy against COVID-19 (3), additional measures, such as vitamin D supplementation, continue to play an important role in managing the disease. Low serum 25-hydroxycholecalciferol [25(OH)D] levels have been linked to increased susceptibility to novel coronavirus infection and greater severity of COVID-19 symptoms (4). Some studies have suggested that vitamin D supplementation may reduce mortality in COVID-19 patients (5, 6), but a previous meta-analyze published in the year 2022 has failed to reach a definitive conclusion due to limited studies and inconsistent study design (7).

With the ongoing spread of COVID-19, the number of clinical studies on the effect of vitamin D supplementation on COVID-19 outcomes has increased (5, 6, 8–13) but the results remain conflicting. Thus, it is necessary to conduct an updated meta-analysis of randomized controlled trials and cohort studies to determine the impact of vitamin D supplementation on mortality, ICU admission, and mechanical ventilation or intubation rates in COVID-19 patients.

Materials and methods

The present meta-analysis was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) statement (14) and has been registered on the international database of prospectively registered systematic reviews, PROSPERO (Registration number: CRD42021299521).

Inclusion and exclusion criteria

Population: COVID-19 patients of all ages and severity levels.

Intervention: Vitamin D supplements of various forms, analogs, doses, and follow-up durations after the diagnosis of COVID-19.

Comparison: Without vitamin D supplements.

Outcomes: mortality, ICU admission rates, and rates of mechanical ventilation or intubation of COVID-19 patients.

Study design: Randomized controlled trials and cohort studies.

Exclusion criteria: (1) Repeated publications; (2) missing outcome data in the literature; (3) lack of definite Vitamin D dose in each study; and (4) the data are wrong or cannot be extracted.

Search strategy

The literature search was conducted across multiple databases including PubMed, Cochrane Library, Embase, CNKI, CBM, WanFang Data, and Cqvip, covering the period from 1 December 2019 to 31 December 2022. Search keywords: Dihydroxyvitamin D, Dihydroxyvitamin, Calcitriol, Alfacalcidol, 24,25-Dihydroxyvitamin D, paricalcitol, Dihydroxycholecalciferol, 1 alpha,25-Dihydroxyvitamin, 1alpha,25-Dihydroxycholecalciferol, 1,25-Dihydroxyvitamin, 25Hydroxyvitamin D3, 1, 25-dihydroxy vitamin D, 25-Hydroxyvitamin D3, 25-hydroxyvitamin D, Calcidiol, Calcifediol, Hydroxycholecalciferol, Ergocalciferol, Cholecalciferol, Vitamin D3, Vitamin D2; COVID-19, COVID19, COVID-19 Virus, COVID-19 Virus Disease, COVID-19 Virus Infection, 2019-nCoV Infection, Coronavirus Disease-19, Coronavirus Disease 19, 2019 Novel Coronavirus Disease, 2019 Novel Coronavirus Infection, 2019-nCoV Disease, Disease 2019, Coronavirus, SARS Coronavirus 2 Infection, SARS-CoV-2 Infection, COVID-19 Pandemic. The search terms are described in the **Supplementary Text 1**.

Study selection and data extraction

Two investigators independently searched the literature, extracted the data, cross-checked the data, and consulted a third party to resolve any disagreements. The titles and abstracts of the literature were initially screened, followed by a full-text review to determine final inclusion based on the established inclusion and exclusion criteria. The extracted data included (1) the first author, year of publication, location, and date of the study; (2) baseline characteristics and interventions of subjects; and (3)outcome indicators and data, including mortality, ICU admission rates, and mechanical ventilation or intubation rats in COVID-19 patients.

Risk of bias assessment

The assessment of the risk of bias in the included literature was carried out independently by two investigators, and the results were verified through cross-checked. The risk of bias in cohort studies was evaluated using the Robin-I tool by the Cochrane guidelines for non-randomized studies (15), and RCTs were evaluated by the Cochrane Collaborations Tool For Assessing Risk of Bias recommended by the Cochrane Manual 5.1.0 (16).

Statistical analysis

RevMan (version 5.3) software (Cochrane Collaboration, UK), Stata (version 15.1) software (Stata Corporation, Lakeway, TX, USA) and R software (version 4.1.3) were used for meta-analysis. The effect size was analyzed using relative risk (RR) and a 95% confidence interval (CI). Hazard ratio (HR) was considered as RR in the study, and the following formula was used to convert odds ratio (OR) into RR: $RR = OR/[(1 - Po) + (Po \times OR)]$, where Po represents the incidence of the outcome of interest in the non-exposed group (17). The standard error of the resulting converted RR was calculated using the formula: $SElog(RR) = SElog(OR) \times log(RR)/log(OR)$. The adjusted HR or RR and 95% CI were utilized to reduce the impact of confounding factors if available. Otherwise, unadjusted HR or RR was adopted.

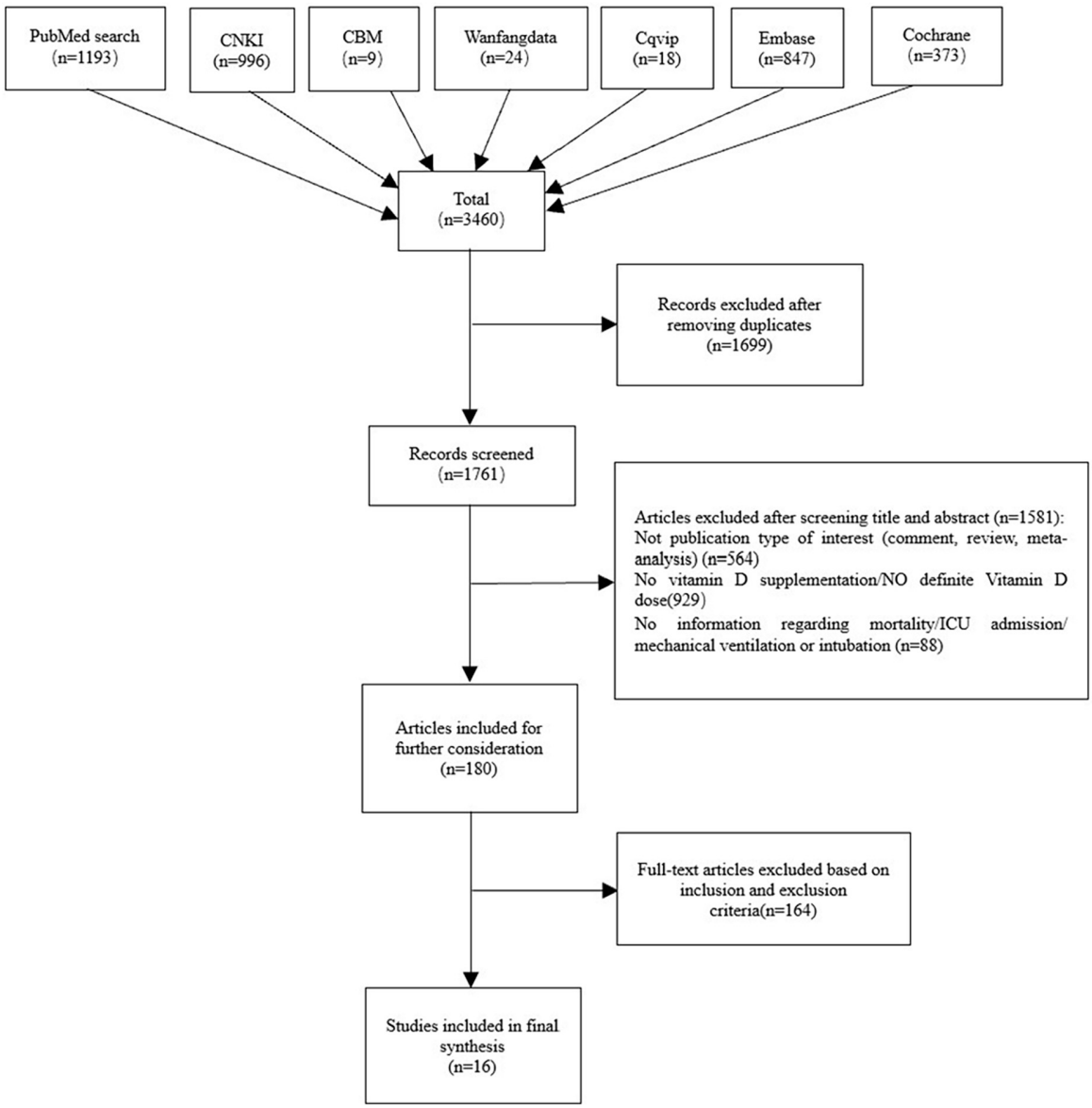

FIGURE 1
Flow chart of literature searching and screening.

The heterogeneity of the included studies was analyzed using the Q test, and if $I^2 < 50\%$ and $P > 0.1$, all studies were considered homogenous and the data were analyzed by a fixed-effect model. In case of $I^2 \geq 50\%$ and $P \leq 0.1$, indicating the presence of heterogeneity, data were analyzed using a random effects model. Potential publication bias was evaluated through funnel plots and Egger's test.

Stratified analyses were performed based on the type of study design, and sensitivity analyses were conducted to test the reliability of the combined analysis of adjusted/unadjusted RR.

Quality of evidence

The quality of the evidence was evaluated using the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) approach (18, 19) and was classified as *high*, *moderate*, *low*, or *very low* based on the following domains: study design, risk of bias, inconsistency, indirectness, imprecision, and other considerations (such as evidence of publication bias). The results are presented in Table 2.

Results

Literature search

A comprehensive literature search was conducted, resulting in the identification of 3,460 citations. Upon manual removal of 1,699 duplicates, screening the remaining titles and abstracts resulted in the selection of 180 articles. Further evaluation of full text resulted in the inclusion of 16 studies in the final analysis (Figure 1),

TABLE 1 The characteristics of eligible studies.

Study and Country	Type of study and patients source	Intervention and Control	Vitamin D supplements		Control		Number of deaths/Intubation or Mechanical ventilation requirement/ICU admission: number of intervention or control
			Age	25(OH)D levels before/after treatment(ng/ml)	Age	25(OH)D levels before/after treatment(ng/ml)	
Elamir et al. (8), Israel	RCT, Hospitalized patients	Oral 0.5 ug calcitriol per day. vs. Without vitamin D supplements	69 ± 18	NA	64 ± 16	NA	0/0/5: 25 vs. 3/2/8: 25
Cannata-Andía et al. (9), Multicentre	RCT, Hospitalized patients	A single oral dose of 100,000 IU cholecalciferol vs. Without vitamin D supplements	59.0(49.0, 70.0)	17.0(11.8,22.0)/29.0 (20.3,35.0)	57.0(45.0, 67.0)	16.1(11.5, 22.0)/16.4(11.8, 23.0)	22/NA/47: 274 vs. 15/NA/44: 269
Javier Mariani et al. (10), Argentina	RCT, Hospitalized patients	A single oral dose of 500,000 IU of vitamin D3 vs. Placebo	59.8 ± 10.7	32.5 (27.2–44.2)/102 (85.2 to 132.2)[a]	58.3 ± 10.6	30.5(22.5–36.2)/30.0 (27.5–31.0)[a]	5/5/9: 115 vs. 2/6/11: 103
IMurai et al. (20), Brazil	RCT, Hospitalized patients	A single oral dose of 200,000 IU cholecalciferol vs. Placebo	56.5 ± 13.8	21.2 ± 10.1/44.4 ± 15.0	56 ± 15	20.6 ± 8.1/19.8 ± 10.5	9/9/19: 119 vs. 6/17/25: 118
Jessie Zurita-Cruz et al. (21), Mexico	RCT, Hospitalized patients	1,000 IU/day of Cholecalciferol for children younger than 1 year and 2,000 IU/day for 1–17 years. vs. Without vitamin D supplements	10.66(4.41–14.62)	13.8(10.75–18.35)/NA	13.95(7.35-14.87)	11.4(8.7–13.1)/NA	1/NA/NA:20 vs. 6/NA/NA:25
Mikhail V. Bychinin et al. (22), Russia	RCT, Hospitalized patients with hypovitaminosis D	60,000 IU cholecalciferol once per 7 days, followed by daily doses of 5,000 IU vs. Placebo	64.5 (57–71)	9.6(5.6–21)/20.6 (11.8–24.8)	63.5 (54–81)	11.2(8.6–14.9)/10.4 (5.8–12.2)	19/33/NA: 52 vs. 27/37/NA: 54
Castillo et al. (23), Spain	RCT, Hospitalized patients	Oral 0.532 mg Calcifediol on day 1, 0.266 mg on days 3 and 7, then weekly. vs. Without vitamin D supplements.	53.14 ± 10.77	NA	52.77 ± 9.35	NA	0/NA/1:50 vs. 2/NA/13: 26
Sophie De Niet et al. (24), Belgium	RCT, Hospitalized patients with hypovitaminosis D	Oral 25,000 IU of Cholecalciferol over 4 consecutive days. Then, 25,000 IU per week up to 6 weeks. vs. Placebo	63.24 ± 14.46	17.87 ± 10.15/NA	68.73 ± 10.97/NA	16.87 ± 9.48/NA	3/NA/5: 22 vs. 4/NA/2: 21
Annweiler C et al. (5), French	Cohort study, hospitalized patients	Oral 50,000 IU cholecalciferol per month, or 80,000 IU or 100,000 IU, or 200,000 IU every 2–3 months, or 800 IU daily. vs. Without vitamin D supplements	87.7 ± 5.4	24.64 ± 14.16/NA	88.6 ± 5.7	29.56 ± 12.84/NA	16/NA/NA:67 vs. 13/NA/NA: 28
Annweiler C et al. (6), French	Cohort Study, COVID-19 patients in the nursing home	Oral 80,000 IU cholecalciferol vs. Without vitamin D supplements	87.7 ± 9.3	NA	87.4 ± 7.2	NA	10/NA/NA: 57 vs. 5/NA/NA: 9
Annweiler G et al. (11), France	Cohort Study, Hospitalized patients	Oral 80,000 IU cholecalciferol within a few hours of the diagnosis vs. Without vitamin D supplements	85 (84–89)	NA	88 (84–92)	NA	3/NA/NA:45 vs. 10/NA/NA: 32

(Continued)

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TABLE 1 (Continued)

Study and Country	Type of study and patients source	Intervention and Control	Vitamin D supplements		Control		Number of deaths/Intubation or Mechanical ventilation requirement/ICU admission: number of intervention or control
			Age	25(OH)D levels before/after treatment(ng/ml)	Age	25(OH)D levels before/after treatment(ng/ml)	
Güven et al. (12), Turkey	Cohort Study, Hospitalized patients	Inject 300,000 IU cholecalcifero in the first 24 h of admission vs. Without vitamin D supplements	74 (60–81)	6.65 (5.06–9.1)/NA	75 (62–83)	7.14 (5.17–8.21)/NA	43/44/NA:113 vs. 30/31/NA:62
Xavier et al. (13), Spain	Cohort Study, Hospitalized patients	Oral 532 μg calcifediol on day 1 plus 266 μg on days 3, 7, 15, and 30. vs. Without vitamin D supplements	61.81 ± 15.5	13(8–24)/NA	62.41 ± 17.2	12 (8–19)/NA	21/NA/20:447 vs. 47/NA/82: 391
Soliman et al. (25), Egypt	Cohort Study, Hospitalized patients with type 2 diabetes	Inject a single dose of 200,000 IU cholecalciferol vs. Placebo	71.30 ± 4.16	10.4 ± 1.3/20.54 ± 3.00	70.19 ± 4.57	21.17 ± 3.96/21.23 ± 3.98	7/14/NA: 40 vs. 3/7/NA: 16
Alcala-Diaz et al. (26), Spain	Cohort Study, Hospitalized patients	Oral 0.532 mg calcifediol at day 0, 0.266 mg on days 3 and 7, and then weekly. vs. Without vitamin D supplements.	69 ± 15	NA	67 ± 16	NA	4/3/NA: 79 vs. 90/26/NA: 458
Jevalikar et al. (27), India	Cohort Study, Hospitalized patients	A single oral dose of 60,000 IU cholecalciferol vs. Without vitamin D supplements.	45.5 ± 18.2	<20/NA	48.8 ± 14.7	<20/NA	1/NA/16:128 vs. 3/NA/13: 69

[a]Only 16 participants from two study sites had their blood samples drawn for measurement of serum 25(OH)D. Calcifediol, 25-hydroxyvitamin D3; calcitriol, 1,25-Dihydroxyvitamin D3; cholecalciferol, vitamin D3; IQR, interquartile range; NA, not available. This table presented data as mean ± SD, or median (IQR).

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consisting of 8 RCTs (8–10, 20–24), and 8 cohort studies (5, 6, 11–13, 25–27).

Study characteristics and risk of bias of the included literature

Table 1 presents the characteristics of the included studies. The RCTs included 1,318 subjects, with 677 in the vitamin D supplementation group and 641 in the control group. The cohort studies included 2,041 subjects, with 976 in the vitamin D supplementation group and 1,065 in the control group. All the studies were carried out in hospitals, except for one which was conducted in a nursing home in France (6). The sample sizes of RCTs ranged from 43 to 543, with mean or median ages ranging from 10.7 to 69 years and follow-up from 7 days to 4 months (8–10, 20–24). Cholecalciferol was administered in the intervention arm of six RCTs (9, 10, 20–22, 24), while calcifediol (23) and calcitriol (8) were used in the remaining two RCTs. The sample sizes of the eight cohort studies ranged from 48 to 785, with mean ages ranging from 45.5 to 87.7 years, and follow-up from 5 days to 3 months. Cholecalciferol was administered in the intervention arm of six cohort studies (5, 6, 11, 13, 25, 27), and calcifediol was administered in the remaining two studies (12, 26). Out of the 16 included studies, only 10 reported the mean baseline levels of serum 25(OH)D, which ranged from 6.65 to 32.5 ng/ml in the intervention groups and 7.14 to 30.5 ng/ml in the control groups (Table 1).

Four RCTs had a low risk of bias (10, 20, 22, 24), one was at a high risk of bias (21) and the rest three studies had an uncertain risk of bias (8, 9, 23) (Supplementary Figures 1, 2). Six cohort studies had a moderate risk of bias (5, 12, 13, 25–27), and the other two had a serious risk of bias (6, 11) (Supplementary Figure 3).

GRADE assessment

The quality of evidence was assessed using the GRADE methods, as presented in Table 2. The certainty of the evidence for mortality (RCTs were very low, cohort studies were low), ICU admission (both RCTs and cohort studies were very low), and mechanical ventilation or intubation (both RCTs and cohort studies were very low) were rated as low to very low due to the heterogeneity in drug type and dosing, population characteristic, and the quality of the included studies.

Outcomes of meta-analyses

Effect of vitamin D supplementation on mortality

All eight RCTs (n = 1,318) and eight cohort studies (n = 2,041) reported the effect of vitamin D supplementation on mortality in COVID-19 patients. The meta-analysis of RCTs indicated no significant difference in mortality between the intervention group and control group (RR = 0.94, 95% CI 0.69–1.29, P = 0.7; fixed effect model; very low-certainty evidence; Figure 2). For the

TABLE 2 The Grading of Recommendation, Assessment, Development, and Evaluation (GRADE).

Outcome	Certainty assessment							No. of patients		Effect	Certainty
	Study design	No. of studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Vitamin D	Control	Relative risk (95% CI)	
Mortality	Cohort studies	8	Serious[a]	Not serious	Serious[b]	Not serious	None	105/976 (10.8%)	201/1065(18.9%)	RR 0.33(0.23–0.47)	⊕⊕◯◯ Low
	Randomized controlled trials	8	Serious[a]	Not serious	Serious[b]	Serious[d]	None	59/677 (8.7%)	65/641(10.1%)	RR 0.94(0.69–1.29)	⊕◯◯◯ Very low
ICU admission	Cohort studies	2	Serious[a]	Serious[c]	Serious[b]	Serious[d]	None	36/575 (6.3%)	95/460 (20.7%)	RR 0.32(0.08–1.29)	⊕◯◯◯ Very low
	Randomized controlled trials	6	Serious[a]	Serious[e]	Serious[b]	Serious[d]	None	86/605(14.2%)	103/562 (18.3%)	RR 0.64(0.38–1.08)	⊕◯◯◯ Very low
Mechanical ventilation or intubation	Cohort studies	3	Serious[a]	Not serious	Serious[b]	Serious[d]	None	64/536 (11.9%)	61/232 (26.3%)	RR 0.93(0.55–1.58)	⊕◯◯◯ Very low
	Randomized controlled trials	5	Serious[a]	Not serious	Serious[b]	Serious[d]	None	49/331(14.8%)	69/325 (21.2%)	RR 0.66(0.39–1.10)	⊕◯◯◯ Very low

CI, confidence interval; RR, risk ratio.

[a]Some do not concern with the method of randomization used/allocation concealment/blinding of participants/blinding of outcome assessment/selective reporting.

[b]There were differences in vitamin D dosages and duration.

[c]I^2=90%;

[d]The confidence interval was not narrow enough for us to be confident that this is the effect or does it reduce and has no effect.

[e]I^2=60%. Grades of evidence: High: Further research is very unlikely to change our confidence in the estimate of effect; Moderate: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; low: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; Very low: Any estimate of effect is very uncertain. The number of plus symbols indicate a higher degree of certainty.

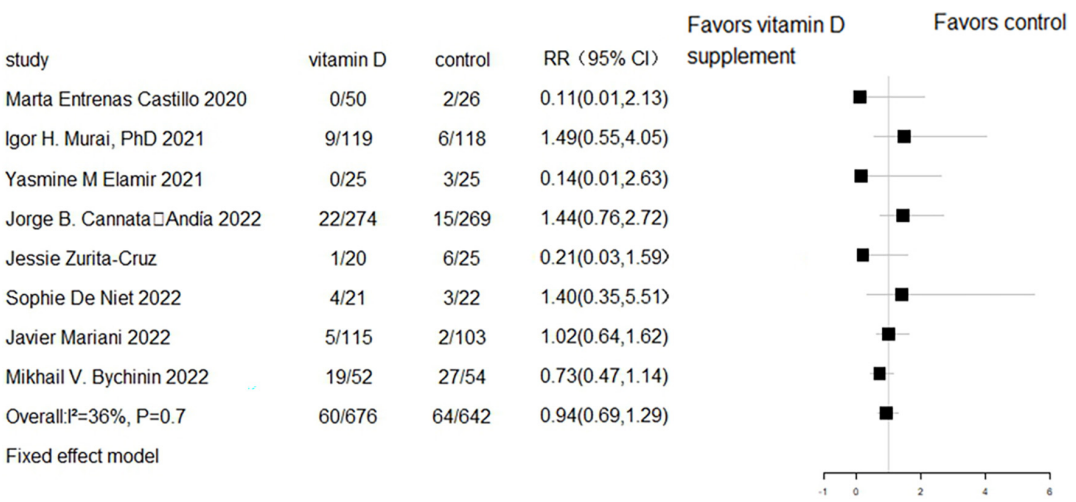

FIGURE 2
Forest plot of RCTs for vitamin D supplementation on mortality.

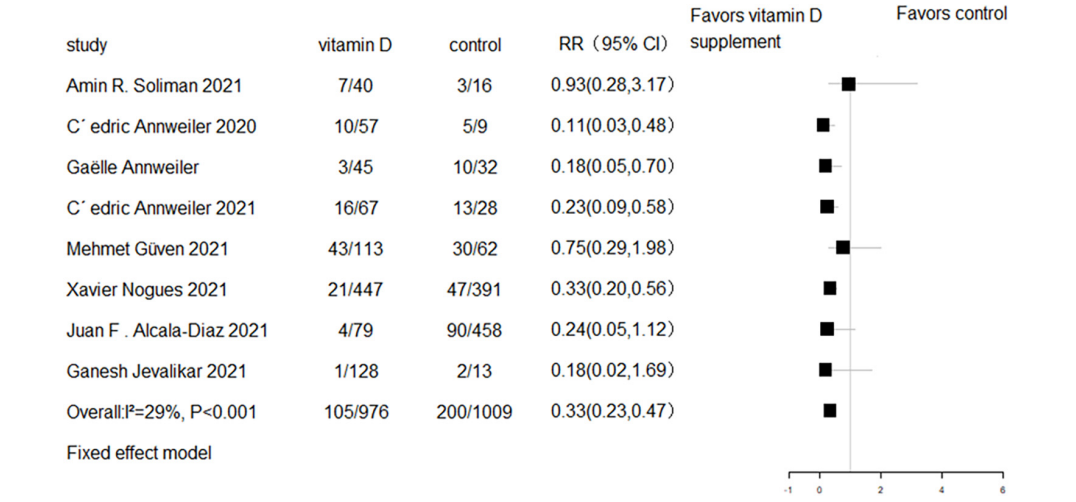

FIGURE 3
Forest plot of cohort studies for vitamin D supplementation on mortality (All cohort studies).

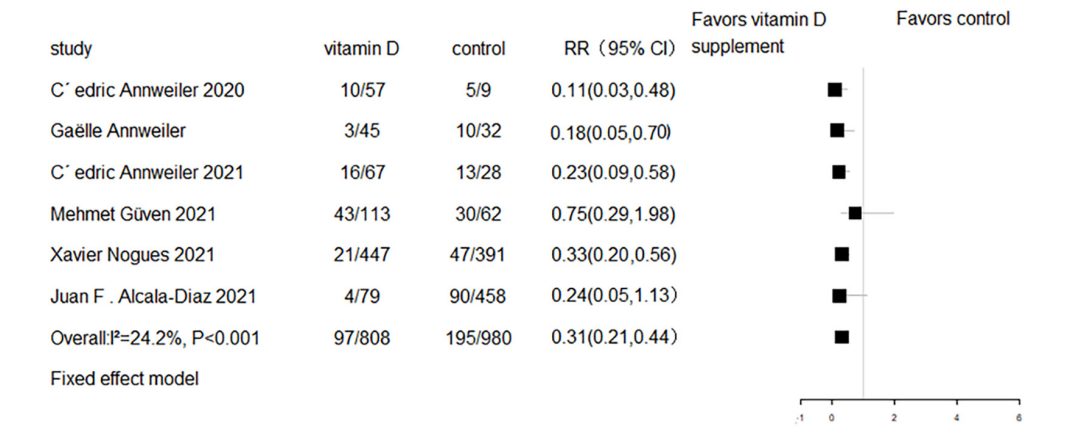

FIGURE 4
Forest plot of cohort studies for vitamin D supplementation on mortality (studies with adjusted RR values only).

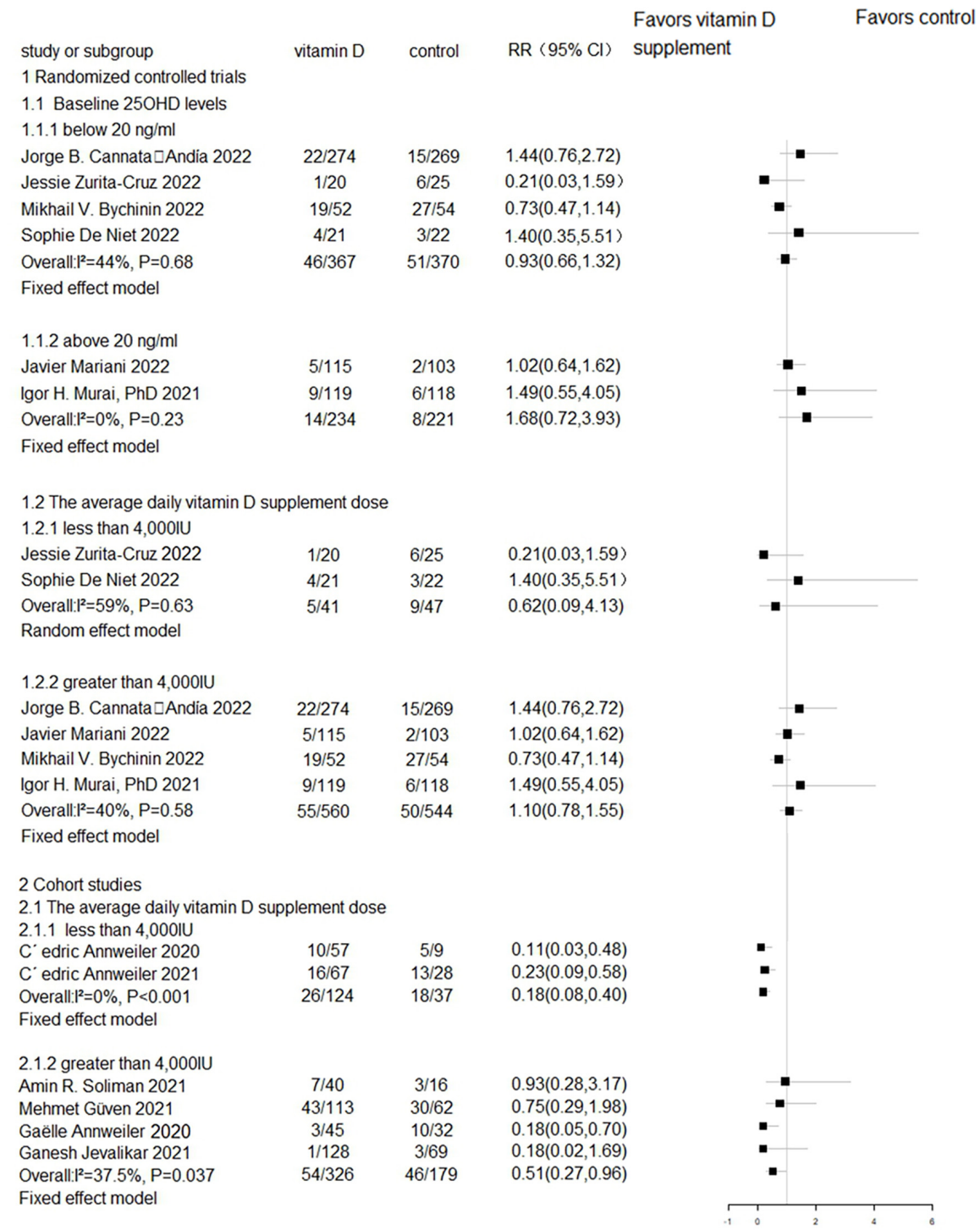

FIGURE 5
Subgroup analyses of mortality.

eight cohort studies, three reported adjusted HRs, another three reported adjusted ORs, and the remaining two studies reported the number of deaths. Subjects with vitamin D supplementation had significantly lower mortality than the control group (RR = 0.33, 95% CI 0.23–0.47, $P < 0.001$; fixed effect model; low-certainty evidence; Figure 3). The results remained consistent even after excluding studies that reported unadjusted RRs or numbers of deaths (RR = 0.31, 95% CI 0.21–0.44, $P < 0.001$; fixed effect model; Figure 4).

We performed subgroup analyses to investigate the association between the average daily vitamin D supplement dose and serum 25(OH)D levels with mortality. The results revealed no significant differences in mortality between individuals with baseline 25OHD levels below 20 ng/ml (RR = 0.93, 95% CI 0.66–1.32, $P = 0.68$) (9,

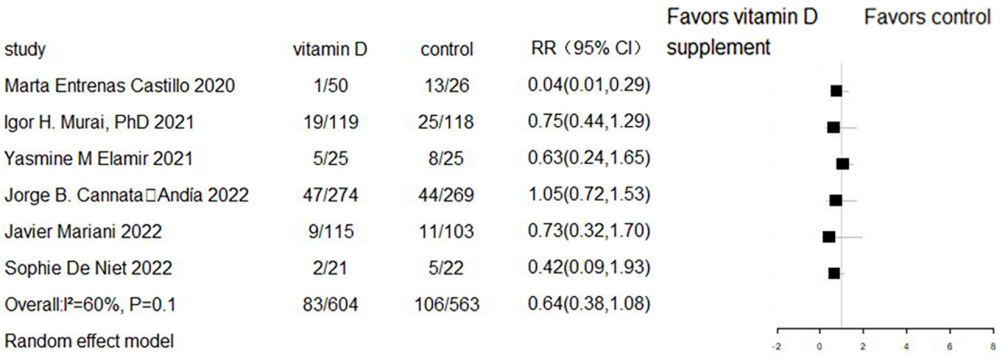

FIGURE 6
Forest plot of RCTs for vitamin D supplementation on ICU admission.

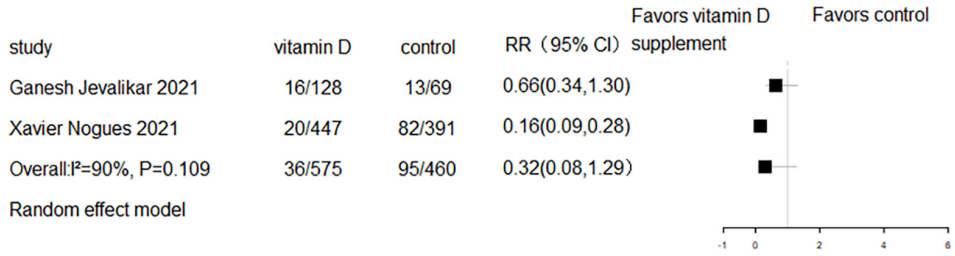

FIGURE 7
Forest plot of cohort studies for vitamin D supplementation on ICU admission.

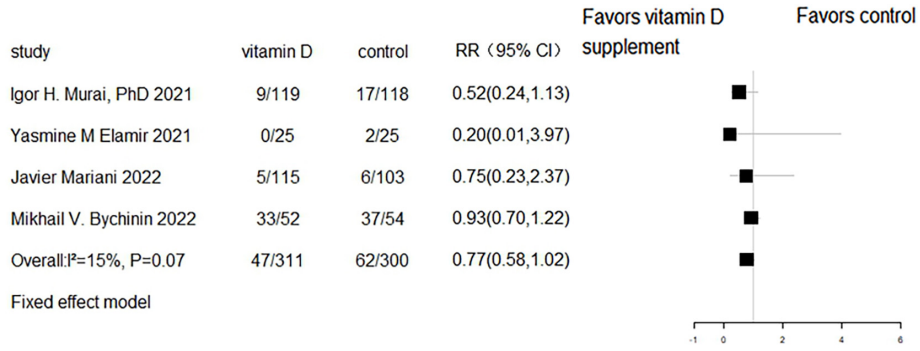

FIGURE 8
Forest plot of RCTs for vitamin D supplementation on mechanical ventilation or intubation.

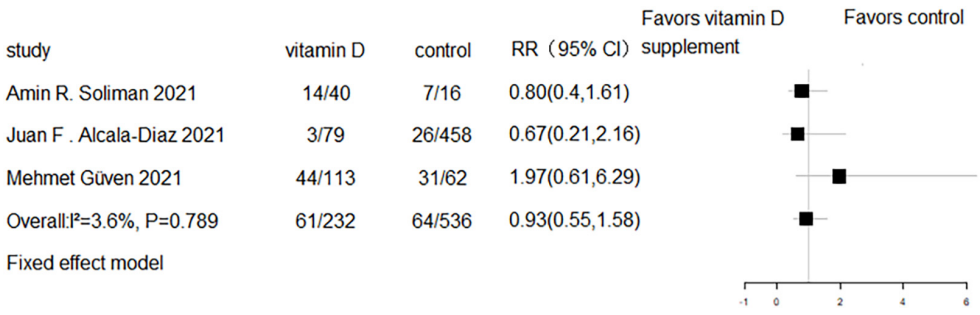

FIGURE 9
Forest plot of cohort studies for vitamin D supplementation on mechanical ventilation or intubation.

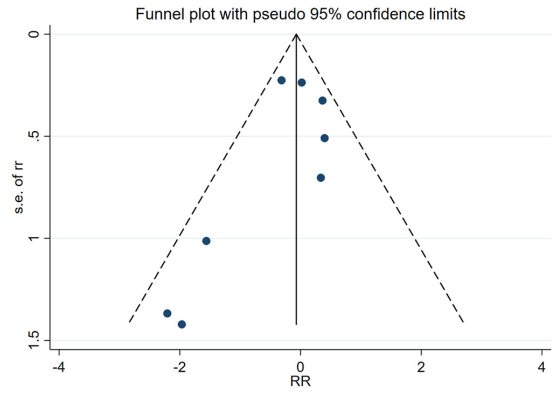

FIGURE 10
Funnel plot of RCTs.

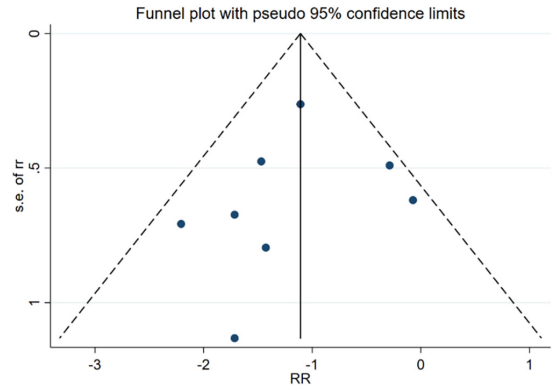

FIGURE 11
Funnel plot of cohort studies.

21, 22, 24) and those with levels above 20 ng/ml (RR = 1.68, 95% CI 0.72–3.93, P = 0.23) (10, 20), or between individuals receiving average daily vitamin D supplementation doses less than 4,000 IU (21, 24) (RR = 0.62, 95% CI 0.09–4.13, P = 0.63) and those receiving doses greater than 4,000 IU (9, 10, 20, 22) (RR = 1.10, 95% CI 0.78–1.55, P = 0.58). However, the results from cohort studies indicated that there was a significant reduction in mortality among individuals receiving average daily vitamin D supplementation doses less than 4,000 IU (5, 6) (RR = 0.18, 95% CI 0.08–0.40, $P < 0.001$) and those receiving doses greater than 4,000 IU (11, 12, 25, 27) (RR = 0.51, 95% CI 0.27–0.96, P = 0.037) (**Figure 5**).

The effect of vitamin D supplementation on ICU admission

Six RCTs and two cohort studies reported the effect of vitamin D supplementation on ICU admission. Meta-analyses showed that there was no difference in ICU admission between the vitamin D supplementation and control groups in either RCTs (RR = 0.64, 95%CI 0.38–1.08, P = 0.10; random effect model; very low-certainty evidence; **Figure 6**) or cohort studies (RR = 0.32, 95% CI 0.08–1.29, P = 0.109; random effect model; very low-certainty evidence; **Figure 7**).

The effect of vitamin D supplementation on mechanical ventilation or intubation

Five RCTs and three cohort studies reported the effect of vitamin D supplementation on mechanical ventilation or intubation. Meta-analyses of RCTs (RR = 0.77, 95% CI 0.58–1.02, P = 0.07; fixed effect model; very low-certainty evidence; **Figure 8**) and cohorts (RR = 0.93, 95% CI 0.55–1.58, P = 0.789; fixed effect model; very low-certainty evidence; **Figure 9**) showed that there was no difference in mechanical ventilation or intubation rate in COVID-19 patients with or without vitamin D supplementation.

Publication bias

No evidence of publication bias was identified through the analysis of the funnel plots (RCTs' Egger's test P = 0.266, **Figure 10**; cohort's Egger's test P = 0.604, **Figure 11**).

Discussion

This present meta-analysis included eight RCTs (8–10, 20–24) and eight cohort studies (5, 6, 11–13, 25–27) involving a total of 3,359 subjects. The results of pooled data indicated that vitamin D supplementation did not significantly reduce mortality, ICU admission, or rates of mechanical ventilation and intubation in COVID-19 patients. The conclusion should be interpreted with caution due to the low quality of the studies included, their small sample sizes, and significant baseline heterogeneity in baseline factors, including drug type and dosing, and population characteristics.

It is widely recognized that vitamin D can regulate the immune system, and its deficiency has been linked to an increased risk of developing the "cytokine storm" associated with COVID-19 (28). Recent reviews of the literature have also suggested that optimizing vitamin D levels in the general population may have served as a protective measure against COVID-19 infection (29, 30). Our study is not the first meta-analysis of vitamin D supplementation in COVID-19 patients. A previous meta-analysis published in 2021 (31) comprising 3 RCTs (20, 23, 32) and 2 cohort studies (6, 11) found that vitamin D supplementation did not result in a significant reduction in mortality, ICU admission rates, or mechanical ventilation (31). Another meta-analysis published in 2021 (33) involving 2 RCTs (20, 23) and 1 case-control study (34) showed that vitamin D supplementation resulted in comparable mortality but lower intensive care unit needs in patients with COVID-19. These two meta-analyses pooled studies with different study types and had much smaller sample sizes than our study. Our meta-analysis was based on a comprehensive search strategy and use established scales to assess the quality of research and strength of evidence. Furthermore, adjusted ORs were used to minimize bias in cohort studies. As a result, our conclusions are more robust and reliable compared to previous meta-analyses.

The pooled analysis found an inconsistent effect of vitamin D supplementation on mortality in cohort studies and RCTs. Although evidence showed that patients receiving higher cumulative doses and average daily doses had a greater decrease in COVID-19 infection rates compared to those receiving lower doses (35), subgroup analysis indicated that there were no significant differences in mortality between individuals with lower or higher baseline 25OHD levels, as well as those receiving small or larger vitamin D supplementation doses in RCTs. Nevertheless, the results from RCTs were more reliable due to the superior methodology.

There are some limitations in this meta-analysis, including the small sample sizes and low quality of the included RCTs and cohort studies, as well as the lack of complete information regarding the study population, such as race, sex, and 25(OH)D level before and after vitamin D supplementation. There was also significant heterogeneity among the included studies in terms of drug type and dosing, population features, and COVID-19 severity and treatment strategies.

In conclusion, while the results of this meta-analysis suggest that vitamin D supplementation may not significantly reduce mortality, ICU admission, and rates of mechanical ventilation intubation in COVID-19 patients, additional well-designed RCTs with large sample sizes are needed to further explore the potential benefit of vitamin D supplementation in this population.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

YZ, JL, and QW designed the review. YZ and JL conducted the systematic review and extracted data. MY and YZ performed the data analysis. JL and QW wrote the manuscript. QW had primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1131103/full#supplementary-material

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Review on the health-promoting effect of adequate selenium status

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Selenium is an essential microelement involved in various biological processes. Selenium deficiency increases the risk of human immunodeficiency virus infection, cancer, cardiovascular disease, and inflammatory bowel disease. Selenium possesses anti-oxidant, anti-cancer, immunomodulatory, hypoglycemic, and intestinal microbiota-regulating properties. The non-linear dose-response relationship between selenium status and health effects is U-shaped; individuals with low baseline selenium levels may benefit from supplementation, whereas those with acceptable or high selenium levels may face possible health hazards. Selenium supplementation is beneficial in various populations and conditions; however, given its small safety window, the safety of selenium supplementation is still a subject of debate. This review summarizes the current understanding of the health-promoting effects of selenium on the human body, the dietary reference intake, and evidence of the association between selenium deficiency and disease.

KEYWORDS

inorganic selenium, organic selenium, selenium intake, health-promoting effects, selenium status

1. Introduction

Selenium is an essential trace element for the human body that was discovered in 1817 by the Swedish chemist Berzelius (1). Numerous studies have demonstrated that selenium possesses anti-oxidant, anti-cancer, immunomodulatory, hypoglycemic, and intestinal microbiota-regulating properties (2–5). Selenium deficiency can result in diminished immunity and increased vulnerability to infections, such as human immunodeficiency virus (HIV) and hepatitis B infections. Long-term selenium deficiency increases the risk of diseases such as Kaschin–Beck disease (KBD), Keshan disease (KD), acquired immunodeficiency syndrome (AIDS), cancer, cardiovascular disease (CVD), and inflammatory bowel disease (IBD) (6). According the World Health Organization (WHO), selenium intake is inadequate in multiple countries, including India, Belgium, Brazil, the United Kingdom, France, Serbia, Slovenia, Turkey, Poland, Sweden, Germany, Spain, Portugal, Denmark, Slovakia, Greece, the Netherlands, Italy, China, Austria, and Ireland (7). Therefore, reasonable selenium supplementation is essential for the human body. However, the safe selenium intake level is limited and not well defined (8).

This review examines the classification, food sources, clinical diseases, health-promoting effects, and dietary reference intake of selenium, as well as its relationships with AIDS, cancer, CVD, and IBD (9–11). Further, we provide recommendations for selenium intake in various populations and for the resolution of health issues caused by selenium deficiency.

2. Selenium classification

In nature, selenium exists in inorganic and organic forms. Inorganic selenium is obtained from metal deposit byproducts, primarily selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) (12, 13). Selenate and selenite are rare in nature and are typically complexed with sodium to form sodium selenite and sodium selenate, respectively (**Figure 1**) (14). Organic selenium is formed through the biotransformation of selenium and amino acids, primarily including selenomethionine (SeM) and selenocysteine (SeC) (**Figure 1**) (15).

As inorganic selenium is hardly absorbed by the human body and highly toxic, only a trace amount of this form is obtained from food (16). Organic selenium is more biocomand patible and more readily absorbed and stored in tissues than inorganic selenium, significantly improves the plasma selenium status in the human body. As a result, organic selenium exhibits greater biological activity and is therefore more widely used in supplement production (17, 18). For instance, when organic selenium is used as a selenium supplement for livestock, the selenium enrichment effect is greater than when inorganic selenium is used (15). Selenium is present primarily in the organic form in the majority of natural and selenium-rich foods. Selenium is primarily found in meat, eggs, bread, and fish (19, 20).

3. Environmental selenium exposure

3.1. Geographical distribution of selenium

Selenium is found in trace amounts in the Earth's crust, typically in the range of 0.05–0.09 mg/kg. Soils can be classified into selenium-deficient (<0.13 mg/kg), selenium-marginal (0.13–0.18 mg/kg), selenium-sufficient (0.18–0.40 mg/kg), selenium-rich (0.40–3.00 mg/kg), and excess-selenium (>3.00 mg/kg) soils (21). Selenium is unequally distributed globally, with the Americas accounting for 52.7% of proven global selenium reserves, followed by Asia and Africa, which account for 15.4% each, Europe, which accounts for 12.2%, and Oceania, which accounts for 4.4% (22). While China's selenium reserves are among the world's largest and at present can meet the national selenium demand, the problem of unequal selenium resource distribution persists (23). Enshi, Hubei Province is dubbed the world's selenium capital because of its widespread and large selenium resources and because it accommodates the world's only independent selenium deposit. However, 22 provinces of China, accounting for 72% of the country's territory, face selenium resource scarcity, with 30% being classified as severe selenium-deficient areas.

3.2. Form and distribution of selenium in foods

The amount of selenium in foods is highly variable and is influenced by the location of crops or the composition of the feed taken by animals. Bread, grains, meat, nuts, fish, eggs, and milk and other dairy products are major sources of selenium (20, 24).

The difference in selenium content between bread and cereals is 0.01–30 mg/kg, with the majority of selenium being in the

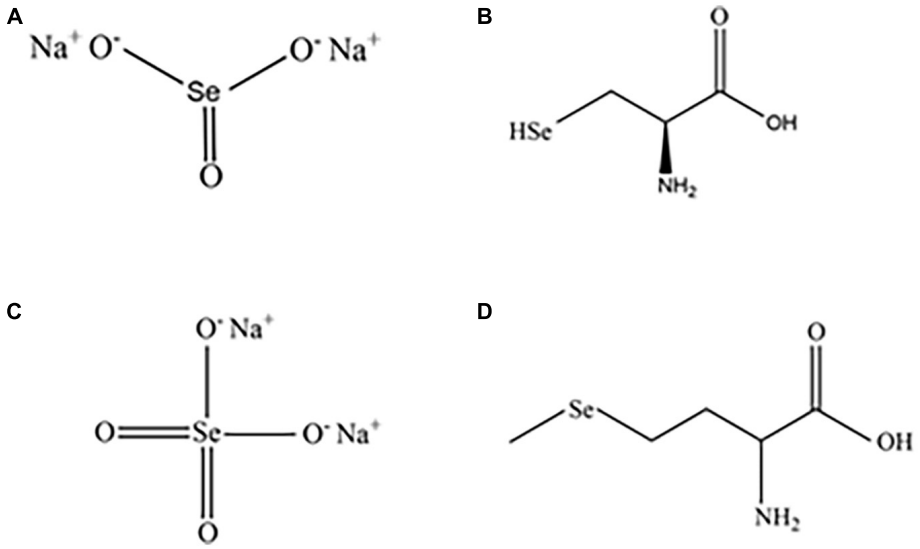

FIGURE 1
Structures of common inorganic and organic selenium compounds. Sodium selenite **(A)**, sodium selenate **(B)**, SeC **(C)**, and SeM **(D)**.

forms of SeM (55–85%), SeC (4–12%), and selenate (25, 26). The selenium amounts of meat, fish, and eggs vary between 3 and 25 g, and the selenium concentration even varies among different sections of meat (19). Internal organs, particularly the liver and kidneys, contain comparatively high levels of selenium. For example, selenium concentrations in beef kidney, liver, and heart tissues are 4.5, 0.93, and 0.55 mg/kg, respectively, whereas muscle concentrations range between 0.2 and 0.55 mg/kg (27). In meat, selenium primarily exists as SeM (50–60%) and SeC. In fish, selenium contents typically range between 0.1 and 5.0 mg/kg, and selenium is primarily in the forms of SeM (29–70%) and selenite or selenate (12–45%) (28, 29). Milk contains selenium primarily in the forms of SeC and selenite. However, when selenium-enriched yeast is used to supplement selenium in milk from dairy cows, the type of selenium in the milk changes. Selenium is currently found mostly in the forms of SeC, SeM, and selenite (30). Fruits and vegetables also contain selenium, and vegetables cultivated in selenium-rich soils can enrich and transform the element. For instance, when onions, garlic, and broccoli are produced in selenium-rich soil, selenium levels can increase from <0.5 mg/kg to 140–300 mg/kg (31).

In addition to supplementation through selenium-enriched foods, selenium supplements are an efficient direct supplementation method. Selenium is currently available as multivitamin and multimineral supplements as well as stand-alone supplements, typically in the form of SeM, selenium-enriched yeast (grown on a selenium-rich medium), sodium selenite, or sodium selenate (23, 32, 33). Selenium-enriched yeast is the most common dietary source of selenium, primarily in the form of cysteine (34).

3.3. Selenium deficiency-related diseases

Selenium is a trace element that plays critical roles in human growth and development. It promotes human health by assisting in metabolism, boosting immunity, increasing physical fitness, and delaying aging. Selenium deficiency can impair body function and result in various diseases, including KBD, KD, neurological system disorders, and immunological deficiency disorders (35–38). Selenium deficiency can be diagnosed by measuring the serum or plasma selenium level, which should be at least 85 μg/L (39).

3.3.1. KBD

KBD is an endemic, chronic, and degenerative osteoarthropathy that occurs in selenium-deficient parts of the world. It is the most prevalent in the diagonal zone extending from northeast to southwest China, but also occurs in Mongolia, Siberia, and Korea. It is a type of osteoarthritis characterized by cartilage tissue atrophy, degradation, and necrosis. It is the most prevalent in youngsters between the ages of 5 and 13 years. The primary signs include swollen joints, shortened fingers and toes, growth retardation, and stunting (35, 40). Patients with KBD have unusually low selenium levels in the hair and whole blood, and markedly decreased glutathione peroxidase levels in the blood (35, 40). A 0.1% sodium selenite aqueous solution is often used to treat children with KBD, with great results (41). A meta-analysis of 10 randomized controlled studies revealed the efficacy of selenium supplementation in the treatment of individuals with KBD; however, the data are limited by the possibility of bias (42). Zou et al.'s meta-analysis of KBD indicates that selenium supplementation is useful for preventing KBD in children (43). In a double-blind, randomized, controlled experiment, Moreno-Reyes et al. reported that supplementation with 100 g of selenium per day reduced clinical symptoms of KBD in children aged 5 to 15 years (44).

3.3.2. KD

KD is a endemic cardiomyopathy that is prevalent in parts of China lacking in selenium. It is the most common in children between the ages of 2 and 10 years and in women of reproductive age. KD occurs across northeast to southwest China. The disease's primary clinical manifestations include acute or chronic heart attacks marked by exhaustion, arrhythmia, and palpitation following limited exertion, inappetence, cardiac insufficiency, cardiac hypertrophy, and congestive heart failure. It is classified into four clinical subtypes: acute, subacute, chronic, and latent. Except for the latent form, case fatality rates are quite high. Pathological changes include numerous foci of cardiac necrosis and fibrosis. Ultrastructural examinations have revealed that membrane organelles, such as the mitochondria, and the sarcolemma are the first to be affected. The disease has a seasonal prevalence and can emerge as soon as three months following exposure to conditions that increase the risk of myocarditis (35, 45).

The mean hair selenium concentration in KD areas is <0.122 mg/kg, whereas it is >0.200 mg/kg in non-KD areas. The selenium concentrations in KD patients' muscle, heart, liver, and kidneys are 10-fold lower than those in healthy people (35). The WHO recommends a minimum selenium intake of 21 mg/d for men and 16 mg/d for women to avoid KD development (46). Oral selenium is a very effective preventative strategy during the first three months of the KD risk period. Oral sodium selenite successfully prevents KD and considerably reduces its incidence rate (41). In a 10-year follow-up study of 302 patients with chronic KD and congestive heart failure, Zhu et al. showed that weekly supplementation with 1 mg of selenium decreased mortality (47). A comprehensive review and meta-analysis of Kawasaki disease indicate that selenium supplementation considerably lowers the incidence of Kawasaki disease (48).

Biochemical and clinical investigations have suggested that reduced glutaminase activity may decrease mitochondrial defense against peroxide-induced membrane damage and thus cause KD (36). KD is caused by selenium deficiency in association with coxsackie enterovirus infection. Inadequate selenium intake results in reduced selenoprotein anti-oxidant activity, and oxidative damage to viral DNA enhances its toxicity (49).

3.3.3. Nervous system disease

Selenium is differentially distributed in various parts of the brain, with peak concentrations in gray matter-rich areas and glands (50). When a diet has insufficient selenium, brain selenium is retained in the organs, interfering with the normal supply route and resulting in the development of severe neurological dysfunction (51). Selenium plays a critical role in the brain and selenium deficit results in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and epilepsy (38, 52). Nervous system diseases can be avoided by supplementation with selenium-rich yeast. Selenium supplementation is effective

in reducing intractable epileptic seizures in children (39). Cardoso et al. conducted a randomized, controlled study of 40 Alzheimer's patients over a period of 24 weeks. The studies demonstrated that supplementation with 30 mg of selenium per day can enhance the selenium concentration in the central nervous system, halt neurodegeneration, and assist Alzheimer's disease patients (53). In a clinical investigation, erythrocyte lipid peroxidation and glutathione peroxidase activity were greater in individuals with refractory epilepsy than in normal adults; nevertheless, supplementation with 200 g/day decreased lipid peroxidation, glutathione peroxidase activity, and morbidity in epileptic patients (54).

3.3.4. Virus infection susceptibility caused by selenium deficiency

The development of early HIV infection has been associated with low plasma selenium levels. Subclinical malnutrition is a significant factor in the development of AIDS. However, there is compelling evidence that the magnitude of selenium deficiency is predictive of the occurrence of AIDS and associated mortality (37, 55). Selenium deficiency also enhances the toxicity of other RNA viruses, such as hepatitis B virus, and of hemolytic anemia. The underlying mechanisms are currently being investigated.

4. Human health-promoting effects of selenium

Selenium is an essential trace element in the human body, and supplementation with selenium has been shown to benefit human health. Selenium has anti-oxidant, anti-cancer, immunomodulatory, hypoglycemic, and intestinal microbiota-regulating properties, and its mechanisms of action have been investigated (**Figure 2**).

4.1. Anti-oxidant effects

When an organism is stressed or diseased, excess free radicals are generated and react with unsaturated lipids in the cell membranes, resulting in lipid peroxidation and severe damage to the biological system. Selenium is a component of glutathione peroxidase, which can convert hydroperoxide into hydroxy fatty acids, limiting lipid peroxidation by free radicals and lowering oxidative stress (56). It is widely recognized that selenium plays a direct or indirect role in removing intracellular free radicals (57, 58). Anti-oxidants prevent or mitigate oxidative DNA damage, and

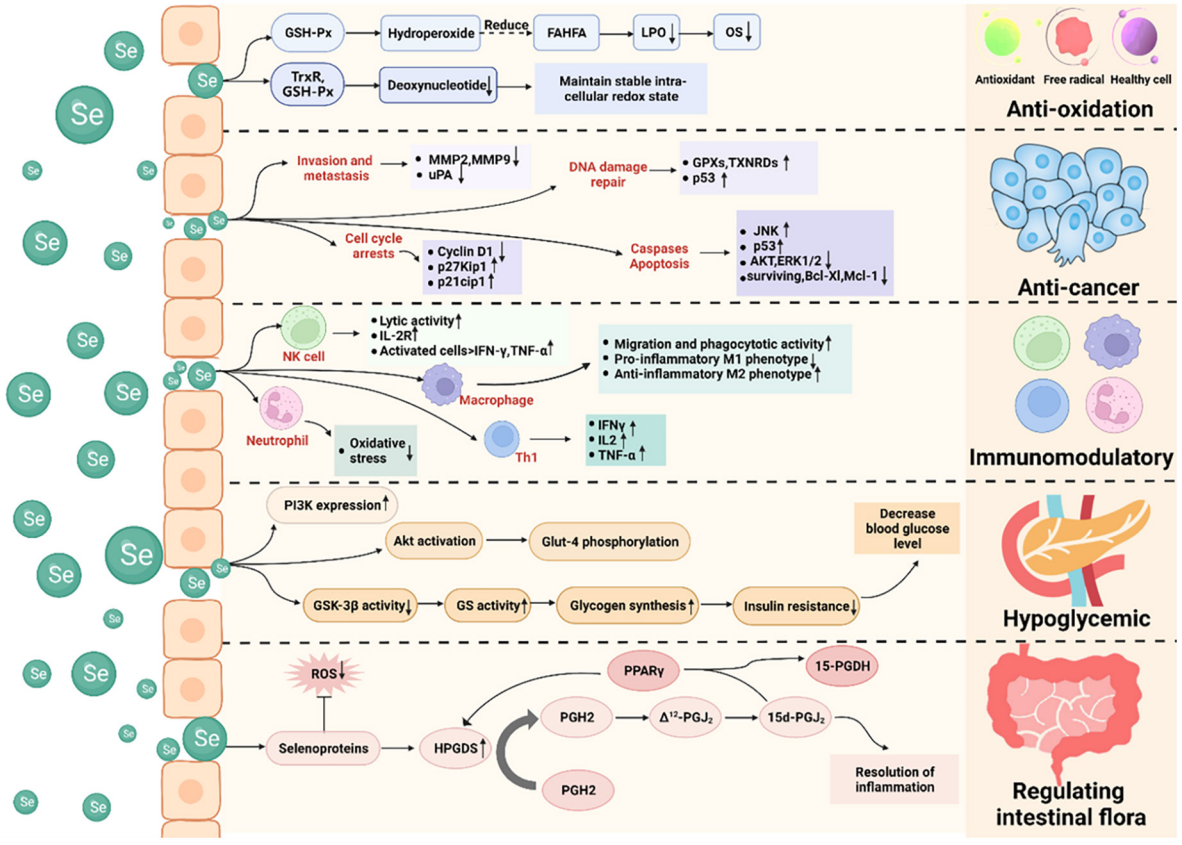

FIGURE 2
Health-promoting effects of selenium and its mechanisms of action. Selenium possesses anti-oxidant, anti-cancer, immunomodulatory, hypoglycemic, and intestinal microbiota-regulating properties. It acts as an anti-oxidant by lowering oxidative stress and deoxynucleotide levels. It functions as an anti-cancer agent by inducing apoptosis and cell-cycle arrest, preventing tumor cell invasion and metastasis, and promoting DNA damage repair. It exerts immunomodulatory effects by affecting non-specific immunity (e.g., macrophages and neutrophils) and specific immunity (e.g., T and B lymphocytes). It exerts hypoglycemic action by regulating the IRS-PI3K-Akt signaling pathway. It regulates the intestinal microbiota by regulating prostaglandins.

anti-oxidant enzymes require mineral cofactors, such as selenium for glutathione peroxidase and zinc and copper for superoxide dismutase (59, 60). Selenium functions as a redox center, protecting tissues from free radical-induced cell damage. Selenase and thioredoxin reductase are both capable of reducing nucleotides in deoxyribonucleic acid and thus maintain intracellular redox homeostasis (39).

An excess amount of reactive oxygen species (ROS) in the bloodstream causes DNA damage and oxidative stress in cells (61). Cells are predisposed to oxidative stress when their anti-oxidant contents are low or their ROS levels high. Selenium can prevent an overabundance of ROS, preserve the redox state of cells, and suppress oxidative stress (62). Fujieda et al. demonstrated that selenium deficiency results in a considerable decrease in glutathione peroxidase activity, which results in an increase in oxidative stress levels, and that sodium selenite treatment is efficient in ameliorating this condition (63). Plasma selenium levels are adversely correlated with oxidative stress levels in children with upper respiratory tract infections, patients with oral orofacial inflammatory disease, and pregnant women (64–66). However, Gać et al. measured plasma selenium concentrations, oxidative stress levels, and total anti-oxidant status in 337 children (mean age: 8.53 ± 1.92 years) and found that the plasma selenium concentration was not negatively correlated with oxidative stress levels, but was positively correlated with the total urine anti-oxidant status. Increased plasma selenium concentrations in healthy children have been shown to improve their overall anti-oxidant status (67). Thus, selenium exhibits anti-oxidant activity and suppresses oxidative stress, hence protecting the human body from oxidative stress-induced damage (4). Studies have demonstrated that high dosages of selenium can elicit cytotoxicity by elevating intracellular ROS, resulting in DNA damage and oxidative stress. High doses of selenium can also cause decreased immunological function and carcinogenic effects (68). Zachariah et al. studied the effects of high dosages of selenium on endothelial cells and reported that high doses of selenium inhibited NO bioavailability and angiogenesis. In addition to inducing ER stress and increasing the generation of ROS, selenium at high dosages can cause endothelial dysfunction (69). Consequently, selenium administration at high dosages induces oxidative stress, resulting in cytotoxicity and endothelial dysfunction. These findings highlight the significance and potential dangers of selenium supplementation as an antioxidant.

Owing to its anti-oxidant action, selenium is frequently employed in product development as a bioactive ingredient. Mileti et al. demonstrated that the addition of sodium selenite greatly improved DPPH clearance and Fe^{2+} chelation during exopolysaccharide synthesis (70). According to Xia et al., adding modest concentrations of selenium (0.5 and 1.0 mmol/L) increased the germination rate of alfalfa seeds and their superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase activities, and decreased their malondialdehyde content (71). Forootanfar et al. reported that selenium-containing nanoparticles and selenium dioxide had DPPH radical-scavenging activities of 23.1 ± 3.4% and 13.2 ± 3.1%, respectively, at the same dose (200 μg/mL). However, findings from reduction capability measurements indicated that selenium dioxide has higher electron donor activity than selenium-containing nanoparticles (72). Xiao et al. developed nanoparticles containing selenium that exhibit strong anti-oxidant activity (73).

In conclusion, selenium, both organic and inorganic, exhibits anti-oxidant effects (63). The organic forms are selenoprotein and SeC, and selenoprotein has a critical physiological role in the body. Approximately 50% of all known selenoproteins have anti-oxidant properties (39). Inorganic selenium acts as an anti-oxidant by lowering oxidative stress and increasing DPPH-scavenging and Fe^{2+}-chelating abilities (63).

4.2. Anti-cancer effects

The association between selenium and cancer has long been a source of controversy. However, in recent years, numerous studies have demonstrated the efficacy of selenium in suppressing carcinogenesis and enhancing immunity and anti-oxidant capacity.

Recently, there has been a surge of interest in the development of nanomaterials with increased anti-cancer activity and less adverse effects on the body as prospective cancer treatment options. In this light, selenium-containing nanoparticles are being investigated as potential cancer treatment agents because selenium is an essential trace element and nanomaterials containing selenium are more biocompatible. Selenium-containing nanomaterials have been found to have anti-ovarian cancer and anti-bone tumor properties. Toubhans et al. demonstrated that inorganic selenium nanoparticles triggered nanomechanical responses, changes in cell-surface roughness and membrane hardness, and cell apoptosis in SKOV-3 and OVCAR-3 ovarian cancer cells, indicating that selenium effectively inhibits the growth of ovarian cancer cells (2). Selenium-doped hydroxyapatite nanoparticles are frequently employed as bone-induction biomaterials. Barbanente et al. demonstrated that hydroxyapatite nanoparticles doped with selenium at low concentrations are biocompatible and may be used to treat bone cancers (74).

Selenium molecules in food undergo metabolic transformations via various pathways, producing a diversity of selenium metabolites with varying biological activities. Redox-active selenium metabolites have improved nucleophilic capabilities and high reactivity, making them powerful anti-cancer agents (75). At present, selenite is the most effective dietary selenium anti-cancer medication licensed by the United States Food and Drug Administration. When selenium is in the + 4 oxidation state as sodium selenite, it can react directly with the cysteine clusters found in the catalytic subunits of enzymes such as protein kinase C. Selenium compounds can oxidize the sulfhydryl groups in the catalytic domain of protein kinase C to disulfide bonds, inactivating the enzyme (76). This is because it oxidizes key thiol-containing enzymes and generates ROS. In addition, selenium compounds exert cytotoxic effects by acting as pro-oxidants, disrupting cellular redox homeostasis, and triggering selenium-induced apoptosis in mutant abnormal cells (57, 77).

Selenium promotes apoptosis, an important anti-cancer mechanism. Methylselenic acid (MSeA) has been found to increase caspase-mediated apoptosis by downregulating survivin, Bcl-xL, and Mcl-1 expression (78, 79). In LNCaP human prostate cancer cells, selenite induced p53 Ser-15 phosphorylation and caspase-mediated apoptosis (80). MSeA exposure induced caspase-mediated apoptosis in DU145 human prostate cancer cells, which

was associated with reduced phosphorylation of protein kinase B (Akt) and extracellular regulated kinase ½ (81). MSeA-induced G1 arrest in DU145 cells was associated with increased p27kip1 and p21cip1 expression (82). Selenium induced cell-growth arrest and death *in vivo*, which was associated with decreased cyclin D1 expression, increased p27kip1 expression, and the activation of c-Jun NH2-terminal kinase (JNK) (83).

Inhibition of tumor cell invasion and metastasis is another important anti-cancer mechanism of selenium. Matrix metalloproteinase (MMP)-2 and MMP-9 degrade the extracellular matrix and basement membrane and play key roles in tumor invasion and metastasis. The urokinase-type plasminogen activator (uPA) system has been associated with tumor invasion, metastasis, and decreased patient survival time (84). Selenite inhibits tumor cell invasion by inhibiting the expression of MMP-2, MMP-9, and uPA (85).

Stimulating DNA damage repair also is an important anti-cancer mechanism of selenium. Given the critical role of selenoproteins (such as glutathione peroxidases and thioredoxin reductases) in anti-oxidant defense and maintaining a reducing cell environment, selenium can accelerate the DNA damage repair response by enhancing selenoprotein production (86). SeM boosts p53 activity and protects cells from DNA damage via its anti-oxidant activity (87). However, Duffield-Lillico et al. discovered in a double-blind, randomized, placebo-controlled clinical study that selenium supplementation did not prevent basal cell carcinoma but increased the incidence of squamous cell carcinoma and non-melanoma skin cancer (82). Algotar et al. observed in a 5-year double-blind, randomized, placebo-controlled experiment that selenium intake of 200 or 400 μg per day increased the incidence of non-melanoma skin cancer (88). In a major clinical research study including 5,345 men, Kristal et al. reported that supplementation with selenium raised the risk of prostate cancer by 91% in men who previously ingested appropriate quantities of selenium (89). In a 22-year follow-up analysis of 4,459 patients with non-metastatic prostate cancer, Kenfield et al. reported that selenium supplementation of 140 μg or more per day may increase prostate cancer mortality (90). Thus, supplementation with selenium raises the incidence of squamous cell carcinoma, non-melanoma skin cancer, and high-grade prostate cancer (91).

In conclusion, selenium functions as an anti-cancer agent by triggering apoptosis and cell-cycle arrest, preventing tumor cell invasion and metastasis, and promoting DNA repair. The anti-cancer effects of selenium in colon, skin, breast, liver, lung, and rectal cancers have since long been documented. Selenium has great clinical potential as an anti-cancer agent (2, 3, 92–96). However, selenium supplementation raises the risk of squamous cell carcinoma, non-melanoma skin cancer, and high grade prostate cancer (91).

4.3. Immunomodulatory effects

The immune system is the most effective barrier against pathogen invasion. It recognizes and eliminates antigenic foreign substances and cooperates with other body systems to preserve homeostasis and physiological balance. Natural killer (NK) cells are vital immune cells that are involved in anti-tumor, anti-viral infection, and immunological control functions. Selenium is required for the regular functioning of the immune system and can affect non-specific immunity (e.g., macrophages and neutrophils) and specific immunity (e.g., T and B lymphocytes). Selenium deficiency results in immune system dysfunction, which harms immunological function. Broome et al. demonstrated that selenium supplementation raised plasma selenium levels, the body's exchangeable selenium pool, lymphocyte phospholipids, and cytosolic glutathione peroxidase activity. Selenium supplementation boosts cellular immune responses and the expression of cytokines by enhancing interferon secretion and increases early peak T cell proliferation and T helper cell counts. Subjects supplemented with selenium exhibited quick poliovirus elimination and the reverse transcriptase-PCR products of polioviruses recovered from their feces had a low number of mutations (97). Selenium supplementation promotes lymphocyte proliferation in response to mitogens, increases the expression of high-affinity IL-2 receptors, and enhances tumor cytotoxicity and NK cell activity mediated by cytotoxic lymphocytes (97, 98). Selenium supplementation has also been found to boost NK cell activity, T cell proliferation, lymphokine-activated killer cell activity, delayed onset of cutaneous allergy reactions, and vaccine-induced immunity in experimental animals (5).

Selenoprotein is thought to play a role in the epigenetic control of pro-inflammatory genes. Narayan et al. demonstrated that selenium supplementation decreased histone H4 acetylation at K12 and K16 in the *COX-2* and *TNF-α* promoters and of the p65 subunit of the redox-sensitive transcription factor nuclear factor kappa B in primary and immortalized macrophages, indicating the critical role of selenoprotein in inhibiting histone H4 acetylation (99). T cell acute lymphoblastic leukemia/lymphoma is a chemotherapy-sensitive hematologic malignancy. Wu et al. demonstrated that ethylene glycol selenoprotein-induced apoptosis in T cell acute lymphoblastic leukemia/lymphoma cells is mediated by caspase activation and increased ROS via the activation of mitochondrial signaling pathways (100). Jiang et al. demonstrated that selenium-enriched chitosan oligosaccharide effectively enhanced phagocytosis, anti-inflammatory cytokine secretion in peritoneal macrophages, phagocytotic, spleen, and thymus indices, and immunity, with no obvious toxicity, in Kunming mice (96). Albumin acts as a carrier of nutrients, whereas globulin is an immunoprotein. The albumin-to-globulin ratio in serum is a useful indicator of animal nutrition and immunological function (101). Interleukin (IL)-2 is a component of cellular immune responses and a critical immunological regulator, regulating cell development, differentiation, and proliferation and contributing to the resolution of viral or bacterial infection. In laying hens, a selenium-enriched earthworm powder containing 1.0 mg/kg selenium increased albumin, globulin, immunoglobulin G, and IL-2 expression levels (101).

Macrophages of the M2 phenotype produce anti-inflammatory cytokines, such as IL-10, which suppress tumor development (102). Selenium supplementation improved migratory and phagocytic activities in selenium-deficient macrophages and promoted the transition from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, thereby lowing pro-inflammatory

action (103). Selenium supplementation also provides protection against endogenous oxidative stress in neutrophils (104). In the elderly, increased serum selenium levels are positively associated with an increase in the number and activity of NK cells. Selenium has been shown to boost the expression of the IL-2 receptor on the NK cell surface, thus increasing the proliferation and clonal expansion of cytotoxic precursor cells and the lytic activity of activated NK cells (105). Activated NK cells exhibit cytotoxicity toward tumor cells and release immunoregulatory molecules such as IFN-γ and TNF-α (106). Selenium supplementation has been shown to have an effect on T cell activation and function (107). For example, a selenium-rich diet can shift the balance of T helper 1/T helper 2 cells toward the T helper 1 phenotype and increase IFN and CD40 ligand levels (108). However, Ivory et al. have shown that selenium administration increases IL-10 release and decreases CD8 T cell granzyme B levels in the blood (91, 109). Not only do perforin and granzyme destroy virus-infected cells and tumors, but they also modulate the immune response to viral infections (110). Immune modulation is impacted both positively and negatively by selenium administration, as demonstrated by the preceding results.

In conclusion, selenium can protect neutrophils from endogenous oxidative stress, increase the migratory and phagocytic activity of macrophages and promote the anti-inflammatory M2 type, and increase the lytic activity of NK cells in order to exert immunomodulatory effects. Selenium can also exert an immunomodulatory effect through the recruitment of T helper 1 cells and the release of pro-inflammatory cytokines. However, selenium can also diminish the number of CD8 T cells and granzyme B, which impacts the control of the immune system.

4.4. Hypoglycemic effects

Diabetes mellitus is a chronic metabolic endocrine disease that affects a large proportion of the global population. Diabetes affects approximately 425 million adults worldwide and this number has been projected to increase to 629 million by 2045 (111). Serum selenium levels do not appear to be related with newly diagnosed type 2 diabetes in humans, although they are considerably increased in individuals with type 2 diabetes. Selenium supplementation has been shown to increase the incidence of type 2 diabetes in elderly people, particularly men with high baseline selenium levels, but not in the general population (112, 113). However, a recent high-quality randomized controlled study showed that supplementation with selenium (200 μg/d) in the form of selenide yeast or L-selenomethionine had no effect on the incidence of type 2 diabetes (114). Therefore, it has been suggestion that increased selenium consumption may be associated with an increased risk of developing diabetes (115). In a study involving in 41,474 subjects, Lin et al. found that dietary selenium intake was positively associated with increased plasma glucose and glycosylated hemoglobin levels, as well as the risk of developing diabetes (116). Additionally, they observed a positive association between serum selenium levels and increased plasma glucose and glycosylated hemoglobin levels. This supports the notion that elevated plasma selenium levels are related with an increased risk of developing diabetes (117, 118). This is primarily because high selenium intake increases the expression of peroxisome proliferator-activated receptor coactivator (PGC-1), a transcriptional coactivator involved in cellular energy metabolism, which may be one of the primary causes of hyperglycemia associated with high selenium intake (119).

However, appropriate selenium supplementation is a critical component in controlling glucose homeostasis in humans (120). El-Borady et al. demonstrated that selenium nanoparticles can help prevent hyperglycemia by lowering plasma glucose levels. Selenium nanoparticles also enhanced insulin levels in the plasma and pancreas of diabetic rats and repaired damaged pancreatic tissue. Additionally, selenium nanoparticles reduced oxidative stress at the transcriptional and cellular levels and enhanced glutathione peroxidase activity (111). Chen et al. demonstrated that supplementing diabetic rats with selenium normalized the glucose-6-phosphatase, lactate dehydrogenase, and glycogen phosphorylase activities and restored glycogen levels to their pre-diabetic levels. Selenium supplementation may enhance glucose uptake and metabolism in the liver by regulating glucose metabolic enzyme activity and mediating insulin-like actions in diabetes (121).

Selenium polysaccharide has a substantial hypoglycemic effect as a particular target of the IRS-PI3K-Akt signaling pathway (122). Polysaccharides may have several hypoglycemic mechanisms. First, polysaccharides have been shown to increase PI3K expression. Second, selenium polysaccharide has been shown to activate Akt and phosphorylate Glut-4. Third, selenium polysaccharide may inhibit GSK-3 action, thus increasing glycogen synthesis and boosting glycogen synthesis (123). However, Zhou et al. have shown that long-term feeding of mice, rats, and pigs with a high-selenium diet (0.4–0.30 mg/kg diet) results in hyperinsulinemia, hyperglycemia, insulin resistance, and glucose intolerance (124). Several studies have demonstrated that a high selenium intake may enhance the activity of GPx1 and other selenoproteins, thereby altering the function of major regulators of glycolysis, gluconeogenesis, and fat synthesis, thereby increasing the prevalence of diabetes (125–130). Ogawa-Wong et al. have demonstrated an increased incidence of type 2 diabetes in individuals with high selenium levels at baseline. Therefore, long-term supplementation with high doses of selenium increases the likelihood of diabetes, and selenium supplementation may have detrimental effects on those who already have adequate selenium levels (131).

In conclusion, selenium has potential as a medicine in the treatment of diabetes, but the optimal dose of selenium requires additional research.

4.5. Regulation of the intestinal microbiota

Intestinal microbes significantly contribute to human physiology by regulating the maturation and proliferation of intestinal cells, aiding food digestion, protecting against harmful bacteria, and regulating the intestinal mucosal immune response (132, 133). Dietary components, particularly trace elements, can affect the colonization of the gastrointestinal tract and the makeup of the microbiota structure. Selenium supplementation increases

the diversity of the microbial community and has various effects on different microbiota categories. Thus, selenium has a unique role in many microbiota (134).

Selenium shows specific antibacterial activity against pathogenic bacteria such as *Escherichia coli* in the complex context of the cecal microbiota, without affecting the abundance of other community members (135). Lin et al. demonstrated that selenium administration improved the diversity and relative abundance of intestinal microbes, restored some intestinal microbiota, and increased methylmercury breakdown and excretion in rats exposed to methyl mercury (136). Approximately a fifth of the intestinal microbiota is capable of expressing selenoprotein, and selenium availability affects selenoprotein expression (137). Selenoproteins are required for various activities in both bacteria and mammalian hosts (138). Dietary selenium has an effect on the composition of the intestinal microbiota and gastrointestinal tract colonization, which in turn affects the host's selenium status and selenoprotein expression (137). Takahashi et al. demonstrated that selenium-methyl SeC and selenocyanate are converted to selenomethionine by intestinal bacteria, indicating that selenium compounds can be converted to selenomethionine by the microbiota and subsequently utilized by the host (134). Using 16S rRNA gene amplicon sequencing to analyze bacterial communities and microbial metabolic pathways, Kang et al. found that the administration of selenium-enriched *Lactobacillus plantarum* significantly increased the metabolization of selenocysteine, selenocystathionine, and selenomethionine, as well as plasma selenium levels and anti-oxidant capability in mice (139).

Selenoprotein affects the intestinal microbiota and increases the expression of hematopoietic PGD_2 synthase (HPGDS), which catalyzes PGD_2 synthesis in immune cells such as macrophages and T cells. PGD_2 dehydrates and isomerizes spontaneously to create prostaglandins J_2 (Δ^{13} -PGJ_2) and Δ^{12}-PGJ_2, respectively, and Δ^{12}-PGJ_2 can be transformed into 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) to alleviate inflammation. As ligands for the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ), PGD_2 metabolites can bind to PPAR-response elements in the *HPGDS* promoter and upregulate its expression, forming a feed-forward loop (140).

In conclusion, selenium can regulate the intestinal microbiota by controlling various prostaglandins.

5. Effects of selenium in different populations

The WHO recommends a selenium consumption of 34 μg/d for men and 26 μg/d for women, taking into account sex and bodyweight differences (141). According to the most recent report on dietary selenium reference intake in China, the recommended daily intake of selenium varies slightly across populations (23). An in-depth study in China and elsewhere revealed that women are deficient in selenium during pregnancy and lactation. Increasing the dietary selenium intake in pregnant and nursing women can successfully prevent miscarriage and reduce fetal teratogenicity (142). International attention has been focused on selenium intake, and recommended selenium intake standards for various populations have been established (**Table 1**) (141, 143–146). Low serum selenium status has been associated with disease risk (**Table 2**). Diseased populations benefit from a moderate selenium intake, and the ingested dose and action mechanism have been investigated (**Figure 3**).

5.1. Effects of selenium in HIV-positive patients

AIDS damages the immune system. It impairs immune function by destroying the human immune system's most vital cells, CD4 + T lymphocytes. As a result, HIV-infected individuals are susceptible to various diseases, are at an increased risk of developing malignant tumors, and have a high mortality rate (147).

TABLE 1 Recommended dietary selenium intakes for various regions and according to the WHO.

Age (years)/pregnancy status	WHO (μg/d) (141)		Northern Europe (μg/d) (144)		Japan (μg/d) (143)		China (μg/d) (145)	USA (μg/d) (146)
	Women	Men	Women	Men	Women	Men		
0–0.5	6		–		–		–	–
0.5–1	10		15		–		–	–
1–3	17		20		8	9	25	20
4–6	22		25		10	10	30	30
7–9	21		30		15	15	40	30
10–13	26	32	35	40	20	22.5	55	40
14–17	26	32	40	50	20	30	60	55
18–49	26	34	40	50	20	32.5	60	55
50–64	26	34	40	50	20	30	60	55
65–79	25	33	40	50	20	30	60	55
≥80	25	33	40	50	20	30	60	55
Pregnant women	28-30	–	55	–	24	–	66	60
Lactating women	35-42	–	55	–	–	–	78	70

"–" indicates that no data are available.

TABLE 2 Relationship between serum selenium status and disease risk.

T	Serum Se concentration (μ g/L)	Effect
HIV	≤85	Increased HIV morbidity and mortality (39, 148)
	–	
Cancer	<130	All-cause mortality and increased cancer mortality (154)
	>150	
Prostate cancer	<130	Increased incidence and mortality of prostate cancer (154, 157)
	>170	
CVD	≤55	Increased morbidity and mortality from CVD (167)
	>145	
IBD	ND	Increased incidence, severity, duration and colon cancer risk of IBD (169, 171)
	ND	

"–" indicates that no data are available.

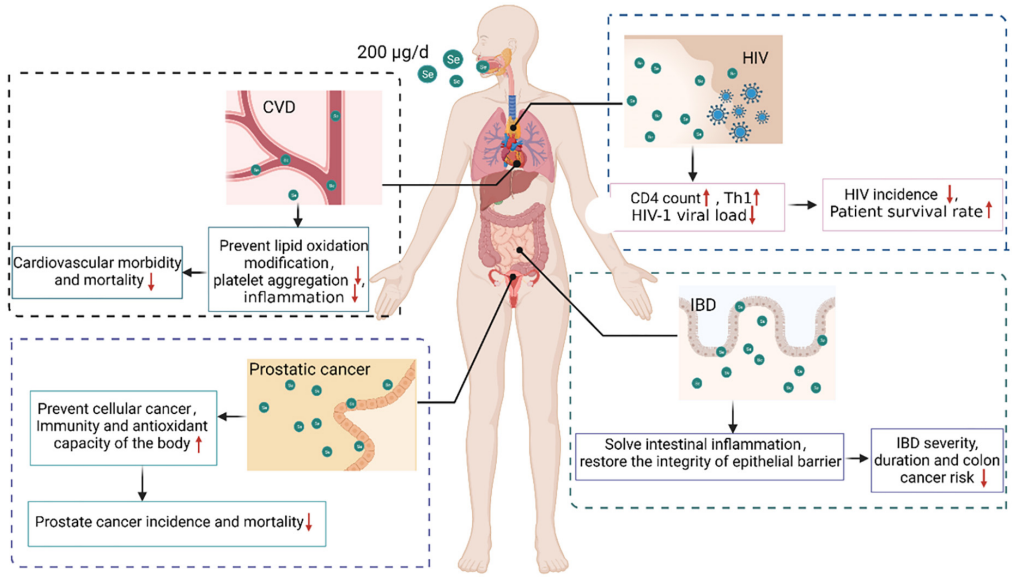

FIGURE 3
Beneficial effects and mechanisms of selenium supplementation in various diseases. Selenium supplementation can provide protection for populations with AIDS, cancer, CVD, and IBD. Supplementing HIV-infected people with 200 μg selenium per day enhanced the CD4 count, stimulated CD4 + T cell differentiation into T helper 1 cells, and decreased the HIV-1 viral load, thus decreasing HIV incidence and enhancing patient survival. Supplementing prostate cancer patients with 200 μg of selenium per day prevented cell carcinogenesis, enhanced the body's immunity and anti-oxidant capacity, and reduced the incidence and mortality of prostate cancer. In a CVD population, supplementation with 200 μg selenium per day reduced lipid oxidation, platelet aggregation, and inflammation, thus decreasing CVD morbidity and mortality. Supplementing an IBD population with 200 μg of selenium per day relieved intestinal inflammation and restored epithelial barrier integrity, thereby decreasing the incidence, severity, duration, and risk of IBD-associated colon cancer.

Selenium is required for normal immune system function and is an essential nutrient for AIDS patients. Selenium deficiency can result in immune system dysfunction, leading to reduced immunological function. Low plasma selenium levels are a strong predictor of HIV infection prognosis, and the degree to which plasma selenium levels drop is predictive of HIV incidence and mortality (6, 7).

CD4 count declines have been associated with decreases in plasma selenium levels in more than 20 publications. HIV patients who are selenium-deficient are 20 times more likely to die of HIV-related causes than those who have sufficient selenium (39). Selenium deficiency is defined as a plasma concentration of selenium that is less than or equal to 85 μg/L (148). Selenium promotes the differentiation of CD4 + T cells into T helper 1 cells, which is related with a decrease in the incidence of hospitalizations for coinfection in HIV-positive individuals (149). Baum et al. conducted a 24-month randomized, placebo-controlled study of 878 HIV-positive people who had never received antiretroviral treatment (150). The study findings indicated that taking a daily multivitamin and 200 μg of selenium in the early stages of HIV disease greatly decreased the risk of immunological decline and the incidence of HIV-related events. In a double-blind, randomized, placebo-controlled study in 450 adult male and female HIV patients, Hurwitz et al. found that nine months of selenium supplementation successfully enhanced blood selenium levels, prevented HIV-1 viral load progression, indirectly improved CD4

counts, decreased morbidity, and improved survival rates (151). Kamwesiga et al. undertook a 24-month, multicenter, double-blind, placebo-controlled, randomized clinical study, including 300 adult HIV patients. The results indicate that supplementation with 200 g of selenium per day can dramatically slow the pace of CD4 cell count reduction in HIV patients (152). In conclusion, supplementation with 200 g of selenium per day can minimize the risk of impaired immunity and the incidence of HIV and enhance the survival rate of HIV patients.

5.2. Effects of selenium in cancer patients

Cancer is a major public health problem worldwide and the second leading cause of death. Each year, an estimated 18.1 million new cases of cancer and 9.6 million cancer deaths occur globally (153). As a result, cancer imposes a significant global economic burden. Current clinical cancer treatments are inadequate in terms of effectiveness and biocompatibility. The association between selenium and cancer has long been a source of debate in the human health field. In recent years, extensive research has been conducted to demonstrate the efficacy of selenium in suppressing cellular carcinogenesis and enhancing the immune system and the body's anti-oxidant capacity.

In a representative sample of the US population, non-linear relationships between serum selenium levels and all-cause and cancer mortality were observed; a negative association was observed at low selenium concentrations (<130 μg/L), whereas a moderate positive correlation was observed at high selenium concentrations (>150 μg/L) (154). The SELECT study revealed that selenium supplementation is related with an increased risk of prostate cancer in men with high baseline selenium levels (122). Supplementation of selenium in selenium-deficient people has been demonstrated to lower the risk of prostate cancer. In a seven-year, double-blind, randomized, placebo-controlled study in 32,400 man, Lippman et al. found that daily supplementation of 200 μg of selenium did not lower the incidence of prostate cancer in men who already consumed an adequate amount of selenium (155). A randomized controlled study by Duffield-Lillico et al. revealed that daily supplentation of 200 μg of selenium per day considerably decreased the incidence of prostate cancer in men with baseline selenium concentrations < 123.2 μg/L (156). Hurst et al. conducted a meta-analysis of blood selenium levels and non-linear dose-response relationships in 13,254 subjects with and 5,007 cases of prostate cancer and found that increasing serum selenium to 170 μg/L lowered the incidence of prostate cancer (157). Thus, selenium supplementation will help reduce cancer incidence and mortality in individuals who are deficient in selenium or have a minor deficiency (158). However, selenium supplementation is harmful to people with enough selenium and increases cancer incidence and death in individuals with high baseline selenium levels.

5.3. Effect of selenium in CVD patients

CVD is the leading cause of death worldwide (159). The burden of CVD is expected to increase with the aging population. Aging, smoking, obesity, elevated cholesterol levels, unhealthy eating habits, level of education, blood pressure, diabetes, and genetics have an effect on the risk of CVD (160).

Selenium has been shown to protect against CVD by suppressing lipid oxidation, platelet aggregation, and inflammation (161, 162). A study on the effect of long-term selenium yeast (200 μg/d) and coenzyme Q10 supplementation on cardiovascular mortality in elderly Swedes revealed that supplementation protected the heart in those with low baseline selenium levels (≤ 85 μg/L), but had no effect in those with plasma selenium levels > 85 μg/L (163). In a 12-year randomized, placebo-controlled study in 443 elderly subjects in good health, Alehagen et al. found that daily intake of 200 mg coenzyme Q10 and 200 μg selenium for four years significantly reduced cardiovascular mortality, and cardiovascular mortality was still decreased by more than 40% eight years after the four-year intervention (164). Yin et al. examined vitamin intake in 39,757 American adults using dietary recall data and found that consuming 207.8 μg selenium daily lowered CVD incidence and mortality (165). Additionally, a negative relation has been observed between selenium and total CVD through weighted quantile sum regression analysis (166). A meta-analysis of prospective observational studies revealed a non-linear relation between CVD risk and plasma selenium concentrations between 30–165 μg/L, but a substantial negative correlation in the range 55–145 μg/L; thus, the relation between baseline selenium status and the incidence of CVD may be non-linear and U-shaped (167). A meta-analysis of randomized controlled trials revealed that daily supplementation of 200 μg of selenium significantly enhanced blood selenium concentrations, whereas daily supplementation of 100 μg had no effect on CVD (167).

In conclusion, persons with low baseline selenium levels may benefit from supplementation, and supplementation with 200 g of selenium per day may reduce CVD morbidity and mortality; however, the preventive effect of selenium against CVD has not been demonstrated. To establish the association between selenium and CVD, larger clinical trials in populations with varying selenium levels are required. Future research should take into account the importance of selenium status, dosing, and safety.

5.4. Effect of selenium in IBD patients

IBD refers to a specific type of chronic inflammatory illness of the intestine, mostly including Crohn's disease and ulcerative colitis (168). The incidence of IBD has been increasing over the last decades, mainly due to nutritional and environmental imbalances (169).

Selenocysteine is a selenoprotein involved in the regulation of inflammation (140). Serum selenium levels have been found to be decreased in patients with IBD (169). In New Zealand, the incidence rate of Crohn's disease is among the highest and the mean plasma selenium levels among the lowest in the world (170). Serum selenium levels have been demonstrated to be adversely associated with the severity and length of IBD and the risk of colon cancer, and selenium may serve as a non-invasive biomarker of IBD activity and severity (171). Additionally, dietary selenium has been shown to be beneficial at resolving intestinal inflammation and reestablishing epithelial barrier integrity (140, 172). Selenium supplementation

decreased colitis-associated inflammation and enhanced mouse survival in mice treated with dextran sodium sulfate (173).

In summary, low plasma selenium levels are associated with an increased risk of IBD. Selenium supplementation may help patients with IBD resolve their intestinal inflammation. The causative link between selenium deficiency and IBD requires further investigation.

6. Conclusions and future perspectives

Numerous studies have established that selenium possesses anti-oxidant, anti-cancer, blood glucose-lowering, and immune system-strengthening properties. Selenium supplementation benefits human health in various ways, most notably in terms of immunological responses and cancer prevention. Selenium supplements can be used to treat conditions such as HIV, IBD, CVD, and cancer. Selenium supplementation is most often accomplished through the use of inorganic sodium selenite, organic selenium, selenium nanoparticles, or selenium-enriched yeast. However, the relationship between selenium and human health is complex, and its "duality" makes research on its health effects difficult. Additionally, the non-linear dose-response relationship between selenium status and health is U-shaped; individuals with low baseline selenium levels may benefit from supplementation, whereas those with acceptable or high selenium levels may experience detrimental effects. Selenium has an extremely narrow range between deficiency and toxicity, and baseline selenium levels vary among populations. Safe methods and doses of selenium intake and the baseline selenium range suited for selenium supplementation remain to be established in future.

Author contributions

YS and ZW drafted the manuscript. PG, WY, QB, and HW drafted and revised the manuscript. All authors contributed to the article and approved the submitted version.

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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.2023.1136458/full#supplementary-material

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Intake of added sugar, fruits, vegetables, and legumes of Portuguese preschool children: Baseline data from SmartFeeding4Kids randomized controlled trial participants

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Introduction: The SmartFeeding4Kids (SF4K) program is an online self-guided intervention for parents with the propose of changing parental feeding practices and children's dietary intake, focusing on the intake of added sugars, fruit, vegetables, and legumes. This paper aims to describe children's dietary pattern at baseline through a 24-h food recall, the SmartKidsDiet24.

Methods: Overall, 89 participants recorded at least one meal of the 3-day food recall. Mean age was 36.22 ± 6.05 years and 53.09 ± 15.42 months old for parents and children, respectively. Of these, 22 participants were considered to have 2 days of near complete 24-h food recalls. Children's dietary intake are reported for these 22 participants based on parents reports and, thus, represent estimations only, as it remains unknown whether children consumed other non-reported foods.

Results: Fruit was the group with the highest daily intake among children (mean 1.77 ± 1.10 portions/day), followed by added sugar foods (mean 1.48 ± 0.89 portions/day), vegetables [median 1.27 (1.64) portions/day] and legumes [median 0.12 (0.39) portions/day]. Fruit intake was positively correlated with vegetable intake ($p = 0.008$). Regarding Dietary Reference Values accomplishment, 13.6% of children exceeded the daily safe and adequate intake of sodium, 77.3% did not meet potassium and fiber recommendations, and 31.8% did not meet vitamin C recommendations.

Discussion: All children did not meet calcium, vitamin B12 and vitamin D intake recommendations. Our findings further justify the need for dietary interventions in this field, to improve young children's diets.

Clinical trial registration: ClinicalTrials.gov, identifier NCT04591496.

KEYWORDS

preschool children, dietary intake, added sugar, fruits, vegetable, legumes

1. Introduction

Childhood obesity has become one of the most challenging public health crises of our time (1, 2). Overall, the worldwide proportion of overweight children and adolescents aged 5–19 years old rose from 1 in 10 to almost 1 in 5 from 2000 to 2016 (3). Nationally, 29.7% of Portuguese children aged 6–8 years old were overweight, and 11.9% were obese (4). The key drivers of this increase in the prevalence of overweight include changing dietary patterns and is accompanied by short-term and long-term consequences, such as an increased risk of developing non-communicable diseases later in life (5). Moreover, the majority of children that are overweight tend to remain in the same Body Mass Index (BMI) category during their adult life, resulting in a significant economic burden on society (6). Hence, early interventions to target childhood obesity are warranted. Mobile health technologies, including mobile apps for weight management, to improve nutrition behaviors and nutrition-related health outcomes are becoming more popular than ever and are reported to represent a highly promising approach for combating childhood obesity and/or inadequate eating patterns (7, 8). We have changed our dietary patterns, leaving behind traditional diets and consuming foods that are frequently low in essential nutrients and fiber, and high in fats and sugars (3, 9). While there is no nutritional requirement for free sugars, the consumption of sugars in the European pediatric population exceeds current recommendations (10). Contrary to a Western style diet, healthier alternatives are higher in plant-based foods, including fresh fruits, vegetables, and legumes (11). Portuguese children and adolescents have been shown to have a higher inadequacy of fruit and vegetable consumption when compared with adults and the elderly (12). This highlights the need to address the intake of these key food groups among the youth. The SmartFeeding4Kids (SF4K) program was developed. This paper aims to describe the children's dietary pattern at baseline of the SmartFeeding4Kids (SF4K) program, focusing on the intake of added sugars, fruits, vegetables, and legumes (13).

2. Materials and methods

2.1. Study design and population

The SF4K randomized controlled trial (RCT) study protocol is described in detail elsewhere (13), namely the characteristics of the intervention, study design, procedures and outcome measures. The SF4K program is an online self-guided intervention for parents with the propose of changing parental feeding practices and children's food intake, focusing on the intake of added sugars, fruit, vegetables, and legumes. The intervention intends to promote positive changes in parental feeding practices and their children's diet through self-regulation strategies and other behavior change techniques (13). The study population was Portuguese preschool children (2–6 years old). The study was approved by the Ethics and Deontology Committee of the Faculty of Psychology, University of Lisbon. Recruitment was conducted nationally, being open to all parents of 2 to 6-year-old children living in Portugal who wanted to participate in this study. Social networks and online groups attended by parents were used to promote and share this trial. Eligibility criteria were being fluent in Portuguese, a parent/caregiver of one 2- to 6-year-old child at baseline and have a mobile phone or computer/tablet with internet access (13). Once parents completed the registration on the SF4K app, they were invited to answer a baseline assessment protocol, including demographic information, parent's and children's weight and height, age and gender, parent's educational level, kinship with the child, number of children and adults in the household, if parents receive child benefits, birth date, childcare attendance and food intolerances and allergies. Baseline assessment also included recordings of their child's food and portion intake for 3 days (two weekdays and one weekend day), though a 24-h food-recall. Both data from the SF4K (intervention group) and psychoeducational control condition groups were included in this analysis.

2.2. Data collection

Data was collected from July 2021 to May 2022. An online 24-h food recall that uses the electronic food composition database by INSA (National Institute of Health Doutor Ricardo Jorge) was developed for this study, theSmartKidsDiet24, where parents recorded all the foods they are sure that their children ate (could be foods prepared by parents or eaten in their presence) in the specific days chosen by the app. Of interest, the database was updated with sugar-sweetened foods/beverages and other processed foods frequent in Portuguese children's diets, as well as vegetarian/vegan alternatives. Parents were guided on adequate measurement of food portions with the child's hand. Foods eaten without the presence/supervision of parents, were not recorded. Therefore, most children did not have fully completed food recalls. As we cannot truly quantify dietary intake without the recording of a full day of eating, we focused this analysis only on the participants that had recorded a total of 5 main meals (breakfast, morning snack, lunch, afternoon snack, and dinner) in at least 2 days of the 3-day 24-h recall. Considering children with at least 2 complete 24-h recall's, mean dietary intake of added sugar foods, fruits, vegetables, and legumes were calculated per meal. For each of these 4 food groups, portions and the following macro and micronutrients were analyzed: energy, protein, carbohydrates, monosaccharides and disaccharides, fiber, total fats, monounsaturated fats, polyunsaturated fats, calcium, potassium, sodium, vitamin B12, vitamin C, and vitamin D. The total daily intake of each of these nutrients was calculated for each food group. The referred micronutrients were chosen, as added sugar foods consumed by Portuguese children are often dairy products, frequently fortified with vitamin B12 and/or vitamin D (e.g., chocolate/flavored milk, yogurt, and ice-cream), hence the evaluation of calcium, vitamin 12 and vitamin D. As foods with added sugar are often processed foods with high sodium content, this micronutrient was also assessed. Since fruits and vegetables are the main sources of dietary potassium and vitamin C, they were also detailed in this paper. To assess whether children met their nutrient requirements, Dietary reference values (DRVs) were collected from the DRV Finder Tool by the European Food Safety Authority, EFSA (14). The average requirement (AR) was considered for energy (kcal), calcium (mg), and vitamin C (mg). The adequate

intake (AI) was considered for fiber (g), potassium (mg), vitamin B12 (μg), vitamin D (μg). Safe and adequate intake was used for sodium (g). Children's and parents' BMI were calculated as weight/height squared (kg/m^2), based on parent's report on their and their children's height and weight. Z-scores of the child's weight, height and BMI for age and sex were calculated using the WHO AnthroPlus software (15). According to BMI, parents were classified as follows: underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), pre-obese (25–29.9 kg/m^2), and obese (≥30.0kg/m^2).

2.3. Data analysis

Statistical analysis was conducted using SPSS version 27.0 (SPSS® Inc., Chicago, IL, USA). Depending on the sample size, the Normal distribution of the variables was verified using the Kolmogorov–Smirnov or Shapiro–Wilk tests. Data from categorical variables were described as frequencies (percentages). Normally distributed continuous variables are presented as mean ± standard deviation (SD). The median (interquartile range) was presented when data from continuous variables were not normally distributed. The hypotheses and statistical analysis were specified before the data were collected. Groups were created based on parental reporting of the 24-h food recall, theSmartKidsDiet24 (high reporting: recorded a total of five meals in at least 2 days of the 3-day 24-h food recall; low reporting: did not record five meals in at least 2 days of the 3-day 24-h food recall). Whole-day dietary intake estimations were calculated for participants with a high reporting on theSmartKidsDiet24 only, by simply averaging the intake of the days with the recording of at least five main meals. Average values were calculated considering 3 days, for participants with recordings of five main meals in a total of 3 days, or considering 2 days, for participants with recordings of five main meals for only 2 days. Reported per meal data also represents averaged data from the referred recorded days. Comparisons between groups were performed using the Students *t*-test for normally distributed data. The non-parametric alternative (Mann–Whitney) was used when data were not normally distributed. Chi-squared test was used for comparisons between categorical variables, and Chi-squared test by Monte Carlo simulation was used when the conditions for the chi-squared test were not met. Fisher's exact test was used in the analysis of contingency tables. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

Overall, 89 participants recorded at least one meal of the 3-day 24-h food recall at baseline. Our sample was constituted mostly by mothers (93.26%, $n = 83$) and sons (56.18%, $n = 50$). The mean age was 36.22 ± 6.05 years and 53.09 ± 15.42 months old for parents and children, respectively. Most children's household included both parents (43.82%, $n = 39$) or both parents and sibling(s) (43.82%, $n = 39$). Regarding education levels, more than half of parents had a university degree (60.67%, $n = 54$). Of these 89 participants, 9 recorded a total of five meals for each of the 3 days and 13 recorded five meals in 2 days, resulting in a total of 22 participants with a high reporting to the theSmartKidsDiet24 at baseline. The meal with the most recordings was breakfast, followed by dinner, afternoon snack and lunch. Morning snack, night snack and extras were the meals with the least recordings. Table 1 shows the number of recordings for each meal of the 3-day 24-h food recalls. Table 2 details children's and parents' characteristics of the whole sample ($n = 89$), and according to reporting to theSmartKidsDiet24. Of the 22 children with a total of 5 main meals in at least 2 days, 54, 55% ($n = 12$) were girls and the mean age was 54.30 ± 17.12 months. No significant differences were found between groups of reporting to the theSmartKidsDiet24 regarding children's age categories ($p = 0.829$), age ($p = 0.673$), gender ($p = 0.243$), weight for age Z-score ($p = 0.244$), height for age Z-score ($p = 0.417$), BMI for age Z-score ($p = 0.499$), household ($p = 0.499$), food intolerances ($p = 1.000$), parent's age ($p = 0.123$), parents education level ($p = 0.488$), parents BMI ($p = 0.281$), or parents nutritional status according to BMI ($p = 0.751$). Regarding the dietary intake of the four food groups evaluated in the sample with high reporting to the theSmartKidsDiet24, fruit represented the group with the highest daily intake among children (mean 1.77 ± 1.10 portions per day), followed by added sugar foods (mean 1.48 ± 0.89 portions per day), vegetables [median 1.27 (1.64) portions per day] and lastly by legumes [median 0.12 (0.39) portions per day]. The caloric intake of monosaccharides and disaccharides (sugar) from added sugar foods was above 10% of daily energy requirements in most children (81.8%). Detailed whole-day dietary intake of macro and micronutrients from each food group according to children's age is shown in Table 3. Dietary intake of macro and micronutrients from each food group discriminated by meal and according to children's age are shown in Tables 4–7. Daily fruit intake was positively correlated with daily intake of vegetables ($p = 0.008$). Children's BMI for age Z-score was positively associated with daily intake of vegetables ($p = 0.007$), as well as the energy ($p = 0.043$), protein ($p = 0.007$), carbohydrates ($p = 0.048$), monosaccharides and disaccharides ($p = 0.003$), fiber ($p = 0.007$), and polyunsaturated fatty acids ($p = 0.007$) prevenient from vegetables. No associations were found for other food groups regarding children's BMI for age Z-score. Parents BMI and education level were not associated with children's intake of these four food groups. Children's age was positively correlated with daily energy ($p = 0.029$), monosaccharides and disaccharides ($p = 0.003$), fats ($p = 0.018$) and saturated fats ($p = 0.034$) intake from added sugar foods, but not from fruit, vegetables, or legumes. Regarding children's DRV accomplishment,

TABLE 1 Number of recordings for each meal of the 3-day 24-h food recalls out of all 89 participants.

Meal/day	Day 1	Day 2	Day 3	Total recordings per meal
Breakfast	83	57	50	190
Morning snack	39	30	20	89
Lunch	43	55	24	122
Afternoon snack	48	47	36	131
Dinner	62	51	43	156
Night snack	25	15	10	50
Extra meal	13	9	8	30

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TABLE 2 Overall children's and parent's characteristics and according to reporting to theSmartKidsDiet24.

	Whole sample (n = 89)	Low reporting to theSmartKidsDiet24 (n = 67)	High reporting to theSmartKidsDiet24 (n = 22)	p-value
Child's age				
≤3 years old [n (%)]	30 (34)	23 (34)	7 (32)	0.83[a]
≥4 years old [n (%)]	59 (66)	44 (66)	15 (68)	
Age (months), mean ± SD	53.1 ± 15.4	52.7 ± 14.9	54.3 ± 17.1	0.67[b]
Age (years), mean ± SD	4.4 ± 1.3	4.4 ± 1.3	4.5 ± 1.4	0.67[b]
Child's gender				
Girl [n (%)]	39 (44)	27 (40)	12 (55)	0.24[a]
Boy [n (%)]	50 (56)	40 (60)	10 (45)	
Child's growth standards				
Weight for age Z-score, median (IQR)	0.36 (1.37)	0.26 (1.40)	0.41 (1.37)	0.24[c]
Height for age Z-score, mean ± SD	0.06 ± 1.31	−0.00 ± 1.35	0.26 ± 1.20	0.42[b]
BMI for age Z-score, median (IQR)	0.45 (1.50)	0.21 (1.83)	0.67 (1.18)	0.50[c]
Child's household				
Lives with both parents [n (%)]	39 (44)	30 (45)	9 (41)	0.40[d]
Lives with both parents and sibling(s) [n (%)]	39 (44)	29 (43)	10 (45)	
Lives with both patents, sibling(s) and others [n (%)]	4 (4)	3 (4)	1 (5)	
Lives with both parents and others [n (%)]	2 (2)	1 (1)	1 (5)	
Lives with father and sibling(s) [n (%)]	1 (1)	0 (0)	1 (5)	
Lives with mother [n (%)]	4 (4)	4 (6)	0 (0)	
Does the child have any food intolerance or allergy?				
Yes [n (%)]	6 (7)	5 (7)	1 (5)	1.00[e]
No [n (%)]	83 (93)	62 (93)	21 (95)	
Parent's age (years), median (IQR)	36.0 (7.0)	37.0 (6.0)	35.0 (8.0)	0.12[b]
Parent gender				
Female [n (%)]	83 (93)	62 (93)	21 (95)	1.00[e]
Male [n (%)]	6 (7)	5 (7)	1 (5)	
Parent's education				
Middle school [n (%)]	7 (8)	5 (7)	2 (9)	0.49[a]
High school [n (%)]	28 (31)	19 (28)	9 (41)	
University [n (%)]	54 (61)	43 (64)	11 (50)	
Parent's BMI*				
Mean ± SD	24.74 ± 4.34	24.45 ± 4.11	25.61 ± 4.98	0.28[b]
Underweight [n (%)]	3 (3)	3 (4)	0 (0)	0.75[d]
Normal weight [n (%)]	46 (52)	34 (51)	12 (55)	
Overweight [n (%)]	30 (34)	23 (34)	7 (32)	
Obese [n (%)]	9 (10)	6 (9)	3 (14)	

BMI, body mass index; IQR, interquartile range; SD, standard deviation. [a]Statistical test used: Pearson's Chi-squared test; [b]Statistical test used: Students t-test; [c]Statistical test used: Mann–Whitney test; [d]Statistical test used: Chi-squared test by Monte Carlo simulation. [e]Statistical test used: Fisher's exact test. *Missing information: 1 parent did not have information on BMI. A p-value of less than 0.05 was considered statistically significant.

13.6% of children exceeded the daily safe and adequate intake of sodium, 77.3% did not meet potassium and fiber AI, and 31.8% did not meet vitamin C AR. Moreover, 100% of children did not meet calcium AR, vitamin B12 and vitamin D AI, considering nutrient intake from the food groups evaluated.

4. Discussion

In our cohort, children showed inadequate consumption of the four key food groups studied. In fact, children showed a higher intake of added sugar foods than vegetables and legumes. Of

TABLE 3 Whole day dietary intake of macro and micronutrients from each food group evaluated of participants with a high reporting to theSmartKidsDiet24 ($n = 22$).

	Children aged ≤3 years old				Children aged ≥4 years old			
	Added sugar	Fruits	Vegetables	Legumes	Added sugar	Fruit	Vegetables	Legumes
Portions	1.2 ± 1.0	2.3 ± 1.2	2.0 (2.0)	0.2 (1.2)	1.6 ± 0.8	1.5 ± 1.0	1.1 (1.3)	0.1 (0.1)
Energy (kcal)	143.8 (406.7)	146.1 ± 76.2	52.5 (62.7)	13.3 (128.4)	481.0 (456.8)	101.6 ± 67.4	39.4 (35.1)	9.0 (16.3)
Protein (g)	2.7 (8.5)	1.6 ± 1.0	2.3 (2.4)	0.9 (11.5)	7.4 (8.9)	1.0 ± 0.7	1.1 (1.3)	0.6 (1.1)
Carbohydrates (g)	19.0 (77.8)	29.9 ± 15.5	5.9 (7.5)	1.8 (6.3)	71.4 (75.5)	21.1 ± 14.2	4.6 (4.7)	1.0 (2.1)
Sugars (g)	14.0 (28.0)	28.9 ± 15.1	2.0 (2.7)	0.2 (1.2)	41.1 (50.2)	20.4 ± 13.8	1.5 (2.2)	0.1 (0.2)
Dietary fiber (g)	0.8 (3.2)	5.0 ± 2.6	1.9 (2.2)	0.5 (1.3)	3.9 (7.8)	3.1 ± 1.9	1.1 (1.3)	0.3 (0.6)
Fats (g)	4.1 (6.6)	0.9 ± 0.5	1.1 (2.3)	0.1 (5.9)	14.4 (15.3)	0.6 ± 0.4	1.1 (1.7)	0.1 (0.5)
MUFA (g)	1.3 (2.8)	0.1 (0.1)	0.2 (1.7)	0.0 (0.5)	2.9 (6.7)	0.0 (0.1)	0.6 (1.2)	0.0 (0.2)
PUFA (g)	0.8 (0.6)	0.3 ± 0.2	0.3 (0.4)	0.0 (0.3)	0.9 (2.0)	0.2 ± 0.1	0.1 (0.2)	0.0 (0.1)
SFA (g)	1.7 (2.8)	0.1 (0.1)	1.1 (0.4)	0.0 (0.8)	5.4 (8.2)	0.1 (0.1)	0.2 (0.3)	0.0 (0.1)
Trans fats (g)	0.1 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Calcium (mg)	57.2 (63.8)	29.3 ± 20.9	36.8 (55.0)	5.1 (27.5)	89.3 (86.2)	13.4 ± 9.1	24.3 (24.3)	3.3 (5.8)
Potassium (mg)	35.5 (148.3)	493.7 ± 269.6	295.0 (359.2)	44.6 (101.3)	187.5 (271.2)	338.2 ± 231.2	165.0 (139.5)	25.0 (41.3)
Sodium (mg)	142.3 (189.4)	15.0 (6.3)	357.3 (388.3)	19.8 (321.3)	155.6 (198.7)	8.0 (7.5)	198.0 (262.1)	18.5 (31.3)
Vitamin B12 (mg)	0.0 (0.2)	0.0 (0.00)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Vitamin C (mg)	0.0 (3.2)	38.9 (49.8)	14.1 (22.4)	0.0 (2.8)	0.0 (2.0)	8.8 (18.3)	6.5 (10.9)	0.0 (1.6)
Vitamin D (mg)	0.0 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)

G, grams; Kcal, kilocalories; Mg, milligrams; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Values are presented as mean ± SD or median (IQR), according to the distribution of data.

interest, consumption was only reported in the presence of parents or if parents were sure about their children's dietary intake, which may indicate that this data could still be undervalued. Moreover, the method used to estimate food portions consumed by the child implies learning a method unknown to the parents (using the child's hand to estimate portions) that may not have been fully assimilated at this early stage in the trial.

The contribution of free sugars to the total energy intake of European children has already been reported to be higher than recommendations (16). This is no surprise, as the preference for sweet taste is a universal characteristic of humans (17, 18). Innate dietary preferences in childhood reflect our basic biology, which predisposes to the preference of sweet foods and the avoidance of bitter-tasting items such as green leafy vegetables (19). This is in line with our findings, as we observed a higher intake of added sugar foods compared to vegetables. This biological predisposition to preferer certain foods over others makes it difficult for parents do effectively guide their children to make healthier choices. Moreover, palatable foods, such as added sugar foods, interfere with normal appetite regulation, that is, with the complex interplay between hunger and satiety signals (20). It has been suggested that excessive consumption of sugar is facilitated by a shift in a hunger-satiety continuum that leads an individual to feel hungry for sugar despite a lack of an actual energy need, and reaches satiety later, ultimately promoting the maintenance of its consumption (21). Eating in the absence of hunger has been shown to be positively associated with an increased weight status among young children (22). Overall, sweetness is a potent stimulus for humans of all ages, and this attraction for sweet foods and beverages may stimulate overeating and induce weight gain in the long term (23). Moreover, school food environments have been shown to affect dietary behaviors of school children, including the consumption of sugar-sweetened beverages and, in fact, children's homes were significant sources of sugar-sweetened beverages consumed at school (24). Intervening in the food environment is, therefore, critical to improve children diets, in both school and community settings (25). Among pre-school children, home availability of sugar-enriched foods were shown to be positively associated with a sweets-and-treats dietary pattern (a dietary pattern high in foods such as sweet biscuits, chocolate, ice cream) and inversely associated with the health-conscious pattern (high in foods such as nuts, natural yogurt, and berries) in the children (26). This highlights the crucial role of parents in managing children's food environment at home and, consequently, highlights the need for parent-targeted interventions in this population.

In Portugal, 16% of children aged 6–8 years old were reported to consume sweet snacks (cookies/biscuits, sweets, cakes, and doughnuts) four or more times a week, and 80% eat these foods up to three times a week (4). Moreover, 14% of children drink sweetened beverages four or more times a week, and 71% drink these up to three times a week (4). Still, regarding the consumption of cakes and sweets, 65% of children of Portuguese 4-year-old children were reported to consume these foods at least once a day (27). On this subject, the WHO recommends reducing the intake of free sugars to less than 10% of total energy intake both for adults and children (28). The fact that in our sample over 80% of children exceeded this recommendation at baseline, and as early-life experiences concerning taste and flavor are relevant for the

TABLE 4 Dietary intake of macro and micronutrients from added sugar foods discriminated per meal of participants aged ≤3 years old ($n = 7$) and aged ≥4 years old ($n = 15$) with a high reporting to theSmartKidsDiet24.

	Added sugar foods											
	Children aged ≤3 years old						Children aged ≥4 years old					
	Breakfast	Morning snack	Lunch	Afternoon snack	Dinner	Night snack	Breakfast	Morning snack	Lunch	Afternoon snack	Dinner	Night snack
Portions	0.0 (0.6)	0.0 (0.5)	0.0 (0.1)	0.5 ± 0.3	0.0 (0.3)	0.0 (0.0)	0.5 (0.8)	0.0 (0.4)	0.0 (0.0)	0.6 ± 0.4	0.0 (0.0)	0.0 (0.0)
Energy (kcal)	0.0 (226.3)	0.0 (209.0)	0.0 (1.6)	39.5 (105.2)	0.0 (14.3)	0.0 (0.0)	0.0 (367.8)	0.0 (163.5)	0.0 (0.0)	91.7 (297.5)	0.0 (0.0)	0.0 (0.0)
Protein (g)	0.0 (4.7)	0.0 (4.2)	0.0 (0.0)	1.9 (2.7)	0.0 (0.0)	0.0 (0.0)	3.0 (7.8)	0.0 (3.2)	0.0 (0.0)	2.6 (3.2)	0.0 (0.0)	0.0 (0.0)
Carbohydrates (g)	0.0 (47.0)	0.0 (28.8)	0.0 (0.4)	5.4 (14.7)	0.0 (3.5)	0.0 (0.0)	39.0 (62.5)	0.0 (27.0)	0.0 (0.0)	16.7 (36.2)	0.0 (0.0)	0.0 (0.0)
Sugars (g)	0.0 (16.2)	0.0 (10.8)	0.0 (0.4)	2.3 (4.2)	0.0 (3.4)	0.0 (0.0)	22.3 (34.7)	0.0 (11.7)	0.0 (0.0)	8.6 (32.0)	0.0 (0.0)	0.0 (0.0)
Dietary fiber (g)	0.0 (2.5)	0.0 (7.3)	0.0 (0.0)	0.0 (0.8)	0.0 (0.0)	0.0 (0.0)	1.4 (2.4)	0.0 (5.3)	0.0 (0.0)	0.7 (0.9)	0.0 (0.0)	0.0 (0.0)
Fats (g)	0.0 (1.6)	0.0 (1.1)	0.0 (0.0)	0.6 (4.1)	0.0 (0.0)	0.0 (0.0)	5.0 (9.6)	0.0 (2.8)	0.0 (0.0)	1.5 (13.4)	0.0 (0.0)	0.0 (0.0)
MUFA (g)	0.0 (0.0)	0.0 (3.7)	0.0 (0.0)	0.2 (1.3)	0.0 (0.0)	0.0 (0.0)	0.1 (2.7)	0.0 (2.4)	0.0 (0.0)	0.3 (3.4)	0.0 (0.0)	0.0 (0.0)
PUFA (g)	0.0 (0.0)	0.0 (1.0)	0.0 (0.0)	0.0 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (1.1)	0.0 (0.0)	0.0 (0.0)	0.1 (0.6)	0.0 (0.0)	0.0 (0.0)
SFA (g)	0.0 (0.4)	0.0 (1.0)	0.0 (0.0)	0.2 (1.7)	0.0 (0.0)	0.0 (0.0)	0.9 (4.1)	0.0 (0.4)	0.0 (0.0)	0.6 (7.1)	0.0 (0.0)	0.0 (0.0)
Trans fats (g)	0.0 (0.0)	0.0 (1.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)
Calcium (mg)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	21.7 (54.0)	0.0 (0.0)	0.0 (0.0)	10.5 (52.0)	0.0 (0.0)	0.0 (0.0)	52.0 (73.7)	0.0 (0.0)	0.0 (0.0)
Potassium (mg)	0.0 (0.0)	0.0 (23.8)	0.0 (0.0)	28.3 (80.0)	0.0 (0.0)	0.0 (0.0)	42.3 (150.8)	0.0 (60.0)	0.0 (0.0)	56.7 (149.7)	0.0 (0.0)	0.0 (0.0)
Sodium (mg)	0.0 (0.0)	0.0 (37.5)	0.0 (4.8)	34.8 (83.1)	0.0 (42.8)	0.0 (0.0)	8.0 (74.3)	0.0 (80.0)	0.0 (0.0)	40.0 (50.9)	0.0 (0.0)	0.0 (0.0)
Vitamin B12 (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Vitamin C (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.5)	0.0 (0.0)	0.0 (0.0)
Vitamin D (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

G, grams; Kcal, kilocalories; Mg, milligrams; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Values are presented as mean ± SD or median (IQR), according to the distribution of data.

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TABLE 5 Dietary intake of macro and micronutrients from fruit discriminated per meal of participants aged ≤3 years old ($n = 7$) and aged ≥4 years old with a high reporting to theSmartKidsDiet24 ($n = 15$).

	Fruit											
	Children aged ≤3 years old						Children aged ≥4 years old					
	Breakfast	Morning snack	Lunch	Afternoon snack	Dinner	Night snack	Breakfast	Morning snack	Lunch	Afternoon snack	Dinner	Night snack
Portions	0.0 (0.5)	0.8 (0.7)	1.0 (1.0)	0.0 (0.6)	0.5 (0.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.4 (0.8)	0.1 (0.4)	0.2 (0.6)	0.0 (0.0)
Energy (kcal)	0.0 (26.5)	52.0 (29.8)	46.0 (75.5)	0.0 (42.1)	35.3 (30.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	23.5 (40.9)	0.0 (35.4)	10.3 (52.0)	0.0 (0.0)
Protein (g)	0.0 (0.2)	0.4 (0.6)	0.9 (1.0)	0.0 (0.2)	0.2 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.5)	0.0 (0.3)	0.1 (0.4)	0.0 (0.0)
Carbohydrates (g)	0.0 (5.6)	10.9 (5.5)	8.2 ± 7.2	0.0 (8.8)	7.1 (5.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.8 ± 5.5	0.0 (7.3)	2.2 (10.9)	0.0 (0.0)
Sugars (g)	0.0 (5.6)	9.8 (6.0)	7.8 ± 6.8	0.0 (8.5)	7.1 (5.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.6 ± 5.2	0.0 (7.2)	1.2 (9.8)	0.0 (0.0)
Dietary fiber (g)	0.0 (1.1)	1.6 (1.2)	1.4 ± 1.2	0.0 (1.4)	1.1 (1.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.9 ± 0.9	0.0 (1.4)	0.3 (1.5)	0.0 (0.0)
Fats (g)	0.0 (0.2)	0.3 (0.3)	0.2 ± 0.2	0.0 (0.3)	0.2 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 ± 0.2	0.0 (0.3)	0.1 (0.3)	0.0 (0.0)
MUFA (g)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PUFA (g)	0.0 (0.1)	0.1 (0.1)	0.1 ± 0.1	0.0 (0.1)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 ± 0.1	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)
SFA (g)	0.0 (0.0)	0.1 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)
Trans fats (g)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Calcium (mg)	0.0 (5.5)	5.4 (9.8)	8.5 (14.9)	0.0 (4.2)	3.0 (11.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	2.8 (7.3)	0.0 (4.1)	0.9 (5.0)	0.0 (0.0)
Potassium (mg)	0.0 (80.0)	140.0 (110.0)	200.0 (280.0)	0.0 (121.3)	112.5 (133.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	65.0 (140.0)	0.0 (107.8)	35.8 (175.0)	0.0 (0.0)
Sodium (mg)	0.0 (2.0)	4.5 (5.0)	3.6 ± 3.0	0.0 (4.0)	3.0 (5.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	3.2 ± 3.0	0.0 (3.8)	1.0 (4.0)	0.0 (0.0)
Vitamin B12 (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Vitamin C (mg)	0.0 (6.0)	4.5 (2.5)	8.0 (21.4)	0.0 (3.9)	3.0 (15.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	2.0 (4.5)	0.0 (7.8)	1.0 (3.8)	0.0 (0.0)
Vitamin D (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

G, grams; Kcal, kilocalories; Mg, milligrams; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Values are presented as mean ± SD or median (IQR), according to the distribution of data.

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TABLE 6 Dietary intake of macro and micronutrients from vegetables discriminated per meal of participants aged ≤3 years old ($n = 7$) and aged ≥4 years old ($n = 15$) with a high reporting to the theSmartKidsDiet24.

	Vegetables											
	Children aged ≤3 years old						Children aged ≥4 years old					
	Breakfast	Morning snack	Lunch	Afternoon snack	Dinner	Night snack	Breakfast	Morning snack	Lunch	Afternoon snack	Dinner	Night snack
Portions	0.0 (0.0)	0.0 (0.0)	1.0 (1.8)	0.0 (0.0)	0.8 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.7 (1.1)	0.0 (0.0)	0.5 (1.0)	0.0 (0.0)
Energy (kcal)	0.0 (0.0)	0.0 (0.0)	30.3 (39.5)	0.0 (0.0)	18.3 (32.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	17.6 (36.7)	0.0 (0.0)	16.5 (24.8)	0.0 (0.0)
Protein (g)	0.0 (0.0)	0.0 (0.0)	1.0 (1.8)	0.0 (0.0)	0.7 (0.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.7 (1.1)	0.0 (0.0)	0.6 (1.0)	0.0 (0.0)
Carbohydrates (g)	0.0 (0.0)	0.0 (0.0)	3.2 (4.9)	0.0 (0.0)	2.2 (4.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	2.5 (5.0)	0.0 (0.0)	2.1 (3.1)	0.0 (0.0)
Sugars (g)	0.0 (0.0)	0.0 (0.0)	0.8 (1.7)	0.0 (0.0)	1.0 (1.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.8 (1.4)	0.0 (0.0)	0.6 (1.3)	0.0 (0.0)
Dietary fiber (g)	0.0 (0.0)	0.0 (0.0)	1.0 (1.5)	0.0 (0.0)	0.7 (0.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.6 (1.4)	0.0 (0.0)	0.7 (0.9)	0.0 (0.0)
Fats (g)	0.0 (0.0)	0.0 (0.0)	0.6 (1.6)	0.0 (0.0)	0.4 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (1.2)	0.0 (0.0)	0.5 (1.1)	0.0 (0.0)
MUFA (g)	0.0 (0.0)	0.0 (0.0)	0.0 (1.1)	0.0 (0.0)	0.1 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.8)	0.0 (0.0)	0.3 (0.7)	0.0 (0.0)
PUFA (g)	0.0 (0.0)	0.0 (0.0)	0.2 (0.3)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)
SFA (g)	0.0 (0.0)	0.0 (0.0)	0.1 (0.2)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.2)	0.0 (0.0)	0.1 (0.2)	0.0 (0.0)
Trans fats (g)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Calcium (mg)	0.0 (0.0)	0.0 (0.0)	20.3 (42.1)	0.0 (0.0)	15.8 (8.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	13.2 (24.8)	0.0 (0.0)	10.3 (24.0)	0.0 (0.0)
Potassium (mg)	0.0 (0.0)	0.0 (0.0)	115.0 (262.5)	0.0 (0.0)	113.8 (130.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	102.5 (155.7)	0.0 (0.0)	77.5 (147.9)	0.0 (0.0)
Sodium (mg)	0.0 (0.0)	0.0 (0.0)	228.8 (325.8)	0.0 (0.0)	125.0 (214.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	126.2 (243.3)	0.0 (0.0)	110.0 (193.5)	0.0 (0.0)
Vitamin B12 (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Vitamin C (mg)	0.0 (0.0)	0.0 (0.0)	5.9 (11.9)	0.0 (0.0)	4.9 (11.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.7 (7.1)	0.0 (0.0)	1.9 (7.3)	0.0 (0.0)
Vitamin D (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

G, grams; Kcal, kilocalories; Mg, milligrams; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Values are presented as median (IQR).

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TABLE 7 Dietary intake of macro and micronutrients from legumes discriminated per meal of participants aged ≤3 years old ($n = 7$) and aged ≥4 years old ($n = 15$) with a high reporting to theSmartKidsDiet24.

	Legumes											
	Children aged ≤3 years old						Children aged ≥4 years old					
	Breakfast	Morning snack	Lunch	Afternoon snack	Dinner	Night snack	Breakfast	Morning snack	Lunch	Afternoon snack	Dinner	Night snack
Portions	0.0 (0.0)	0.0 (0.0)	0.2 (0.3)	0.0 (0.0)	0.0 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)
Energy (kcal)	0.0 (0.0)	0.0 (0.0)	13.3 (33.4)	0.0 (0.0)	0.0 (74.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (9.3)	0.0 (0.0)	0.0 (16.3)	0.0 (0.0)
Protein (g)	0.0 (0.0)	0.0 (0.0)	0.7 (2.8)	0.0 (0.0)	0.0 (5.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.2)	0.0 (0.0)	0.0 (1.1)	0.0 (0.0)
Carbohydrates (g)	0.0 (0.0)	0.0 (0.0)	1.3 (1.9)	0.0 (0.0)	0.0 (5.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (1.4)	0.0 (0.0)	0.0 (2.1)	0.0 (0.0)
Sugars (g)	0.0 (0.0)	0.0 (0.0)	0.2 (0.3)	0.0 (0.0)	0.0 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)
Dietary fiber (g)	0.0 (0.0)	0.0 (0.0)	0.0 (0.4)	0.0 (0.0)	0.0 (1.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.2)	0.0 (0.0)	0.0 (0.6)	0.0 (0.0)
Fats (g)	0.0 (0.0)	0.0 (0.0)	0.1 (1.8)	0.0 (0.0)	0.0 (3.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.2)	0.0 (0.0)	0.0 (0.3)	0.0 (0.0)
MUFA (g)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)
PUFA (g)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)
SFA (g)	0.0 (0.0)	0.0 (0.0)	0.0 (0.2)	0.0 (0.0)	0.0 (0.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Trans fats (g)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Calcium (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (6.5)	0.0 (0.0)	0.0 (9.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (2.8)	0.0 (0.0)	0.0 (5.8)	0.0 (0.0)
Potassium (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (42.2)	0.0 (0.0)	0.0 (41.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (16.7)	0.0 (0.0)	0.0 (33.8)	0.0 (0.0)
Sodium (mg)	0.0 (0.0)	0.0 (0.0)	0.9 (19.8)	0.0 (0.0)	0.0 (319.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (12.5)	0.0 (0.0)	0.0 (31.3)	0.0 (0.0)
Vitamin B12 (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Vitamin C (mg)	0.0 (0.00)	0.0 (0.0)	0.0 (1.6)	0.0 (0.0)	0.0 (0.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Vitamin D (mg)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

G, grams; Kcal, kilocalories; Mg, milligrams; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Values are presented as median (IQR).

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promotion of healthy dietary patterns later in life (29), further justifies interventions in this field.

In our cohort, fruit intake was higher than vegetable intake. Nationally, daily consumption of fruits has been reported more frequent (63%) than vegetable soup (57%) (4). Moreover, previous data reports 92% of Portuguese 4-year-old children consume soup at least once a day, 45% consume vegetables daily, cooked or in salads, 86% consume fresh fruit daily and 59% consume it two or more times a day (27). Of interest, parent-targeted interventions have been shown to result in significant increase in fruit and vegetable intake in children (30). Increasing parent's knowledge is relevant as pressure-to-eat is counterproductive and can have negative effects (31). Regarding strategies for changing children's eating behaviors, evidence suggests that hands-on approaches such as gardening and cooking, as well as providing children with free, accessible fruits and vegetables may encourage a greater consumption of these foods (32).

In our sample, children's sodium intake was high, especially considering total dietary intake was not evaluated and, thus, children's actual daily intake of sodium may be even higher. Having 13.6% of children exceed the recommendation just through four food groups that exclude the recording of key sodium sources, such as processed and fast foods (apart from those that have added sugar) may be a strong indicator of a nutritionally poor dietary pattern. Current WHO recommendation on sodium consumption for adults is 2 g sodium/day (33). This recommendation has already been shown to be largely exceeded by Portuguese adults (34). As for children, WHO states that the recommended maximum level of intake of 2 g/day sodium in adults should be adjusted downward based on the energy requirements of children relative to those of adults (33). In our sample children's sodium intake was high, which is in line with current evidence that suggests that Portuguese children also have high sodium intake (35). As for potassium, we found that 77.3% of our sample had potassium intakes below the recommended. Although we haven't considered children's whole dietary pattern, since vegetables, fruits and legumes are some of the main dietary sources of potassium, these findings support previous national and international data that report that young children do not consume enough potassium (36–38). Similarly, these food groups are among the main dietary sources of fiber and vitamin C. In our sample, 73.3 and 31.8% of children showed low compliance to the fiber and vitamin C intake recommendations, respectively. An adequate fiber intake in children and adolescents might be associated with a lower risk of obesity, constipation, metabolic syndrome, insulin resistance, and high blood pressure (39). Low vitamin C, an essential nutrient that must be obtained through the diet in adequate amounts, is thought to be both a cause and a consequence of various communicable and non-communicable diseases (40). Hence, considering their low intake in our cohort, dietary interventions aimed at increasing the consumption of fiber and vitamin C rich foods are warranted. As expected, all children did not meet their calcium, vitamin B12 and vitamin D intake recommendations, most likely due to the lack of information regarding their consumption of the main dietary sources of these nutrients, such dairy, meats and fish.

This study has several limitations. Collected data was self-reported, making it more vulnerable to errors. As previously mentioned, parents were instructed to register only the foods they were sure the child ate, meaning there may be missing meals/snacks eaten by the kids in the absence of parents. On the other hand, children with a low reporting to the without recordings of at least five meals per day in at least 2 days were excluded from the detailed dietary analysis, although we do not know if they did not have all five meals recorded due to actual missing information on their diet or if they did not eat these meals at all. This is major limitation of our study, as we cannot be sure if the nutrient intakes represent true whole-day dietary intakes. This weaknesses of the app and study protocol could have been solutioned by allowing more than one log-in per child, that is, both parents and other family members/caregivers (when applicable) could register the child's dietary intake. This may have prevented some missing data on the theSmartKidsDiet24. The app should also have included an option so parents (or others) could specify whether the child did not have the meal in question or if they are not sure because they were not present. This way, some of the considered incomplete theSmartKidsDiet24 reports could have been included in the analysis, if we knew that the child didn't actually eat and it was not the case of missing data.

5. Conclusion

Our findings suggest that the dietary intake of key components of a healthy dietary pattern of Portuguese preschool children is inadequate, with a high consumption of sugary foods and low intake of vegetables and legumes. As current literature on diet in overall health strongly states that dietary patterns rich in processed foods with low nutritional value and high in calories, and low in nutrient dense foods like vegetables and legumes are linked to poor health throughout the life course, the establishment of healthy dietary patterns from a young age is warranted.

Children's dietary intake was assessed based on parents reports and, thus, represent estimations only, as it remains unknown whether children consumed other non-reported foods. Nevertheless, our results further justify the need for interventions in this field, such as the SmartFeeding4Kids program, designed to be an intervention for parents who want to improve their feeding practices and develop a healthy diet in their young children. There is no doubt that consistent systemic changes are needed to fully address this problematic, namely with regard to the promotion of an environment where the availability and access to healthy foods is improved. Nevertheless, when considering in young children, it is recognized that parent targeted interventions are valuable strategies to promote healthier eating. Mobile apps have the potential to share information in a flexible, easy, and intuitive format in a cost-effective manner. Moreover, they are interesting tools in the fast-paced world we live in, being suitable for time-constrained and overwhelmed parents, as they are easily accessible and can be self-paced.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics and Deontology Committee of the Faculty of Psychology, University of Lisbon. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

JS, AG, DB, TG, and LB gave scientific support, critically revised the draft, clarified concepts, conceptualized the study, and collected and processed the data. SC and JS interpreted the data, drafted the manuscript, and wrote the manuscript in its final format. All authors contributed to the article and approved the submitted version.

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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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The association between oxidative balance score and periodontitis in adults: a population-based study

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Introduction: The pathogenesis between oxidative stress and periodontitis was correlated. The Oxidative Balance Score (OBS) is a systematic tool to assess the effects of diet and lifestyle in relation to oxidative stress. However, the association between OBS and periodontitis has not been reported previously.

Methods: Sixteen dietary factors and four lifestyle factors were selected to score the OBS. Multivariate logistic regression and sensitivity analysis were used to investigate the relationship between OBS and periodontitis based on data from the National Health and Nutrition Examination Survey (NHANES) 1999–2018. Subgroup analysis and interaction tests were used to investigate whether this association was stable across populations.

Results: This study included 3,706 participants. There was a negative linear association between OBS and periodontitis in all participants [0.89 (0.80, 0.97)], and after converting OBS to a quartile variable, participants with OBS in the highest quartile had a 29% lower risk of periodontitis than those with OBS in the lowest quartile [0.71 (0.42, 0.98)]. This negative association differed with respect to age and diabetes.

Conclusion: There is a negative association between OBS and periodontitis in US adults. Our results suggest that OBS may be used as a biomarker for measuring periodontitis.

KEYWORDS

Oxidative Balance Score (OBS), periodontitis, oxidative stress, NHANES, antioxidants

1. Introduction

Periodontitis is a common inflammatory disease of the oral cavity (1, 2), with the majority of cases occurring in people aged 55–59 years old (3), which is an important cause of tooth loss in adults (4). Plaque biofilm initiates the process, influencing the host's immunological function and inflammatory response (5, 6). Bacterial and their metabolite-produced inflammatory mediators cause immunological dysfunction and periodontal tissue

Abbreviations: OBS, Oxidative Balance Score; NHANES, National Health and Nutrition Examination Survey; NCHS, National Center for Health Statistics; AAP, Academy of Periodontology; AL, attachment loss; PD, pocket depth; LDL-C, low-density lipoprotein cholesterol; PIR, income-to-poverty ratio; CKD, chronic kidney disease.

damage (7). Several previous epidemiological studies have demonstrated that periodontitis is a risk factor for various systemic illnesses (8, 9), with low-grade inflammation in the peripheral circulation being linked to the genesis and development of several diseases (10). Periodontitis has been linked to depression (11), Alzheimer's disease (12), metabolic syndrome (13), and cardiovascular disease in various studies (14, 15).

An increasing body of research shows that the inflammatory response to periodontitis is linked to elevated local and systemic oxidative stress, as well as decreased antioxidant capacity (16, 17). Reactive oxygen species, which are primarily created in excess by hyperactive neutrophils as periodontitis develops, are not counterbalanced by the antioxidant defense system and cause tissue damage (18), increased metabolites of protein damage (19), DNA damage (20), and lipid peroxidation are characteristics of the process (21). Periodontitis may potentially have an impact on the antioxidants' local and systemic actions (19).

The relationship between oxidative stress and periodontitis has attracted the interest of researchers. Over the past 40 years, studies have been reported on the association between several antioxidants and the prevalence of periodontitis (22–24). However, there are differences in data collection methods for dietary intake in many studies, which may explain the conflicting results of some studies (25). More importantly, antioxidants work systematically in concert, so measuring individual species in isolation can present limitations (26). For example, in addition to dietary factors, a number of lifestyle factors, including smoking (27), alcohol consumption (28), physical activity, and obesity (29, 30), also have an impact on organismal inflammation and oxidative stress.

The Oxidative Balance Score (OBS) is a composite indicator that assesses the oxidative balance of an individual (31). Generally, a higher OBS indicates a preference for antioxidants over pro-oxidants (32). The negative associations between OBS and a number of inflammation-related diseases has been found in several epidemiological studies, including cardiovascular disease (33), type 2 diabetes (34), chronic kidney disease (35), and osteoarthritis (36). However, no studies have assessed the association between OBS and periodontitis. Therefore, I conducted a cross-sectional study to examine the association between dietary and lifestyle integrated OBS and periodontitis according to the National Health and Nutrition Examination Survey (NHANES) 1999–2018.

2. Materials and methods

2.1. Study population

The NHANES is a continuous nationwide survey that investigates the nutrition and health condition of adults and children in the United States (37, 38). The National Center for Health Statistics (NCHS) Research Ethics Review Board authorized the study protocol. At the time of recruiting, all participants provided written consent at the time of recruitment. This study utilizes the most recent five survey cycles of data from the last decade to conduct the survey. We excluded 69,441 participants without complete OBS data, 43,516 participants with missing periodontal examination data, and 215 extreme dietary intakes. The study eventually included 3,706 participants (**Figure 1**).

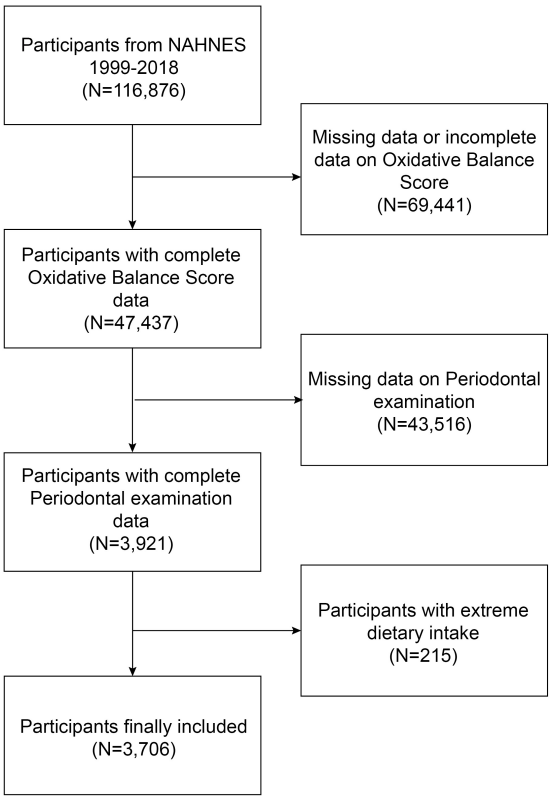

FIGURE 1
Flow chart of participants selection. NHANES, National Health and Nutrition Examination Survey.

2.2. Oxidative Balance Score

Based on past research and experience, the OBS calculation combines the contributions of 16 dietary factors and 4 lifestyle factors, including 15 antioxidants and 5 pro-oxidants (39–42). **Table 1** demonstrates the detailed scoring scheme of the OBS, with the first through third quartiles assigned a score of 0–2 for dietary antioxidants and 0 for pro-oxidants in the highest tertile and 2 in the lowest tertile. For lifestyle factors including physical activity (0 points for <400 MET-minute/week; 1 point for 400–1,000 MET-minute/week; 2 points for >1,000 MET-minute/week), alcohol intake (0 points for >30 g/d; 1 point for 0–30 g/d; 2 points for None), BMI (0 points for obesity. 1 for overweight; 2 for normal weight) and serum cotinine level (0 points for >0.038 ng/mL; 1 point for 0.038–1.13 ng/mL; 2 points for <1.13 ng/mL) (43, 44).

2.3. Periodontitis

All participants in this study were examined by experienced dentists, and the specific examination procedures are described in the operating manual available on the NHANES website (45–47). For the classification of periodontal disease, we used the 2012 CDC/American Academy of Periodontology (AAP) case definition of periodontitis. Mild periodontitis was classified as two interproximal sites with attachment loss (AL) of three

TABLE 1 Oxidative Balance Score assignment scheme.

OBS components	Property	Scoring assignment		
		0	1	2
Dietary components				
Dietary fiber (g/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Carotene (RE/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Riboflavin (mg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Niacin (mg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Vitamin B6 (mg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Total folate (mcg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Vitamin B12 (mcg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Vitamin C (mg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Vitamin E (ATE) (mg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Calcium (mg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Magnesium (mg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Zinc (mg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Copper (mg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Selenium (mcg/d)	Antioxidant	Tertile 1	Tertile 2	Tertile 3
Total fat (g/d)	Prooxidant	Tertile 3	Tertile 2	Tertile 1
Iron (mg/d)	Prooxidant	Tertile 3	Tertile 2	Tertile 1
Lifestyle components				
Physical activity (MET-minute/week)	Antioxidant	<400	400–1,000	>1,000
Alcohol (g/d)	Prooxidant	>30	0–30	None
Body mass index (kg/m^2)	Prooxidant	>30	25–30	<25
Cotinine (ng/mL)	Prooxidant	>0.038	0.038–1.13	<1.13

OBS, Oxidative Balance Score; RE, retinol equivalent; ATE, alpha-tocopherol equivalent; MET, metabolic equivalent.

millimeters and two interproximal sites with pocket depth (PD) of four millimeters (not on the same tooth) or one site with PD of five millimeters. Moderate periodontitis was defined as two interproximal sites with AL 4 mm (not on the same tooth) or two interproximal sites with PD 5 mm (not on the same tooth). A total of 2 interproximal sites with AL 6 mm (not on the same tooth) and 1 interproximal site with PD 5 mm were categorized as severe periodontitis. The final number of periodontitis cases was the sum of mild, moderate, and severe cases (48).

2.4. Covariables

Covariates included age, gender, LDL-C (low-density lipoprotein cholesterol), race, diabetes, family income-to-poverty ratio (PIR), cancer, waist circumference, triglycerides, education level, and serum klotho levels. Detailed information on variable collection methods can be found in the NHANES Survey Methods and Analysis Guide.[1]

1 https://wwwn.cdc.gov/nchs/nhanes/analyticguidelines.aspx

2.5. Statistical analysis

All analyses were performed with R (version 4.2) or Empowerstats (version 4.1). The chi-square test and *t*-test were used to assess the demographic characteristics of the participants by OBS quartile. Weighted multivariate logistic regression analyses were used to investigate the linear associations between OBS and periodontitis. After transforming OBS from a continuous variable to a categorical variable (quartile) a trend test was used to investigate the trend of linear association between OBS and periodontitis. Subgroup analysis was used to investigate the association between OBS and periodontitis in people of different gender, race, education, and diabetes status, and interaction tests were used to investigate whether the associations were consistent across subgroups. Statistical significance defined as two-sided $p < 0.05$.

3. Results

3.1. Baseline characteristics

At the time of assessment, the mean (SD) age of the 3,706 participants was 53.37 (14.89) years, 53.13% participants were females, and a total of 2,598 participants (70.08%) were diagnosed with periodontitis. In comparison to the bottom OBS quartile, participants in the top OBS quartile are more likely to be males, non-Hispanic white people and younger; In terms of socioeconomic status, higher OBS participants were more likely to have higher educational attainment and higher income; in terms of lifestyle, a lower proportion of higher OBS participants drank alcohol. In addition, participants in the lowest OBS quartile were more likely to with cancer and diabetes; to have lower BMI and waist circumference in terms of body size, and lower serum cotinine levels (Table 2). Supplementary Table 1 depicts the differences in clinical characteristics of participants with and without periodontitis.

3.2. Association between Oxidative Balance Score and periodontitis

Table 3 shows the association between OBS and periodontitis. We found higher OBS was negatively correlated with periodontitis both in crude model [0.79 (0.65, 0.92)] and adjusted model [0.82 (0.70, 0.93)]. After adjusted all covariables, each one-unit increase in the OBS score was found to be associated with an 11% decrease in the risk of periodontitis [0.89 (0.80, 0.97)]. After changing the OBS from a continuous to a categorical variable, sensitivity analyses were carried out. In the fully adjusted model, participants in the highest quartile had a 29% lower risk of periodontitis compared to those in the lowest quartile of OBS [0.71 (0.42, 0.98)]. Supplementary Tables 2, 3 demonstrate the association between OBS and periodontitis-related variables, with results showing that higher OBS scores are associated with lower C-reactive protein levels and with higher grades of self-reported oral health.

TABLE 2 Basic characteristics of participants by Oxidative Balance Score quartile.

Characteristics	Oxidative Balance Score				*P*-value
	Q1 *N* = 927	Q2 *N* = 926	Q3 *N* = 926	Q4 *N* = 927	
Age (years)	54.80 ± 15.29	53.43 ± 14.49	52.97 ± 14.39	52.61 ± 14.24	0.018
Periodontitis, (%)					<0.001
Yes	75.13	70.58	63.44	52.67	
No	24.87	29.42	36.56	47.33	
Sex, (%)					<0.001
Male	36.57	47.08	55.83	62.89	
Female	63.43	52.92	44.17	37.11	
Race/Ethnicity, (%)					<0.001
Non-Hispanic white	47.03	48.16	51.51	54.37	
Non-Hispanic black	21.47	17.93	15.01	11.87	
Mexican American	15.53	17.49	19.33	19.20	
Other race/Multiracial	15.97	16.42	14.15	14.56	
Education level, *n* (%)					<0.001
Less than high school	37.22	30.67	27.11	22.33	
High school	25.89	21.60	23.33	18.55	
More than high school	36.89	47.73	49.56	59.12	
Smoking, (%)					0.206
Ever	50.05	46.44	47.30	45.31	
Never	49.95	53.56	52.70	54.69	
Drinking alcohol, (%)					0.006
Ever	71.81	61.12	62.35	58.37	
Never	28.19	38.88	37.65	41.63	
Cancer, (%)					0.341
Yes	10.79	9.83	9.18	12.51	
No	89.21	90.17	90.82	87.49	
Diabetes, (%)					0.001
Yes	15.43	15.77	10.04	10.25	
No	82.31	81.97	87.58	86.95	
Borderline	2.26	2.26	2.38	2.80	
BMI (kg/m^2)	30.02 ± 6.99	29.98 ± 6.84	29.29 ± 6.21	28.69 ± 6.06	<0.001
Waist circumference (cm)	101.30 ± 15.91	101.69 ± 16.10	100.37 ± 14.98	98.99 ± 14.52	0.002
PIR	2.18 ± 1.51	2.46 ± 1.58	2.70 ± 1.63	3.00 ± 1.66	<0.001
Triglycerides (mg/dL)	141.84 ± 129.14	134.99 ± 106.87	139.92 ± 107.54	132.09 ± 143.62	0.514
Klotho (pg/mL)	813.23 ± 303.24	817.82 ± 277.08	831.34 ± 271.82	815.73 ± 313.40	0.823
LDL-C (mg/dL)	119.76 ± 34.45	120.93 ± 35.04	121.05 ± 35.01	116.19 ± 33.77	0.184
Serum cotinine (ng/ml)	98.92 ± 153.72	51.32 ± 122.73	36.25 ± 101.03	29.92 ± 95.40	<0.001

Mean ± SD for continuous variables: the *P*-value was calculated by the weighted linear regression model. (%) for categorical variables: the *P*-value was calculated by the weighted chi-square test. Q, quartile; PIR, ratio of family income to poverty; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol.

3.3. Subgroup analyses

Subgroup analyses and interaction tests stratified by age, sex, race, BMI, and diabetes were performed to assess whether the relationship between OBS and periodontitis was consistent in the general population and to identify any potentially different population settings. Our findings showed that the associations were inconsistent. There was no statistical significance for gender, race, or BMI, as shown in Table 4, but we did find a significant interaction between age and diabetes (*P* for interaction <0.05).

TABLE 3 The associations between Oxidative Balance Score and periodontitis.

Exposure	Model 1 [OR (95% CI)]	Model 2 [OR (95% CI)]	Model 3 [OR (95% CI)]
Oxidative Balance Score (continuous)	0.79 (0.65, 0.92)	0.82 (0.70, 0.93)	0.89 (0.80, 0.97)
Oxidative Balance Score (quartile)			
Quartile 1	reference	reference	reference
Quartile 2	0.91 (0.79, 1.03)	0.90 (0.81, 0.99)	0.93 (0.88, 0.98)
Quartile 3	0.83 (0.72, 0.94)	0.81 (0.72, 0.91)	0.84 (0.70, 1.00)
Quartile 4	0.53 (0.35, 0.72)	0.62 (0.39, 0.85)	0.71 (0.42, 0.98)
P for trend	<0.001	<0.001	<0.001

Model 1: No covariates were adjusted. Model 2: Age, gender, and race were adjusted. Model 3: Age, gender, race, diabetes, cancer, PIR, triglycerides, klotho, and LDL-C were adjusted. PIR, ratio of family income to poverty; LDL-C, low-density lipoprotein cholesterol.

TABLE 4 Subgroup analysis of the association between Oxidative Balance Score and periodontitis.

Subgroup	Oxidative Balance Score [OR (95%CI)]	*P* for interaction
Sex		0.182
Male	0.85 (0.75, 0.95)	
Female	0.93 (0.90, 0.96)	
Age		0.046
<60 years	0.91 (0.84, 0.98)	
≥60 years	0.83 (0.76, 0.91)	
Race/Ethnicity		0.089
Non-Hispanic white	0.88 (0.81, 0.95)	
Non-Hispanic black	0.79 (0.63, 0.95)	
Mexican American	0.94 (0.85, 1.03)	
Other race/Multiracial	0.92 (0.89, 0.96)	
Education level, *n* (%)		0.579
Less than high school	0.85 (0.72, 0.98)	
High school	0.91 (0.82, 1.00)	
More than high school	0.81 (0.70, 0.92)	
Diabetes, (%)		0.028
Yes	0.75 (0.56, 0.94)	
No	0.98 (0.95, 1.02)	
Borderline	0.93 (0.91, 0.95)	

Age, gender, race, diabetes, cancer, PIR, triglycerides, klotho, and LDL-C were adjusted. PIR, ratio of family income to poverty; LDL-C, low-density lipoprotein cholesterol.

The negative association effect of OBS with periodontitis was significantly greater in older adults older than 60 years [0.83 (0.76, 0.91)] than in those younger than 60 years [0.91 (0.84, 0.98)]. In addition, this negative association effect was significantly greater in participants with diabetes [0.75 (0.56, 0.94)] than in those without diabetes [0.98 (0.95, 1.02)]. Although there were inconsistent effect values for the association between OBS and periodontitis in some subgroups, our results suggest that a negative association between OBS and periodontitis was maintained in all subgroups.

4. Discussion

In the cross-sectional study that enrolled 3,706 representative participants, we observed a negative association between the OBS and periodontitis, and there was significant dependence of age and diabetes on this association, indicating that an increased OBS may contribute to a decreased risk of periodontitis. Our results suggest that the management of OBS in dietary intake and lifestyle may alleviate the occurrence of periodontitis.

To our knowledge, this is the first study to assess the relationship between OBS and periodontitis, and it highlights the negative association between OBS levels derived from dietary intake and lifestyle and the risk of periodontitis. Previous studies have found that oxidative stress has a negative impact on periodontitis risk and oral health (49). Tamaki et al. (50) investigated the association between serum oxidative stress levels and periodontitis in 200 adult participants from the community with periodontitis. The results of the age-adjusted logistic analysis showed a statistically significant association between high ROM levels and clinical attachment loss (50). In a cohort study that included 770 participants with chronic kidney disease (CKD), Sharma et al. (51) attempted to investigate the causal association between oxidative stress, periodontitis, and renal function using a mediator analysis model, and the authors found a bidirectional negative association between periodontitis and renal function, with oxidative stress providing the pathobiological basis for this relationship. Li et al. investigated the association between four serum antioxidant vitamins (vitamins A, C, D, and E) and periodontitis in a cross-sectional study that included 6,158 Americans. The results showed a significant negative association between vitamins C and D and periodontitis, and in addition, the authors concluded that periodontitis increased the level of systemic inflammation in the obese population. In our analysis, we detected a linear negative association between OBS and periodontitis (48). A trend test considering OBS as a quartile also demonstrated a dose-response relationship between OBS and periodontitis. It has been widely reported that OBS can be used as an indicator of inflammatory diseases (52), and the association between OBS and periodontitis was also recognized in our study.

The results of the subgroup analysis showed significant differences in the association between OBS and periodontitis with respect to age and diabetes, which is partially consistent with previous studies. Ebersole et al. (53) evaluated the association between five antioxidants (folate, vitamin D, vitamin E, *cis*-beta-carotene, and β-cryptoxanthin). The authors found an interaction between age and periodontitis-related levels of these nutrients, with reduced levels of these antioxidants increasing with age in moderate and severe periodontitis (53). Our interaction test showed that the negative association effect between OBS and periodontitis was more significant in the elderly. Furthermore, diabetes and periodontitis share a common pathogenesis associated with altered immune inflammatory responses at the systemic level (54). An animal study showed that periodontitis exacerbates oxidative stress levels in rats with diabetes (55). Our results also suggest that participants with diabetes are more prominent in the negative association between OBS and periodontitis.

The role of oxidative stress and inflammation in the pathogenesis of periodontitis has attracted the attention of

researchers for decades (56, 57). Although many past observational cross-sectional studies have confirmed the association of oxidative stress with periodontitis, the low specificity of oxidative stress markers requires caution in interpreting the results, and a meta-analysis that included 16 observational studies outlined the substantial heterogeneity introduced by differences in patient populations and analytical tools (58). In fact, with the exception of vitamin C, which is considered a well-known strong antioxidant, the associations between other dietary components and inflammatory diseases are often conflicting (59, 60). Therefore, the introduction of a comprehensive scoring system reflecting dietary and non-dietary antioxidant and pro-oxidant exposures to assess the relationship between oxidative stress and periodontitis is warranted (61). OBS, which combines dietary and lifestyle factors, has been shown to be a useful marker for inflammatory diseases in studies in different countries and regions (43, 62, 63).

The strengths of our study include the simultaneous consideration of multiple dietary and lifestyle factors for the oxidative potential of periodontitis; secondly, the use of a complex multi-stage probability sampling design and appropriate covariate adjustment increased the reliability and representativeness of our study. Our study has some limitations. First, due to the design of the cross-sectional study, a causal relationship between OBS and periodontitis could not be inferred (64). In addition, database limitations prevented the inclusion of all covariates that have an impact on oxidative stress, such as environmental pollution, flavonoid intake, and Oxidative markers (65). Nevertheless, the correlation between periodontitis and current OBS was stable enough to be less likely to be significantly influenced by unincluded factors.

5. Conclusion

In conclusion, higher OBS indicates that dietary and lifestyle antioxidant exposure is superior to prooxidant exposure and is associated with a lower risk of periodontitis. Our results suggest that OBS may serve as a biomarker for periodontitis in adults. However, further studies are still needed to validate our findings.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: www.cdc.gov/nchs/nhanes/.

Ethics statement

The studies involving human participants were reviewed and approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HQ read and approved the final manuscript, performed the analysis, wrote a draft of this manuscript, conceived the study design, contributed to the interpretation of the results, and critically revised the manuscript for important intellectual content.

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Conflict of interest

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

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Supplementary material

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Associations between serum trace elements and the risk of nasopharyngeal carcinoma: a multi-center case-control study in Guangdong Province, southern China

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Background: Associations between trace elements and nasopharyngeal carcinoma (NPC) have been speculated but not thoroughly examined.

Methods: This study registered a total of 225 newly diagnosed patients with NPC and 225 healthy controls matched by sex and age from three municipal hospitals in Guangdong Province, southern China between 2011 and 2015. Information was collected by questionnaire on the demographic characteristics and other possibly confounding lifestyle factors. Eight trace elements and the level of Epstein–Barr virus (EBV) antibody were measured in casual (spot) serum specimens by inductively coupled plasma–mass spectrometry (ICP-MS) and enzyme-linked immunosorbent assay (ELISA), respectively. Restricted cubic splines and conditional logistic regression were applied to assess the relationship between trace elements and NPC risk through single-and multiple-elements models.

Results: Serum levels of chromium (Cr), cobalt (Co), nickel (Ni), arsenic (As), strontium (Sr) and molybdenum (Mo) were not associated with NPC risk. Manganese (Mn) and cadmium (Cd) were positively associated with NPC risk in both single-and multiple-element models, with ORs of the highest tertile compared with the reference categories 3.90 (95% CI, 1.27 to 7.34) for Mn and 2.30 (95% CI, 1.26 to 3.38) for Cd. Restricted cubic splines showed that there was a linear increasing trend between Mn and NPC risk, while for Cd there was a J-type correlation.

Conclusion: Serum levels of Cd and Mn was positively related with NPC risk. Prospective researches on the associations of the two trace elements with NPC ought to be taken into account within the future.

KEYWORDS

cadmium (Cd), manganese (Mn), nasopharyngeal carcinoma (NPC), head and neck cancer, logistic regression, restricted cubic splines, odds ratio, trace element (TE)

1. Introduction

Nasopharyngeal carcinoma (NPC) is a malignant epithelial tumor arising from the nasopharyngeal mucosal lining (1) and has a high incidence in southern China (2). 133,354 new cases of NPC were diagnosed in 2020 around the world, and around half of the patients are from southern China (3). Epstein–Barr virus (EBV) infection is a major risk factor for undifferentiated NPC, which is responsible for over 90% of the total patients in the areas with high incidence (4). EBV is a ubiquitous herpesvirus that is carried by more than 90% of people worldwide, but only a small division suffer from NPC (5), which could not explain the unique regional characteristics of NPC. Therefore, cofactors must mediate the effect of EBV on NPC.

Trace elements are widely present in the environment and can influence the human body complexly by accumulating through water or contaminated food intake. Although in the normal range some trace elements may be essential in metabolism, they can become potentially toxic and produce negative effects on health at higher concentrations or a long-term exposure (6). This may be due to the ability of trace elements to increase oxidative stress or change the repair of DNA damage (7, 8). Several studies have reported that certain trace elements are closely related to the regional endemic tumors. For example, the regional high prevalence of liver cancer in Taiwan and lung cancer in the miners of Gejiu, Yunnan Province are associated with excessive exposure of arsenic (As) in water and Tin in mine dust, respectively (9, 10).

As a regional endemic tumor, the relationship between trace elements and NPC have been doubted for several decades (11). Several ecological studies have shown that the concentrations of As and cadmium (Cd) in rice and some plant-based food in Guangdong exceeded the food safety threshold (12). In addition, the concentrations of nickel (Ni) in rice, drinking water or local residents' hair are significantly higher in high-risk regions than those in low counterparts (12–14). Epidemiological surveys have reported that trace elements Cd and zinc were found to be positively associated with NPC, while for strontium (Sr), calcium and magnesium were negatively associated (15). However, previous studies have not provided convincing evidence for the impact of trace elements on NPC, in part because those were mainly ecological studies, or limited by their small sample sizes, or focused on individual trace element, or in the occupational populations (16–18). A well-designed case-control study is still required to explore the associations of commonly exposed trace elements with NPC risk in the general population of endemic regions.

In the present study, a case–control study in three municipal hospitals in NPC-endemic southern Chinese province was conducted to explore the serum concentrations of eight trace elements (chromium, Cr; manganese, Mn; cobalt, Co; Ni; As; Sr; molybdenum, Mo and Cd) with NPC risks, with the possible dose–response relationships and the interactions between some trace elements and other potential risk factors on NPC risks were also explored.

Abbreviations: As, Arsenic; Cd, Cadmium; CI, Confidence interval; Co, Cobalt; Cr, Chromium; EBV, Epstein–Barr virus; Mo, Molybdenum; Mn, Manganese; Ni, Nickel; NPC, Nasopharyngeal carcinoma; OR, Odds ratio; Sr., Strontium.

2. Materials and methods

2.1. Study population

Two hundred and forty newly diagnosed NPC cases were selected consecutively in three municipal hospitals in Guangdong Province, southern China from 2011 to 2015 years. 15 cases were excluded, including 4 samples collected after treatment, 5 cases with insufficient serum volume, and 6 cases with incomplete information, leaving 225 eligible cases for this study. The control group was recruited from the health check-ups of the same hospital in the same period and was randomly matched to cases at 1:1 ratio by age (±5 years old), sex and city of residence. The Institutional Research Ethics Committee of Sun Yat-sen University Cancer Center (SYSUCC) approved this study.

2.2. Sample collection

After fasting for 12 h, 4 mL of venous blood were collected from the subjects in an inert separation gel coagulant tube in the morning. Samples were separated and packed within 12 h to obtain serological samples, and immediately transferred to the laboratory for storage in a refrigerator at −80°C until assay.

Interviewers were trained and an electronic organized questionnaire was utilized by interviewers to face-to-face interviews. To minimize interviewer bias, each questioner was assigned a roughly same number of cases and controls. The collected information contains the following factors: demographic characteristics; current occupation; education levels; residential history; chronic sinusitis history; family history of NPC; cigarette smoking and alcohol consumption.

2.3. Analytical procedures

Serum concentrations of the eight concerned trace elements (i.e., Cr, Mn, Co, Ni, As, Sr., Mo, and Cd) were determined by using inductively coupled plasma–mass spectrometry (ICP-MS, Thermo Fisher Scientific company, Germany). The detection limits of each element were demonstrated (Supplementary Table S1). In brief, each 200 µL of serum samples was weighted into a 5 mL pressure vessel, adding 1 ml 65% HNO_3 and 1 ml 30% H_2O_2. Three parallel tests were carried out for each sample, and the average value of the three tests was taken. Samples were treated as a half value of the lower detection limit when they below the detection limit and were excluded when they higher than or lower than three standard deviations of the control group (log-transformed).

The serum samples were detected blindly by the inorganic and element analysis platform in the Test Center of Sun Yat-sen University. 5% serum samples were randomly selected for retest, and Intra-class Correlation Coefficients (ICC) was calculated to assess the test–retest reliability. Supplementary Table S2 shows that the reliability is good, with most of the trace elements having an ICC greater than 0.7. The detection limit of specific elements and the calibration equation show

a good linear relationship between the concentration of each element and the signal strength of the instrument (all of $R^2 > 0.999$; data not shown).

Viral capsid antigen to IgA (VCA-lgA) was measured by using the commercial enzyme-linked immunosorbent assay (ELISA) kits (EUROIMMUNAG, Lübeck, Germany) at the central laboratories of SYSUCC. Our previous study has described the detailed procedure of the EBV serological test (19). In our study, according to the median of antibody level of all subjects, the group with higher levels of VCA-lgA was ≥1.212.

2.4. Statistical analysis

The characteristics between cases and controls were compared by Chi-square tests or Mann–Whitney *U* tests. And the relationships between each trace elements were evaluated by using Spearman's rank correlation. We used restricted cubic splines to explore nonlinear relationships between trace elements (log-transformed) and the NPC risk (OR). Knots within the splines were set at 5th, 35th, 65th and 95th percentile, respectively. Conditional logistic regression models with single and multiple-element models were applied to estimate the associations between each trace element and NPC. The concentration of trace elements related with the lowest NPC risk was the concentration with the lowest OR on the spline curve. Considering that the lowest OR of nonlinear trace elements is located in the second tertile of the serum trace element concentration of the controls, the associations between three predefined trace elements concentrations categories and NPC risks were examined: three equally distributed categories of trace elements concentration of controls were defined by the 33rd and 67th centiles. The reference criterion for these studies was the trace elements level associated with the lowest NPC risk. Adjusted factors included gender (male, female), age (years), chronic rhinitis status (yes, no), first-degree family history of NPC (yes, no), drinking (ever, never), smoking status (ever, never) and the levels of VCA-lgA (higher, lower). Ever smokers were defined as those who reported having smoked more than 1 cigarette every 1–3 days for 6 consecutive months, and ever drinkers were defined as those who consumed more than 1 glass of alcoholic beverage (including wine, beer and liquor) per day for an equal period. Respondents who had quit smoking or alcohol for less than 1 year were considered 'current' in our study; Those who had quit for longer were considered "former." Both "former" and "current" consumers were collectively called "ever" consumers (20, 21). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to evaluate NPC risk. For linear trace elements, *p*-values for the trend were treated by the median of each element tertiles as continuous variables.

We used likelihood ratio tests to compare interaction term between each serum trace element and potential modifier in logistic regression model. Stratification analysis by smoking and the levels of EBV antibody was applied to estimate the relationship between the selected serum trace element and NPC risk. In the multiple-elements model, we used the Bonferroni correction to make multiple comparisons, and values of $p < 0.01$ were considered statistically significant. For other analyses, a two-tailed $p < 0.05$ was considered statistically significant. Data were analyzed by R statistical packages (The R Foundation; http://www.r-project.org; version 4.1.1).

3. Results

3.1. Characteristics of study population

Table 1 displayed the distribution of demographic variables and possible risk factors for NPC among the 225 NPC patients and

TABLE 1 Characteristics of nasopharyngeal carcinoma (NPC) cases and controls.

Characteristics	Cases (*N*=225)	Controls (*N*=225)	*P*-value
Age (years, Mean ± standard deviation)	50.00 ± 11.03	48.91 ± 11.93	0.491
Residential area, *n* (%)			0.970
Zhongshan	59 (26.22)	57 (25.33)	
Sihui	56 (24.89)	58 (25.78)	
Zhaoqing	110 (48.89)	110 (48.89)	
Gender, *n* (%)			0.363
Male	158 (70.22)	148 (65.78)	
Female	67 (29.78)	77 (34.22)	
Chronic rhinitis status, *n* (%)			<0.001
No	192 (85.33)	213 (94.67)	
Yes	33 (14.67)	12 (5.33)	
Cigarette smoking, *n* (%)			0.072
Never	95 (42.22)	115 (51.11)	
Ever	130 (57.78)	110 (48.89)	
Alcohol consumption, *n* (%)			0.006
Never	142 (63.11)	170 (75.56)	
Ever	83 (36.89)	55 (24.44)	
First-degree family history of NPC, *n* (%)			<0.001
No	202 (89.78)	220 (97.78)	
Yes	23 (10.22)	5 (2.22)	
EBV antibodies (VCA-lgA), *n* (%)			<0.001
Lower level	27 (12.00)	198 (88.00)	
Higher level	198 (88.00)	27 (12.00)	
Education levels, *n* (%)			0.977
None or primary school	91 (40.44)	87 (38.67)	
Secondary school	89 (39.56)	90 (40.00)	
High school	36 (16.00)	38 (16.89)	
University or more	9 (4.00)	10 (4.44)	
Current occupation, *n* (%)			0.271
Unemployed	8 (3.56)	7 (3.11)	
Farmer	103 (45.78)	100 (44.44)	
Blue collar	65 (28.89)	69 (30.67)	
White collar	24 (10.67)	25 (11.11)	
Other/unknown	25 (11.11)	24 (10.67)	

Student's *t*-tests and Chi-square tests were used to estimate differences of variables between the case and control group according to their distributions.

225 controls. NPC patients, compared with controls, were more likely to have chronic rhinitis, to have cigarette smoking, to have consumed alcohol, to have higher EBV antibody levels and to have a first-degree NPC family history. The mean age was 50.00 ± 11.03 years old in NPC patients and 48.91 ± 11.93(SD) years old in control group, accordingly ($p > 0.05$). In addition, 70.22% of patients with NPC were male ($N = 158$). There was no statistical difference in occupational types and education levels between the case and control groups.

3.2. Concentration levels of the trace elements in the case and control group

A total of eight trace elements in serum samples were tested between cases and controls. The detection limits of each element were displayed in Supplementary Table S2. Except Sr., the levels of other trace elements between NPC patients and controls were significantly distinct ($p < 0.05$). The median concentrations of five elements were higher in the NPC patients than in the control group, including Co (0.66 vs. 0.32 μg/L), Ni (4.17 vs. 1.77 μg/L), Cr (2.99 vs. 2.15 μg/L), Mn (12.93 vs. 4.52 μg/L) and Cd (0.91 vs. 0.20 μg/L), whereas As (1.99 vs. 2.59 μg/L) and Mo (0.79 vs. 1.14 μg/L) values were lower in the cases (Table 2). Most of the elements showed significant correlations (Supplementary Table S3), with the highest Spearman's rank correlation 0.720 between Mn and Co.

3.3. Association between serum trace elements and the risk of nasopharyngeal carcinoma

We used restricted cubic splines to flexibly model and visualize the relation of serum trace elements (log-transformed) with NPC risk (Figure 1). After adjusting for potential confounders, the restricted cubic spline showed U-shaped correlations between Co and Sr concentrations on a continuous scale and NPC risk, J-shaped associations for Cd and As and linear relationships for Cr, Mn, Ni, and Mo. The adjusted ORs for the risk of NPC related with linear and nonlinear serum trace elements in the single-element models were shown in Tables 3, 4, respectively. After adjusting different potential confounders, no significant associations were found between serum As, Sr. and Mo and the risk of NPC. Serum Cr, Mn, Co, Ni and Cd were positively associated with the risk of NPC, with the OR of the highest tertile of trace elements vs. the reference category 3.90 (95%CI, 1.27 to 7.34) for Mn and 2.30 (95%CI, 1.26 to 3.38) for Cd (Figure 2) after further adjusting the five metals in the multiple-elements models. The variance inflation factor (VIF) was <1.5 for all the five trace elements indicating collinearity was not a concern.

Restricted cubic spline displayed the association between levels of Cd concentration on a continuous scale and NPC risk was J-shaped (P for nonlinearity < 0.001); when we compared with the reference category (after transformed;1.004–1.017 μ g/L; 33rd–67th centiles), the multivariable adjusted OR for NPC risks were 2.30 (95%CI, 1.26 to 3.38) for the highest tertile (after transformed; >1.017 μg/L; 68th–100th centiles), 1.09 (95%CI, 0.88 to 2.53) for the lowest tertile (log-transformed; <1.003 μg/L 1st–32nd centiles; Figure 1). And there was a linear relationship between serum Mn and NPC risk (P for nonlinearity = 0.519). When the concentration of serum Mn was lower than 8.73 μg/L [log (Mn + 10) = 1.27 μg/L], serum Mn showed a weak protective effect for this cancer, with OR < 1; After exceeding this value, the OR of NPC risk became >1 and increased linearly with the increase of serum Mn concentration.

Stratified analyses by potential effect modifiers (Supplementary Table S4) showed that NPC risk associated with serum Mn and Cd were slightly higher in the ever-smokers and those with high level of VCA-lgA. The risk of NPC associated with the highest tertile of Mn compared with the reference category was 4.41 (95%CI, 2.04 to 9.80) in ever-smokers vs. 3.53 (95%CI, 1.32 to 9.99) in never-smokers, and 2.55 (95%CI, 1.20 to 5.48) in ever-smokers vs. 1.47 (95%CI, 0.54 to 4.05) in never-smokers for Cd. Similarly, the risk of NPC associated with the highest tertile of Mn compared with reference category was 6.28 (95%CI, 1.82 to 11.02) in high antibody level group vs. 5.29 (95%CI, 1.60 to 9.27) in low antibody level group, and 2.43 (95%CI, 1.02 to 6.04) in high antibody level group vs. 1.55 (95%CI, 0.63 to 3.62) in low antibody level group for Cd. We further explored the other risk factors,

TABLE 2 Concentrations of trace elements in serum of controls and cases (N=450; μg/L).

Elements	Controls (N=225)				Cases (N=225)				P-value
	Mean (SD)	Percentile 25	Median	Percentile 75	Mean (SD)	Percentile 25	Median	Percentile 75	
Cr	3.28 (4.01)	0.93	2.15	3.92	4.33 (4.39)	1.58	2.99	6.05	<0.001
Mn	7.06 (7.70)	2.11	4.52	9.59	15.12 (10.10)	7.38	12.93	21.96	<0.001
Co	0.43 (0.35)	0.17	0.32	0.53	0.68 (0.41)	0.38	0.66	0.92	<0.001
Ni	5.24 (10.45)	0.50	1.77	4.37	6.39 (8.08)	0.92	4.17	8.94	<0.001
As	5.55 (7.00)	1.86	2.59	5.22	5.46 (8.05)	1.42	1.99	4.24	<0.001
Sr	30.34 (10.12)	23.13	28.88	35.26	31.55 (12.74)	23.20	29.13	38.15	0.519
Mo	1.62 (1.87)	0.13	1.14	2.14	1.13 (1.49)	0.05	0.79	1.63	0.004
Cd	0.42 (0.71)	0.06	0.20	0.58	1.82 (2.11)	0.05	0.91	3.21	<0.001

Cr, Chromium; Mn, Manganese; Co, Cobalt; Ni, Nickel; As, Arsenic; Sr, Strontium; Mo, Molybdenum; Cd, Cadmium. P-values are used for Mann–Whitney U-tests for continuous variables according to the distribution.

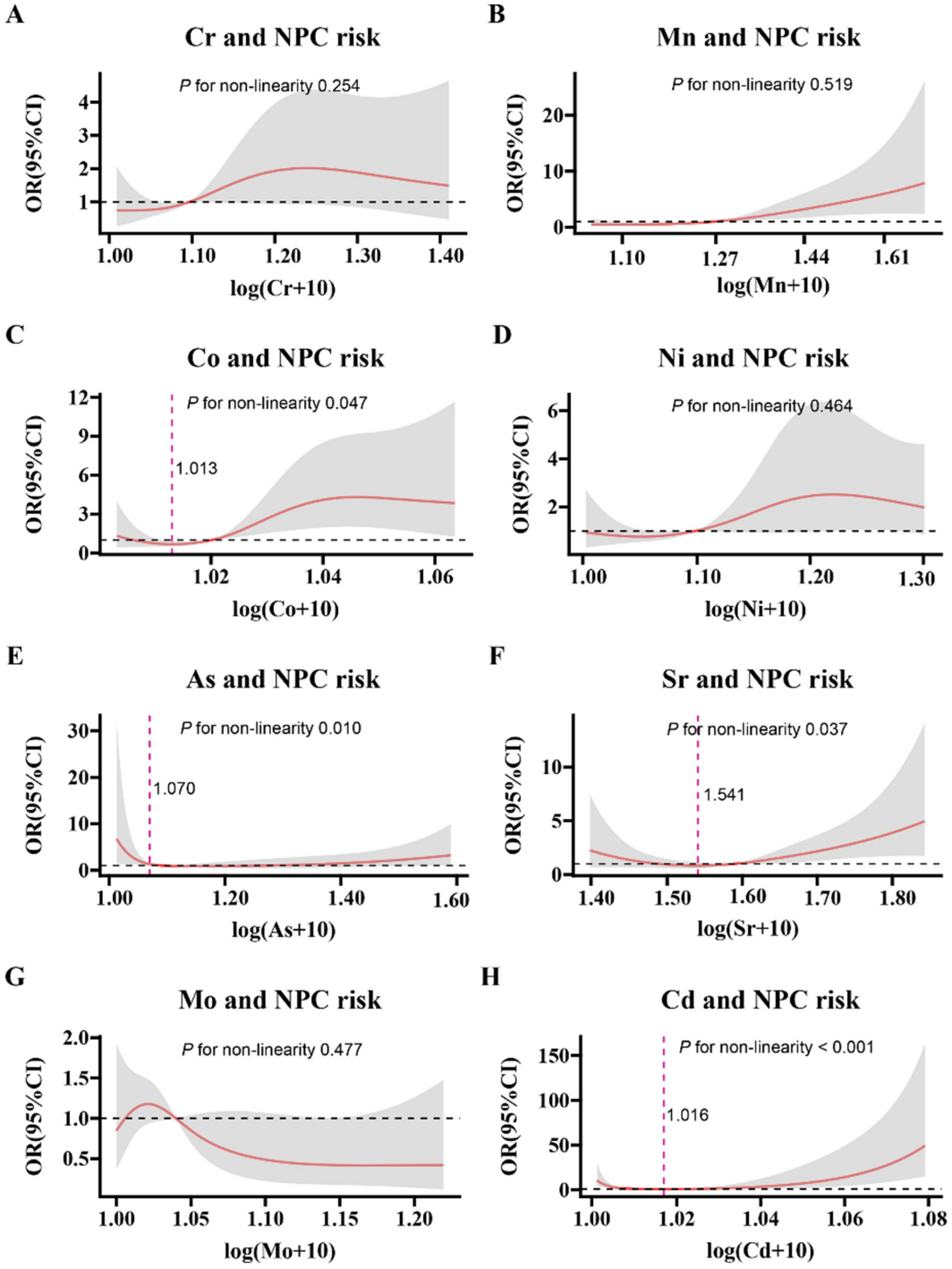

FIGURE 1
Nonlinear associations between trace elements in serum (log-transformed) and the risk of NPC (Odds Ratio) in the single-element models. Nonlinear associations were presented by the restricted cubic splines, and adjusted by age (years), gender (male, female), chronic rhinitis status (yes, no), first-degree family history of NPC (yes, no), drinking (ever, never), smoking status (ever, never) and the levels of VCA-IgA (higher, lower). Shading in the plots indicated the confidence interval (95%CI). The knots in the plots were set at 5th, 35th, 65th, and 95th percentile, respectively. Dashed red lines indicate the concentration of serum trace elements with the lowest risk of NPC for nonlinear trace element. Figure A-H represented the risk of NPC with the concentration of serum chromium, manganese, cobalt, nickel, arsenic, strontium, molybdenum and cadmium, respectively.

including chronic rhinitis status, alcohol consumption and first-degree family history of NPC, and the potential synergistic effects of trace elements in the carcinogenesis of nasopharyngeal epithelial cells, and no obvious interactions have been found, with all of the $p > 0.05$.

4. Discussion

This multi-center case–control study probed into the relationship between trace elements and NPC risk in Guangdong Province, a region

TABLE 3 Odds ratios for the association between serum levels of trace element (Linear) and the risk of nasopharyngeal carcinoma based on the single-element model (95% confidence intervals).

Serum trace elements (μg/L)		Tertiles of serum trace elements (μg/L)			*P*-trend*
		Q1	Q2	Q3	
Cr					
	N (controls/cases)	148 (74/74)	156 (74/82)	146 (77/69)	
	Model 1	Reference	1.30 (0.78–2.19)	2.21 (1.36–3.62)	0.012
	Model 2	Reference	1.31 (0.78–2.19)	2.27 (1.39–3.72)	0.009
	Model 3	Reference	1.17 (0.54–2.54)	2.38 (1.14–5.06)	0.048
Mn					
	N (controls/cases)	92 (74/18)	116 (74/42)	242 (77/165)	
	Model 1	Reference	2.52 (1.32–4.95)	9.54 (5.22–12.25)	<0.001
	Model 2	Reference	2.63 (1.38–5.19)	9.90 (5.40–12.98)	<0.001
	Model 3	Reference	1.72 (0.70–4.29)	5.13 (2.23–8.16)	<0.001
Ni					
	N (controls/cases)	129 (74/55)	119 (74/45)	202 (77/125)	
	Model 1	Reference	0.72 (0.42–1.23)	1.99 (1.25–3.17)	<0.001
	Model 2	Reference	0.69 (0.40–1.18)	1.99 (1.25–3.18)	<0.001
	Model 3	Reference	1.21 (0.55–2.71)	2.22 (1.11–4.49)	0.015
Mo					
	N (controls/cases)	138 (74/64)	190 (74/116)	122 (77/45)	
	Model 1	Reference	0.78 (0.50–1.23)	0.40 (0.24–0.66)	0.006
	Model 2	Reference	0.79 (0.50–1.25)	0.40 (0.24–0.66)	0.007
	Model 3	Reference	0.96 (0.48–1.92)	0.61 (0.44–1.03)	0.105

*P-trend was estimated from the conditional logistic regression models according to the median value of each quartile of elements as a continuous variable.
Model 1 Adjusted for age, gender. Chronic rhinitis status, drinking and first-degree family history of NPC. Model 2 Further adjusted by smoking status (never/ever). Model 3 Further adjusted by the levels of VCA-lgA (lower/higher). Trace elements were added 10 and transformed by log.

with high NPC incidence. Of the 8 common trace elements investigated, our results showed that serum Mn and Cd were both significantly positive associated with NPC risk in both single-and multiple-element models; and restricted cubic splines showed that there was a linear increasing trend between Mn and NPC risk, while for Cd there was a J-type correlation. But no associations with other trace elements (Cr, Co, Ni, As, Sr, and Mo) were found.

First of all, the reason for studying the relationship between these 8 trace elements and NPC is that all of them play important physiological and metabolic roles in the human body. In addition, previous studies have shown a deficiency or excessive intake of the trace elements of As, Cd, Ni and Sr. had a close ecological relationship with the occurrence of NPC, or some trace elements were closely associated with other types of tumors, such as Cr for lung, thyroid and exocrine pancreatic cancers (22–24), Mn for respiratory cancer and respiratory disease mortality (25), Co for respiratory toxicity (26) and Mo for breast cancer (27). Therefore, we conducted this case–control study for an in-depth exploration of the relationships between these trace elements and NPC. Of the limitation in our laboratory testing ability, we did not test for Zn and Tin, although there have been reported positive associations of NPC or regional liver cancer with these two trace elements (15).

Generally, the main routes of general populations to Cd exposure are water and food intake (1). Although some NPC patients may reduce their dietary intake due to physical discomfort, such as nasal congestion, tinnitus, nasal discharge stained with blood, in theory, this can only reduce the level of serum trace elements in patients with NPC. However, in our study, we still found a positive correlation between higher serum trace elements and NPC risk. Existing studies have reported that the Cd level in the soil of Guangdong Province exceeded Chinese food safety threshold (12), which might result in elevated serum Cd concentration in the local residences (28) and the high prevalence in southern China. Smoking is confirmed as another source of Cd exposure (29), and might also increase the risk of NPC in this population. However, we cannot distinguish the exposure sources for each individual, such as trace elements in the workplace, and a more detailed study should be conducted in the future.

Our result of a moderate positive correlation between Cd and NPC risk in southern China is consistent with another case–control study in Tunisia, an area with low incidence of NPC (18). Our study, however, has a slightly higher OR than that in Tunisia (Average OR = 2.30 and 1.31, respectively), the potential cause of which might be that people in high NPC incidence are more sensitive to Cd exposure, which might be due to the NPC susceptibility genes in NPC endemic regions. Several epidemiologic investigations of genetic susceptibility to NPC have discovered SNPs in relation to genetic polymorphisms in cytochrome P450 (CYP) and glutathione-S-transferase (GST) gene families encode phase I and II xenobiotic metabolism enzymes

TABLE 4 Odds ratios for the association between serum levels of trace element (nonlinear) and the risk of nasopharyngeal carcinoma based on the single-element model (95% confidence intervals).

Serum trace elements		N controls/ cases	Model 1		Model 2		Model 3	
			OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Co								
	Q1 (<1.009)	100 (72/28)	0.70 (0.38–1.29)	0.261	0.70 (0.38–1.30)	0.263	1.07 (0.45–2.56)	0.880
	Q2 (1.009–1.019)	115 (76/39)	Reference		Reference		Reference	
	Q3 (>1.019)	225 (77/158)	3.96 (2.43–6.57)	<0.001	3.99 (2.45–6.63)	<0.001	2.88 (1.43–5.91)	0.003
As								
	Q1 (<1.060)	146 (73/73)	2.69 (1.63–4.49)	<0.001	2.76 (1.67–4.63)	<0.001	1.91 (0.91–4.06)	0.087
	Q2 (1.060–1.133)	162 (74/88)	Reference		Reference		Reference	
	Q3 (>1.133)	142 (78/64)	1.19 (0.75–1.90)	0.458	1.20 (0.76–1.92)	0.434	1.06 (0.53–2.10)	0.869
Sr								
	Q1 (<1.538)	141 (74/67)	1.15 (0.70–1.88)	0.587	1.14 (0.70–1.88)	0.590	0.98 (0.46–2.07)	0.964
	Q2 (1.538–1.624)	141 (74/67)	Reference		Reference		Reference	
	Q3 (>1.624)	168 (77/91)	1.47 (0.91–2.36)	0.113	1.46 (0.91–2.36)	0.115	2.29 (1.10–4.90)	0.028
Cd								
	Q1 (<1.004)	154 (74/80)	2.39 (1.35–4.29)	0.003	2.46 (1.38–4.44)	0.003	1.99 (0.87–4.59)	0.105
	Q2 (1.004–1.017)	150 (74/76)	Reference		Reference		Reference	
	Q3 (>1.017)	146 (77/69)	3.61 (2.28–5.79)	<0.001	3.61 (2.28–5.78)	<0.001	2.34 (1.19–4.61)	0.013

Model 1 Adjusted for age, gender. Chronic rhinitis status, drinking and first-degree family history of NPC. Model 2 Further adjusted by smoking status (never/ever). Model 3 Further adjusted by the levels of VCA-IgA (lower/higher). Trace elements were added 10 and transformed by log.

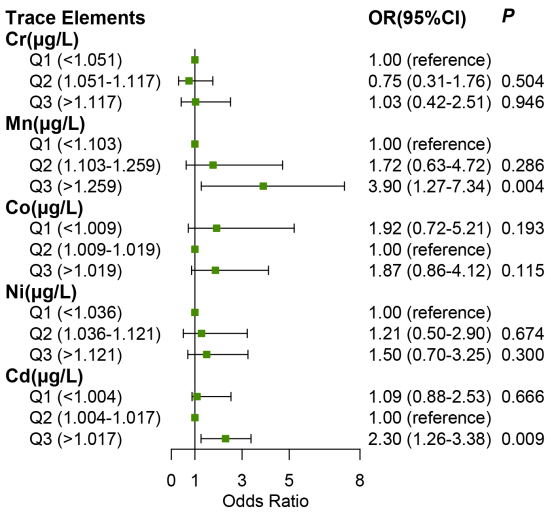

FIGURE 2
Associations between trace elements in serum (log-transformed) and the risk of NPC (Odds Ratio) in the multiple-element models. Adjusted by age, gender, chronic rhinitis status, smoking, drinking, first-degree family history of NPC and VCA-IgA. OR: odds ratio. CI: confidence interval. Trace elements were added 10 and log transformed.

involved in the biotransformation of chemicals such as the toxin of Cd (30–32). However, more biological mechanisms for the sensitivity of trace element in cancer patients or people in endemic areas are still needed. Cd has been confirmed as the first group of human carcinogen by the International Institute for the Classification of Cancer and closely related to a variety of cancers, including prostate and hormone-related cancer (33). According to etiological studies of other cancers, it can be related to its function in promoting hypermethylation (34–36), malignant transformation and DNA repair inhibition (37).

Mn is an essential trace element for the human body, but excessive intake will also be harmful to human health. In our study, the median concentration of serum Mn in healthy people of Guangdong province (4.52 μg/L) was obviously higher than those in other studies from China (3.30 μg/L) (38) and European countries (2.32 μg/L) (39). This may relate by serious Mn pollution of groundwater in the Pearl River Delta (40). Some studies have shown that Mn in three types of drinking water in Zhaoqing area of Guangdong Province has exceeded the standard, and the over-standard rate of well water is as high as 26.2% (41, 42). In addition to exposure from water, some living habits such as drinking tea (43) and ingesting herbal plants (44) also affect the level of Mn in the human body. Cantonese people have the habit of drinking herbal tea and herbal soup. Early symptoms of NPC are often confused with influenza, prompting patients to consume herbal diets more frequently to manage these symptoms prior to a clinical diagnosis, which may relate with a higher Mn content in NPC patients (45). Although there is no definite evidence for the relationship between Mn levels and tumors, it is speculated that a high Mn level may affect DNA replication and repair courses for cell mutations, DNA damage and chromosome aberrations (46). This analysis is the first to report a strong positive relationship between Mn and NPC risk and a linear-relationship toward higher risk (25, 47). The role of Mn in the NPC development, however, needs additional investigation.

It is worth mentioning that some studies have shown that Ni also correlates with NPC (16). An epidemiological survey found a

significantly higher Ni level in rice, drinking water and hair of local inhabitants in high-risk regions than in low counterparts. In addition, in high-risk regions, there was also a higher Ni content in NPC patients than in controls (15). In our study, only single-element models showed a moderate correlation between Ni and NPC. This correlation was not shown in the multiple-element models, which might be because of the insufficient sample size, or Ni was affected by other known or unknown confounding factors. For example, our study found that the correlation coefficients between Ni and Mn, Ni and Cd are 0.363 (p-value < 0.001) and 0.278 (p-value < 0.001), respectively. However, this does not rule out the possibility that there is no correlation between Ni and NPC, which needed to be studied with a larger sample size.

In the stratified analysis, the NPC risk associated with Mn and Cd were slightly higher in the ever-smokers and the high antibody level group than those in the never-smokers and the low EBV antibody level group. This may be due to the synergistic effect (21, 48, 49) between trace elements and the two risk factors in the carcinogenesis of nasopharyngeal epithelial cells, although the p value of interactions were higher than 0.05.

Until now, the cumulative evidence powerfully displays a causal role of EBV in the incident of NPC; However, EBV alone is not an adequate cause of NPC. Environmental cofactors prompt NPC development. It is believed that the accumulation of genome instability induced by environmental factors can facilitate EBV persistent infection in the precancerous nasopharyngeal epithelium. Once infected, EBV latent genes give growth and survival benefits that lead to NPC occurrence (50, 51). According to this etiological model, we postulate that long-term exposure to the trace elements, e.g., Cd (34–36), Ni (52), and Mn (46), might impose various genetic damage or alterations in nasopharyngeal epithelial cells, which further mediates EBV infection and promotes NPC development.

There are several strengths of this study. First of all, the intensity of our research demonstration is high. We comprehensively compared the relationship between eight serum trace elements and NPC with a multi-center sample collection. Strict quality control was applied and the quality of laboratory testing was high. Moreover, our research results are relatively robust. Both single and multiple-element models were utilized and restricted cubic splines were also used to analyze the nonlinear relationship between the concentration of serum trace elements and the risk of NPC. We found a linear increasing trend between Mn and NPC risk, while for Cd there was a J-type correlation.

However, our study has obvious limitations. First of all, our study is based on retrospective data; Secondly, trace elements in serum do not reflect long-term exposure levels; Thirdly, our research lacks occupational exposure information and specific amounts of alcohol consumption and pack years of smoking for the participants; Finally, the results cannot be directly extended to those in the low-risk areas; Consequently, further studies with larger cohorts and systematic clinical evidence are expected to shed light on the causal relationship between them.

5. Conclusion

In conclusion, Mn and Cd were positively related with NPC risk. Mn and Cd in certain concentration elevate the risk of NPC. In addition, prospective studies on the relationship between trace elements and NPC should be considered in the future.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: information that could compromise research participant privacy or consent. Explicit consent to deposit raw data was not obtained from the participants. Requests to access these datasets should be directed to S-MC, caosm@sysucc.org.cn.

Ethics statement

The studies involving human participants were reviewed and approved by Sun Yat-sen University Cancer Center. The patients/participants provided their written informed consent to participate in this study.

Author contributions

X-YG, S-MC, and A-HL contributed to conception and design of the study. S-HX and WC organized the database. X-YG, HW, and XL performed the statistical analysis. X-YG wrote the first draft of the manuscript. S-MC, H-NZ, and XY wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1142861/full#supplementary-material

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The effect of L-carnitine supplementation on lipid profile in adults: an umbrella meta-analysis on interventional meta-analyses

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Introduction: Previous meta-analyses investigating the therapeutic effects of L-carnitine on lipid profiles have demonstrated inconsistent results. The present umbrella meta-analysis aimed to investigate the impact of efficacy of L-carnitine on lipid profiles in adults.

Methods: Databases including PubMed, Scopus, and Embase, Web of Science, and Google Scholar were searched up to June 2023. Meta-analysis was performed using a random-effects model.

Results: Our results from thirteen meta-analyses indicated that L-carnitine supplementation significantly total cholesterol (TC) (ES = −1.05 mg/dL, 95% CI: −1.71, −0.39; $p = 0.002$), triglycerides (TG) (ES = −2.51 mg/dL; 95% CI: −3.62, −1.39, $p < 0.001$), and low-density lipoprotein-cholesterol (LDL-C) (ES = −4.81 mg/dL; 95% CI: −6.04, −3.59; $p < 0.001$). It also increased high-density lipoprotein-cholesterol (HDL-C) (ES: 0.66 mg/dL, 95% CI: 0.20, 1.12, $p = 0.005$) levels.

Conclusion: The present umbrella meta-analysis suggests supplementation with L-carnitine in a dosage of more than 2 g/day can improve lipid profile. Thus, L-carnitine supplementation can be recommended as an adjuvant anti-hyperlipidemic agent.

KEYWORDS

L-Carnitine, lipid profile, dyslipidemia, umbrella meta-analysis, systematic reviews

Introduction

Chronic heart disease (CHD) is a serious problem in public health in the world. The prevalence of this disease has enhanced considerably in developed and developing countries (1). CHD is a leading cause of death in the world, claiming the lives of up to 17.5 million people each year (2). The underlying cause of CHD is atherosclerosis, an inactive, progressive condition characterized by the deposition of excess cholesterol in the sub endothelial space (3, 4). Dyslipidemia, as one of the most important modifiable risk factors for atherosclerosis, is

determined by a disturbance in circulating amounts of triglycerides (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels (5, 6). Therefore, dyslipidemia is associated with atherosclerosis leading to CHD. Statins are pharmacological drugs used to prevent CHD by reducing plasma LDL-C levels; however, statins do not significantly alter other lipid indices (7, 8). Also, these common medications can cause poisoning and side effects such as myotoxicity, intracranial hemorrhage, and coenzyme Q10 deficiency in patients (3, 4, 9). Currently, natural compounds are used for the prevention and treatment of chronic diseases, and the use of natural compounds with antioxidant, anti-inflammatory, lipid profile modulating, and blood pressure lowering properties as oral supplements are one of the new ways to prevent and treat chronic diseases (10, 11).

L-carnitine is an ammonium cation, either obtained from dietary or synthesized in the liver, kidney, and brain. Animal sources like fish, meat, milk, and poultry are the best sources of L-carnitine (12). L-carnitine is necessary for importing long-chain fatty acids (LCFA) into the mitochondrial matrix for beta-oxidation (13) and depleting the acyl groups out of the mitochondria in all tissues (14). Furthermore, L-carnitine can improve adipokines concentrations (15) and decrease the repletion of detrimental metabolites generated in coronary embolism and thrombosis (16–18). Overall, these mechanisms may be improved lipid profile and prevent related diseases.

Several meta-analyses have been conducted to investigate the therapeutic effects of L-carnitine on lipid profiles (19, 20); nevertheless, the results are still inconsistent (21–23). Thus, the present umbrella meta-analysis study was conducted to impart accurate and deterministic data regarding supplementation with L-carnitine on lipid profiles including TG, TC, LDL-C, and HDL-C levels.

Methods

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines in this study to analyze the articles (PROSPERO registration number: CRD42022307425) (24).

Search strategy

To find the relevant studies, five electronic databases, including Scopus, Web of Science, PubMed, Embase and Google Scholar were searched systematically up to June 2023. Search strings were relevant to the L-carnitine on lipid profiles (Supplementary file). In addition, we used the sign of "*" to improve the search literature sensitivity. Besides, a manual search of the references of qualified studies was performed to minimize the risk of missing relevant papers.

Study selection

Following are the PICOTS criteria: Population/Patients (P: adults of 18 > years of age); Intervention (I: L-carnitine); Comparison (C: control or placebo group); Outcome (O: lipid profile) (TG, HDL-C, LDL-C, and TC); Time (T: studies with a duration of follow-up of ≥2 weeks); and Study design (S: Meta-analyses of RCTs). We included meta-analysis studies examining the impacts of L-carnitine supplementation on lipid profile with their effect sizes (ES) and their corresponding confidence intervals (CI). In addition, other typologies of research studies including *in vivo*, *in vitro* and *ex-vivo* studies, observational studies, case reports, meta-analyses of non-randomized controlled trials or non-controlled trials, and quasi-experimental studies were excluded from the present study.

Methodological quality and quality of evidence

The methodological quality was evaluated using the Assessing the Methodological Quality of Systematic Reviews (AMSTAR) tool (25). The AMSTAR2 checklist was categorized into "critically low quality," "low quality," "moderate quality," and "high quality." We evaluate the overall strength and quality of evidence using GRADE according to the Cochrane Handbook of systematic reviews of interventions and based on five factors: precision, consistency of results, bias risk, publication bias, and directness, and. The quality of a level decreases when one of the above factors is not met (26).

Data extraction

Data that were extracted included the outcomes (ESs and CIs of TG, HDL-C, LDL-C, and TC), information regarding the year of publication, the study's first author, number of placebo and intervention groups, study location, sample sizes, supplement dosage, and duration.

Statistical analysis

To evaluate the combined ES of the intervention, ESs, and CIs for lipid parameters were used. Cochran-Q test and I^2 index were applied to assess the heterogeneity of the meta-analysis. Significant heterogeneity of data was defined as $I^2 > 50\%$ or a significant Cochran-Q test ($p < 0.10$). Subgroup analyses were done according to the duration, the dose of L-carnitine, sample size, age, and health status to identify potential sources of heterogeneity. The sensitivity analysis was conducted by the one-study exclude method, to determine the effect of each meta-analysis on the overall ES. Egger's and Begg's tests were used to assess the effects of a small study. The funnel plot was evaluated by visual inspection to identify the publication bias. The trim and fill methods were performed if there was a publication bias. All statistical analyses were executed using Stata software (version 16, Stata Corp. College Station, TX, US).

Results

Study selection and study characteristics

Figure 1 shows the PRISMA flow diagram for the studies. There were 95 articles total after searching electronic databases. After

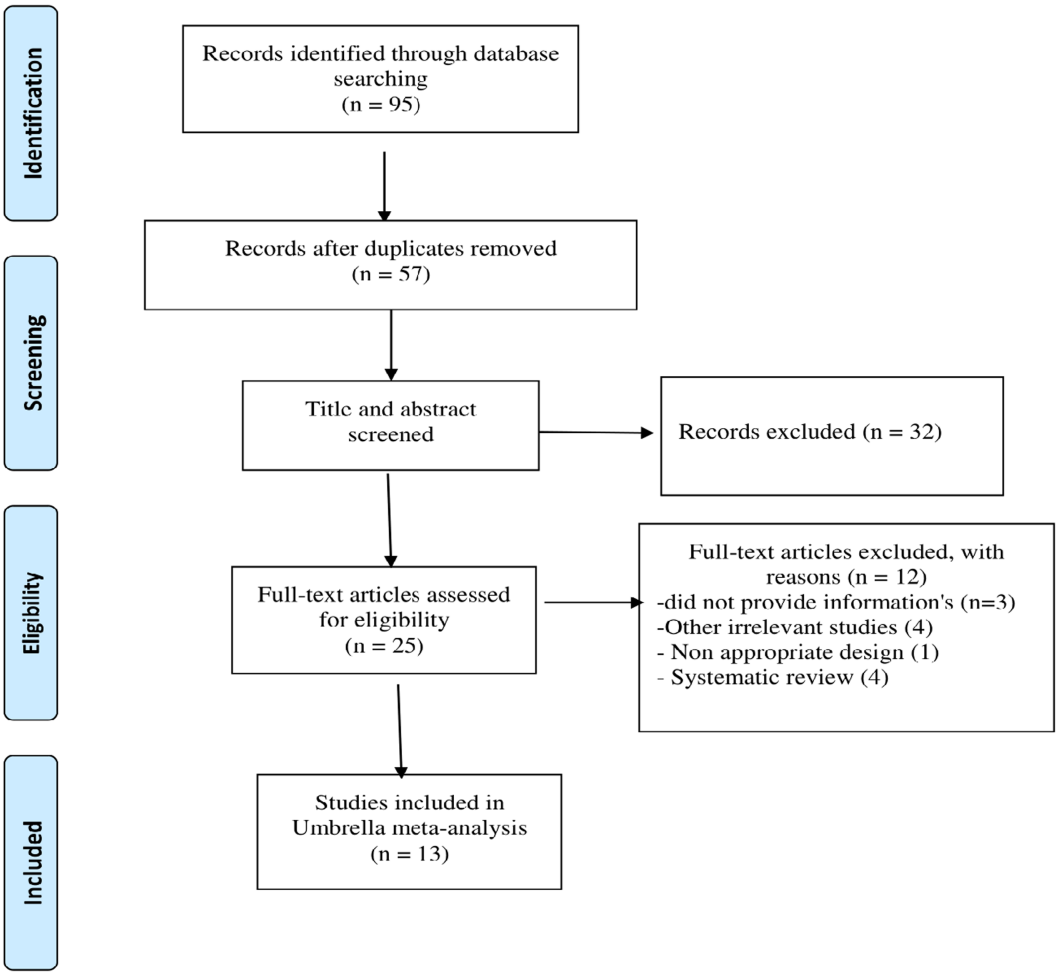

FIGURE 1
Flow diagram of study selection.

eliminating 38 duplicate articles, 57 papers were carefully evaluated based on titles and abstracts, with 25 being chosen for full-text evaluation. 13 meta-analyses eventually met our inclusion criteria and were included in the present umbrella meta-analysis. The features of the qualified articles are shown in Table 1. The total number of effect sizes identified was 11 for TG, and TC, and 12 for LDL-C, and HDL-C, depending on the type of variable studied. The included studies were published between 2013 and 2021, and the participants' average age ranged from 26 to 53. L-carnitine administration ranged from 0.54 g/day to 2.4 g/day on average across studies. From 14 to 25 weeks were spent taking L-carnitine supplements Table 1 illustrates the quality of the RCTs incorporated in the current umbrella meta-analysis.

Methodological quality and GRADING-of-evidence

Table 2 shows the results of the quality assessment of qualified studies using the AMSTAR2 questionnaire. Out of 13 studies, ten studies had high-quality and three studies had moderate-quality. Grade assessment revealed low quality for TC and TG, but LDL-c and HDL-c have a moderate, and very low quality of evidence, respectively, (Table 3).

L-carnitine on TC

L-carnitine supplementation had a significant lowering effect on TC level (ES = −1.05 mg/dL, 95% CI: −1.71, −0.39; $p = 0.002$), with a significant between-study heterogeneity ($I^2 = 87.1\%$, $p < 0.001$) (Figure 2). In subjects with a mean age of under 50 years old and a supplement of L-carnitine >2 g/day resulted in a notable reduction of TC (Table 4).

L-carnitine on TG

According to the pooled estimate, subjects who took supplements of L-carnitine had significantly decreased levels of TG (ES = −2.51 mg/dL; 95% CI: −3.62, −1.39, $p < 0.001$;12 meta-analyses) (Figure 3). The studies had significant between-study heterogeneity ($I^2 = 92.8\%$, $p < 0.001$). Subgroup analysis revealed that L-carnitine supplementation has a more pronounced effect on

TABLE 1 Study characteristics of included studies.

Citation (First author et al., year)	No. of studies in meta-analysis	Location	no. of participants in meta-analysis	Mean age (year)	dose(g)	Health condition/ duration(wk)	Quality assessment scale and outcome
Casariego et al. 2013	4	Spain	284	NR	2.2	T2DM 25 week	Yes (Jadad) 4/4 high
Abbasnezhad et al. 2020	6	Iran	468	53	1.9	Liver disorders 20 week	Yes (Cochrane) 6/6 high
Abolfathi et al. 2020	4	Malaysia	254	53	0.84	NAFLD 15 week	Yes (Jadad) 4/4 high
Asadi et al. 2020	23	Iran	1,533	NR	1.8	Metabolic disorders 16 week	Yes (Cochrane) 18/23 high
Asbaghi et al. 2020	8	Iran	508	53	2.4	T2DM 19 week	Yes (Cochrane) 8/8 high
Askarpour et al. 2019	56	Iran	3,004	50	1.6	Metabolic disorders 18 week	Yes (Cochrane) NR
Chen et al. 2014	21	China	637	52.5	0.73	ESRD 15 week	Yes (Cochrane) 6/21 high
Choi et al. 2020	6	Korea	375	53	1.6	Metabolic syndrome 14 week	Yes (Cochrane) 4/6 high
Huang et al. 2013	12	China	391	49	0.54	ESRD 18 week	Yes (Jadad) 8/12 high
Liao et al. 2021	3	China	496	26	2.08	PCOS 12 week	Yes (Cochrane) 3/3 high
Yang et al. 2014	11	China	397	50	1.18	ESRD 20 week	Yes (Cochrane) 1/11 high
Gholipur et al.2018	10	Iran	466	NR	1	CKD 21 week	Yes (Cochrane) 5/10 high
Fathizadeh et al. 2019	NR	Iran	NR	NR	NR	NR	NR

lowering TG levels in subjects with metabolic disorders who have a mean age of less than 50 years and an intervention duration of less than 18 weeks (Table 4).

L-carnitine on LDL-C

Data from four meta-analyses indicated that L-carnitine supplementation significantly reduced LDL-C levels (ES = −4.81 mg/dL; 95% CI: −6.04, −3.59; $p < 0.001$;11 meta-analyses) (Figure 4). Studies showed a significant degree of heterogeneity ($I^2 = 96.8\%$, $p < 0.001$). In studies with a dosage of more than 2 g/day on subjects with type 2 diabetes mellitus (T2DM), and an intervention duration of less than 18 weeks, subgroup analysis also revealed a strong impact (Table 4).

L-carnitine on HDL-C

L-carnitine supplementation significantly increased HDL-C levels (ES = 0.66 mg/dL, 95% CI: 0.20, 1.12, $p = 0.005$; 11 meta-analyses) (Figure 5). Also, a high degree of heterogeneity was detected ($I^2 = 72.2\%$, $p < 0.001$). Subgroup analysis revealed that subjects with metabolic disorders who received an 18-week intervention had a more pronounced effect of L-carnitine supplementation on HDL-C levels (Table 4).

Sensitivity analysis

Sensitivity analysis for TG showed that the elimination of studies by Liao et al. (19) had an effect on the pooled ES, resulting in non-significance (ES = −0.29 mg/dL, 95% CI: −0.81, 0.22; $p > 0.05$). Also, the sensitivity analysis revealed no significance for TC, LDL-C, and HDL-C.

Publication bias, trim and fill

Egger's unlike Begg's test has revealed a significant small-study effect on TC ($p = 0.001$ and 0.193), LDL-C ($p = 0.011$ and 0.999), and HDL-C ($p = 0.013$ and 0.755) levels. In contrast to other parameters, no evidence of a significant small study effect was found for TG ($p = 0.945$ for Begg's and $p = 0.055$ for Egger's test). Publication bias was identified through a visual assessment of the funnel plot (Supplementary file). We conducted the trim and fill test since the

TABLE 2 Results of assess the methodological quality of meta-analysis.

Study	Q 1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Quality assessment
Casariego et al. 2013	No	Partial Yes	Yes	Partial Yes	Yes	Yes	No	Yes	No	No	Yes	No	No	Yes	No	Yes	Moderate
Abbasnezhad et al. 2020	No	Partial Yes	Yes	Partial Yes	Yes	Yes	Partial Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Abolfathi et al. 2020	Yes	Yes	Yes	Partial Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	High
Asadi et al. 2020	No	Yes	Yes	Partial Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	High
Asbaghi et al. 2020	Yes	Yes	Yes	Partial Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	High
Askarpour et al. 2019	Yes	Partial Yes	Yes	Partial Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	High
Chen et al. 2014	No	Yes	Yes	Partial Yes	Yes	Yes	Partial Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Moderate
Choi et al. 2020	No	Yes	Yes	Partial Yes	No	Yes	Yes	Partial Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Moderate
Huang et al. 2013	No	Yes	Yes	Partial Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Moderate
Liao et al. 2021	No	Yes	Yes	Partial Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	High
Yang et al. 2014	No	Yes	Yes	Partial Yes	Yes	Yes	Partial Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	Moderate
Gholipur et al.2018	Yes	Yes	Yes	Partial Yes	Yes	Yes	Partial Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	Moderate
Fathizadeh et al. 2019	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Critically low

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TABLE 3 Summary of results and quality of evidence assessment using the GRADE approach.

Outcome measure	Summary of findings		Quality of evidence assessment (GRADE)					
	No of patients (trials)	Effect size (95% CI)	Risk of bias [a]	Inconsistency [b]	Indirectness [c]	Imprecision [d]	Publication bias [e]	Quality of evidence [f]
Lipid profile								
LDL-C (mg/dl)	6,751 (51)	−4.81 (−6.04, −3.59)	Not Serious	Not Serious	Serious	Not Serious	Not Serious	Moderate
HDL-C (mg/dl)	7,010 (54)	0.66 (0.20, 1.12)	Not Serious	Not Serious	Serious	Serious	Serious	Very Low
TG (mg/dl)	8,075 (59)	−2.51 (−3.62, −1.39)	Not Serious	Not Serious	Serious	Serious	Not Serious	Low
TC (mg/dl)	8,006 (58)	−1.05 (−1.71, −0.39)	Not Serious	Not Serious	Serious	Serious	Not Serious	Low

LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; TC, total cholesterol.

[a]Risk of bias based on the AMSTAR2 results.

[b]Downgraded if there was a substantial unexplained heterogeneity ($I2 > 50\%$, $P < 0.10$) that was unexplained by meta-regression or subgroup analyses.

[c]Downgraded if there were factors present relating to the participants, interventions, or outcomes that limited the generalizability of the results.

[d]Downgraded if the 95% confidence interval (95% CI) crossed the minimally important difference (MID) for benefit or harm. MIDs used for each outcome were: 3.87 mg/dL for LDL, HDL, and TC, 8.86 mg/dL for TG.

[e]Downgraded if there was an evidence of publication bias using funnel plot.

[f]Since all included studies were meta-analyses of randomized control trials, the certainty of the evidence was graded as high for all outcomes by default and then downgraded based on prespecified criteria. Quality was graded as high, moderate, low, very low.

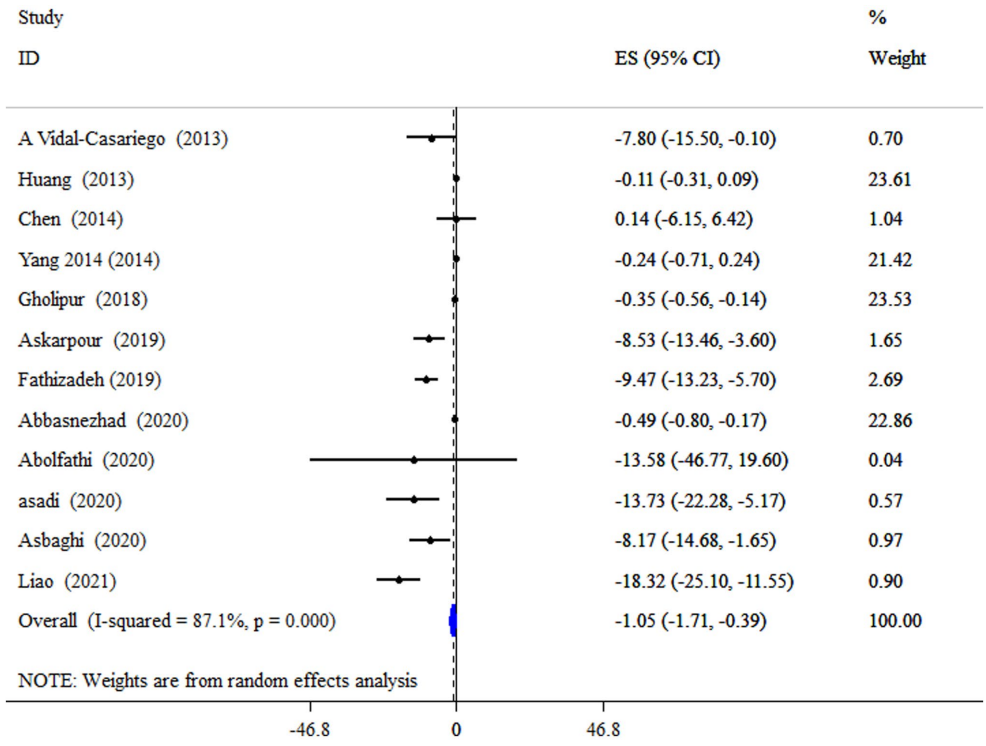

FIGURE 2
Forest plot detailing mean difference and 95% confidence intervals (CIs), the impacts of L-carnitine supplementation on TC levels.

funnel plot assessment's visual inspection revealed an uneven distribution of studies for all outcomes. Unlike other parameters, trim and fill analysis was performed on HDL-C level with 16 studies (five imputed studies); thus, correction for potential publication bias altered the effect of L-carnitine on HDL-C (ES = 0.31 mg/dL; 95% CI: −0.13, 0.74, $p > 0.05$).

TABLE 4 Subgroup analyses for the effects of L-Carnitine supplementation on lipid profile.

	Effect size, *n*	ES (95% CI)[1]	P-within[2]	I^2 (%)[3]	P-heterogeneity[4]
L-Carnitine on TC levels					
Overall	12	−1.05 (−1.71, −0.39)	0.002	87.1	<0.001
Age(year)					
≤50	4	−1.56 (−3.02, −0.09)	0.037	92.3	<0.001
>50	4	−2.23 (−6.23, 1.77)	0.275	49.7	0.113
NR	4	−7.25 (−14.19, −0.31)	0.041	91.5	<0.001
Intervention duration (week)					
<18	3	−16.48 (−21.72, −11.23)	<0.001	0.0	0.701
≥18	8	−0.38 (−0.74, −0.02)	0.040	72.0	<0.001
NR	1	−9.47 (−13.23, −5.70)	<0.001	–	–
Study population					
T2DM	2	−8.02 (−12.99, −3.04)	0.002	0.0	0.943
Metabolic disorders	2	−9.91 (−14.40, −5.41)	<0.001	6.1	0.302
CKD	4	−0.22 (−0.36, −0.08)	0.002	0.0	0.459
NAFLD	1	−13.58 (−46.76, 19.60)	0.423	–	–
Liver disorders	1	−0.49 (−0.81, −0.18)	0.002	–	–
PCOS	1	−18.32 (−25.10, −11.55)	<0.001	–	–
NR	1	−9.47 (−13.23, −5.70)	<0.001	–	–
Dosage (g/day)					
≤ 2	8	−0.37 (−0.73, 0.00)	0.051	72.9	<0.001
> 2	3	−11.51 (−18.36, −4.66)	<0.001	65.4	0.056
NR	1	−9.47 (−13.23, −5.70)	<0.001	–	–
L-Carnitine on LDL-C levels					
Overall	11	−4.81 (−6.04, −3.59)	<0.001	96.8	<0.001
Age(years)					
≤50	4	−8.79 (−19.73, 2.16)	0.116	98.5	<0.001
>50	3	−3.12 (−7.39, 1.14)	0.151	77.1	0.013
NR	4	−5.68 (−11.14, −0.22)	0.042	97.1	<0.001
Intervention duration (week)					
<18	4	−11.28 (−19.76, −2.81)	0.009	89.9	<0.001
≥18	6	−1.83 (−2.71, −0.95)	<0.001	94.8	<0.001
NR	1	−6.25 (−9.30, −3.20)	<0.001	–	–
Study population					
T2DM	2	−7.56 (−10.90, −4.23)	<0.001	55.2	0.135
Metabolic disorders	2	−6.26 (−8.68, −3.83)	<0.001	0.0	0.392
CKD	3	−0.34 (−0.58, −0.09)	0.007	45.4	0.160
NAFLD	1	−14.85 (−45.43, 15.73)	0.341	–	–
Liver disorders	1	−0.20 (−0.57, 0.17)	0.296	–	–
PCOS	1	−18.91 (−21.58, −16.25)	<0.001	–	–
NR	1	−6.25 (−9.30, −3.20)	<0.001	–	–
Dosage (g/day)					
≤ 2	7	−0.57 (−1.07, −0.07)	0.025	79.1	<0.001
> 2	3	−11.08 (−18.73, −3.43)	0.005	95.6	<0.001
NR	1	−6.25 (−9.30, −3.20)	<0.001		–
L-Carnitine on HDL-C levels					
Overall	11	0.66 (0.20, 1.12)	0.005	72.2	<0.001
Age (years)					

(Continued)

TABLE 4 (Continued)

	Effect size, *n*	ES (95% CI)[1]	P-within[2]	I^2 (%)[3]	P-heterogeneity[4]
≤50	4	1.18 (−0.38, 2.75)	0.139	78.2	0.003
>50	4	0.12 (−0.25, 0.49)	0.511	5.2	0.367
NR	3	0.89 (0.52, 1.25)	<0.001	0.0	0.546
Intervention duration (week)					
<18	5	1.19 (0.21, 2.17)	0.017	26.9	0.242
≥18	5	0.23 (−0.18, 0.65)	0.272	57.6	0.051
NR	1	1.39 (0.21, 2.57)	0.021	–	–
Study population					
T2DM	2	0.36 (−1.91, 2.63)	0.754	38.6	0.202
Metabolic disorders	3	1.04 (0.51, 1.57)	<0.001	18.3	0.294
CKD	2	0.02 (−0.35, 0.40)	0.907	0.0	0.541
NAFLD	1	1.36 (−0.96, 3.68)	0.251	–	–
Liver disorders	1	0.08 (−0.02, 0.17)	0.099	–	–
PCOS	1	10.27 (1.67, 18.88)	0.019	–	–
NR	1	1.39 (0.21,2.57)	0.021	–	–
Dosage (g/day)					
≤ 2	7	0.54 (0.09, 1.00)	0.018	75.7	<0.001
> 2	3	2.00 (−1.87, 5.87)	0.310	70.4	<0.001
NR	1	1.39 (0.21, 2.57)	0.021	–	–
L-Carnitine on TG levels					
Overall	12	−2.51 (−3.62, −1.39)	<0.001	92.8	<0.001
Age (years)					
≤50	4	−4.25 (−6.48, −2.03)	<0.001	97.7	<0.001
>50	5	−5.59 (−14.45, 3.27)	0.216	61.5	0.034
NR	3	−7.69(−12.23, −3.15)	<0.001	0.0	0.388
Intervention duration (week)					
<18	5	−13.22 (−18.12, −8.32)	<0.001	21.5	0.278
≥18	6	−0.15 (−0.42, 0.12)	0.277	46.0	0.099
NR	1	−10.35 (−16.43, −4.27)	<0.001	–	–
Study population					
T2DM	2	−0.05 (−7.45, 7.36)	0.990	0.0	0.484
Metabolic disorders	3	−9.00 (−14.23, −3.76)	<0.001	0.0	0.473
CKD	3	−0.09 (−0.29, 0.12)	0.421	0.0	0.850
NAFLD	1	−14.51 (−17.06, −11.97)	<0.001	–	–
Liver disorders	1	−22.13 (−38.92, −5.34)	0.010	–	–
PCOS	1	−0.23 (−0.41, −0.05)	0.012	–	–
NR	1	−10.35 (−16.43, −4.27)	<0.001	–	–
Dosage (g/day)					
≤ 2	8	−0.19 (−0.61, 0.23)	0.371	65.7	0.005
> 2	3	−5.64 (−17.40, 6.12)	0.347	85.3	<0.001
NR	1	−10.35 (−16.43, −4.27)	<0.001	–	–

ES, effect size; CI, confidence interval; 1Obtained from the Random-effects model, 2Refers to the mean (95% CI), 3Inconsistency, percentage of variation across studies due to heterogeneity, 4Obtained from the Q-test, NR, Not reported; T2DM, Type 2 diabetes mellitus; MetS, Metabolic syndrome, CKD, Chronic kidney disease; NAFLD, Non-alcoholic fatty liver disease; PCOS, Polycystic Ovary Syndrome.

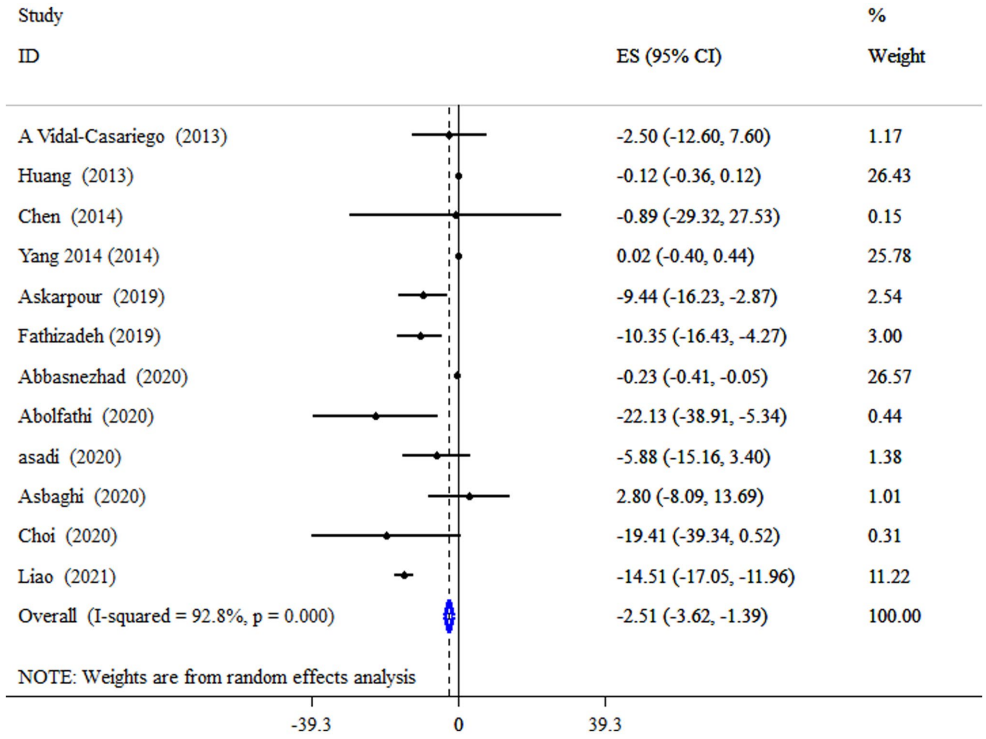

FIGURE 3
Forest plot detailing mean difference and 95% confidence intervals (CIs), the impacts of L-carnitine supplementation on TG levels.

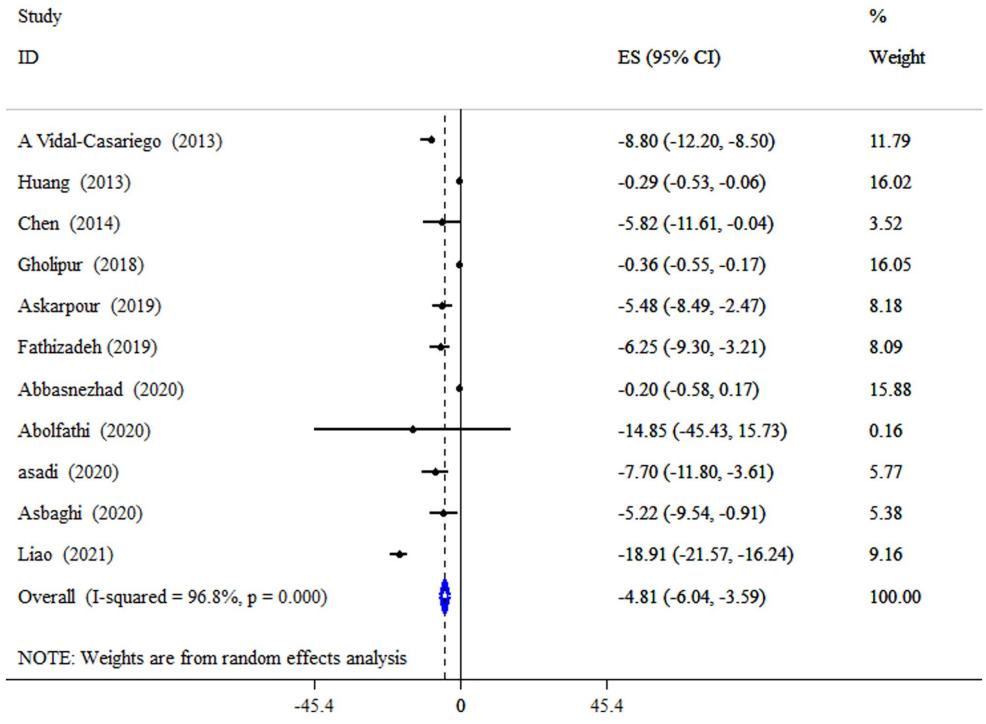

FIGURE 4
Forest plot detailing mean difference and 95% confidence intervals (CIs), the impacts of L-carnitine supplementation on LDL-C levels.

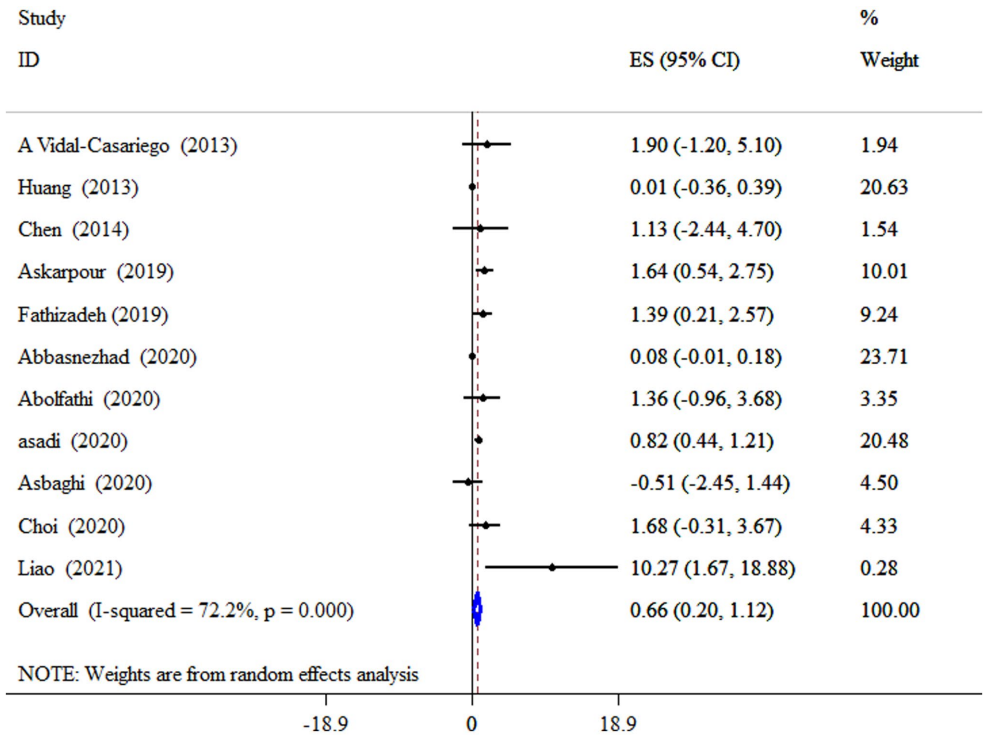

FIGURE 5
Forest plot detailing mean difference and 95% confidence intervals (CIs), the impacts of L-carnitine supplementation on HDL-C levels.

Discussion

L-carnitine supplementation could have antihyperlipidemic effects according to our pooled analysis on 13 meta-analyses consisting of 51, 54, 58, and 59 separate clinical trials for LDL-C, HDL-C, TC and TG, respectively. As far as we know, this is the first umbrella meta-analysis of RCTs in the realm of the clinical benefits of L-carnitine supplementation on lipid parameters. Based on the results, TC, TG, LDL-C were significantly decreased after L-carnitine supplementation. Also, results from our study demonstrated that consuming carnitine supplements improved HDL-C levels.

Previous reports have proven that dyslipidemia are independent predictors of cardiovascular disease (CVD) risk. Accumulating evidence has suggested potentially beneficial properties of L-carnitine as nutraceutical for managing dyslipidemia and prevent of CVD. L-carnitine as a non-protein amino acid is the known carrier of fatty acids (FAs) across the inner mitochondrial membrane and plays an important role in the metabolism of FAs and activation of β-oxidation via regulating long-chain FAs transport from the mitochondrial membrane. Endogenous production of L-carnitine can be done in kidneys and liver from lysine, methionine ascorbate, niacin, pyridoxine, and iron (27).

Scientific evidences associating L-carnitine and disturbances of glucose and lipid metabolism has been reported recently and have suggested L-carnitine as a potential therapeutic agent for some diseases such as T2DM, non-alcoholic fatty liver disease (NAFLD), end-stage kidney disease (ESKD), atherosclerosis, etc. (28, 29). A large number of studies suggested that L-carnitine consumption is associated for normalizing the blood concentrations of TC, TG and LDL-C (22, 30). As shown in Figure 6 the potential positive effects of L-carnitine on the lipid parameters might be explained by several mechanisms. Considering recently published investigations, carnitine deficiency impairs insulin-sensitivity. L-carnitine enhanced the mitochondrial oxidation (beta-oxidation) of long chain-Acyl CoA, which its accumulation triggers insulin resistance in muscle cells and hepatocytes (31). Beyond that, L-carnitine can reduce the availability of free fatty acids (FFAs), diminish conversion of FFAs to TGs and prevent excess TG accumulation in hepatocytes (32). Interestingly, L-carnitine also can affect cholesterol synthesis pathway (mevalonate pathway) via inhibition of β-hydroxy β-methylglutaryl (HMG)-CoA reductase activity (33, 34). In addition to the benefits of lipids control by L-carnitine in the prevention of CVD, several studies also shown the cardio-protective effects of L-carnitine in terms of reduction of infract size and amelioration of cardiac dysfunction (35). Furthermore, L-carnitine can improve lipid profile by alteration the activity of lipid oxidation enzymes *via* modifying the expression of genes associated with lipid metabolism signaling including peroxisome proliferator activated receptor (PPAR α & γ), and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (36–39). Also, it is evident that oxidative stress and inflammation can trigger initiation of hyperlipidemia in animals and humans. L-carnitine with anti-oxidant and anti-inflammatory properties can modulate dyslipidemia (40–42). It is important to mention that, protection of LDLs from oxidative stress by L-carnitine can be described *via* few mechanisms, including: oxygen concentration reduced due to enhancement of the β-oxidation of long chain-Acyl CoA (by cause of a large amount of oxygen consumption rates in β-oxidation) and consequently reactive oxygen species formation is decreased (43). Also, L-carnitine can inhibit the activity of enzymes involved in free radical generation and by induction of antioxidant mechanisms (44). Moreover, L-carnitine has been indicated

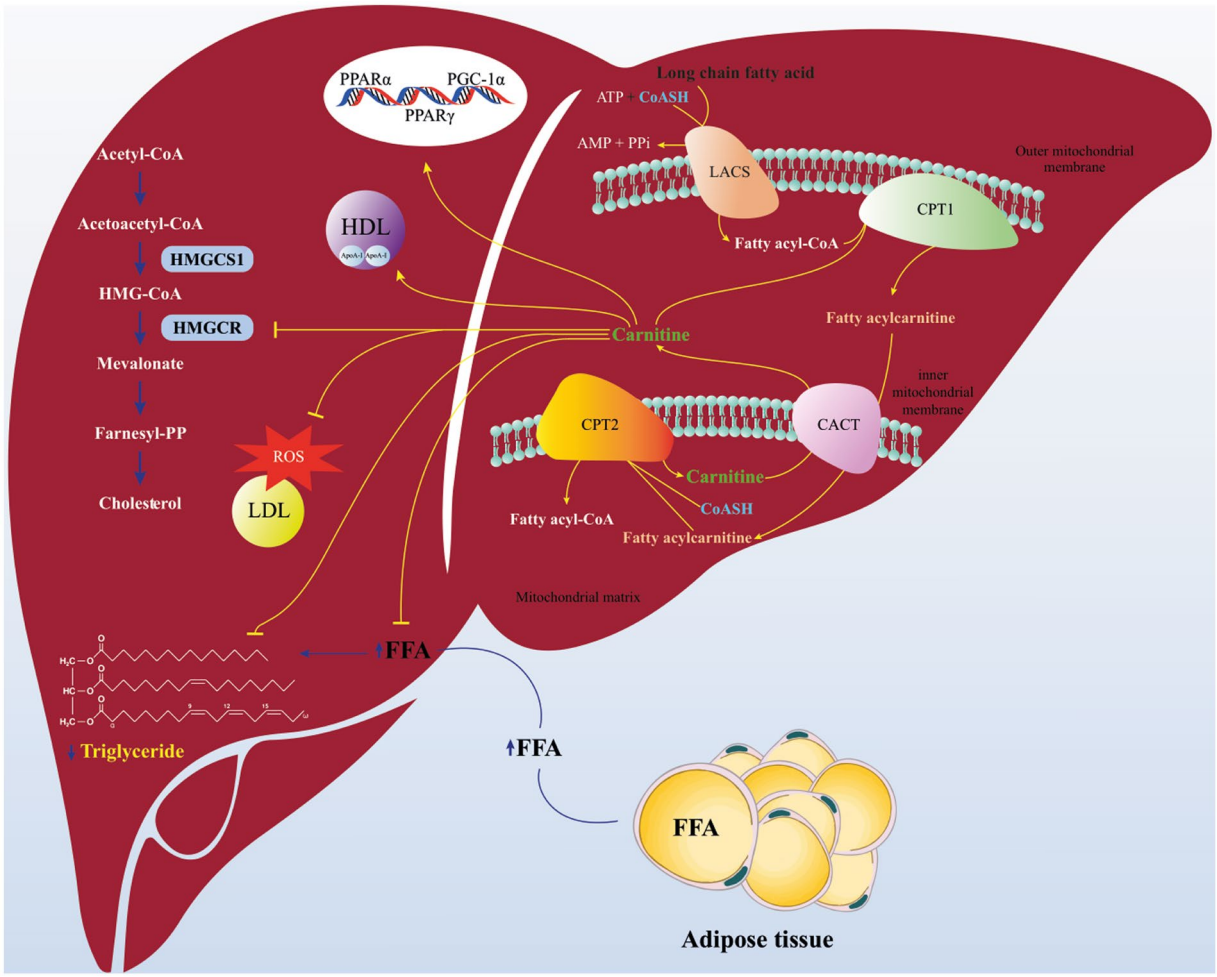

FIGURE 6
Schematics of proposed pathway for beneficial effects of L-carnitine on lipid parameters. L-carnitine enhanced the mitochondrial oxidation (beta-oxidation) of long Chain-Acyl CoA. Carnitine can reduce the availability of free fatty acids (FFAs), diminish conversion of FFAs to triglycerides. L-carnitine can affect cholesterol synthesis pathway (mevalonate pathway) *via* inhibition of β-hydroxy β-methylglutaryl (HMG)-CoA reductase activity. Carnitine can improve lipid profile by modifying the expression of genes associated with lipid metabolism signaling including peroxisome proliferator activated receptor (PPAR α & γ), and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α). Also, carnitine can protect LDLs from oxidative stress and promotes the synthesis of HDL *via* increment in Apo-A1 level. Carnitine Acylcarnitine translocase (CACT); Carnitine palmitoyi transferase II (CPT2); Carnitine palmitoyi transferase I (CPT1); Long Acyl-CoA synthetase (LACS).

that has a potent for scavenging superoxide anion (45). Additionally, evidence suggests that L-carnitine promotes the synthesis of HDL-C *via* increment in Apo-A1 level (46).

Even though the findings suggest that L-carnitine supplementation may be efficacious for controlling lipid profile, it must be stated that, the effects of L-carnitine on all mentioned lipid parameters were heterogeneous. This heterogeneity may be explained by differences in treatment dosage, gender, mean age, study population, and duration of intervention. Also, the evidence from this study implies that L-carnitine supplementation >2 g/day in subjects with mean age ≤ 50 can meaningfully decrease TC. Similarly, supplementation with L-carnitine in subjects with metabolic disorders with mean age ≤ 50 years and duration of <18-weeks can meaningfully reduce TG levels. In line with TG reduction, L-carnitine intervention in a dose of >2 g/day with duration <18-weeks and with a sample size of >400 participants significantly reduced LDL-C levels, and in people with T2DM, this effect was more significant. Also, L-carnitine consumption had beneficial associations with HDL-C levels in subject with metabolic disorders and intervention duration of <18-weeks.

Aforementioned, the reducing effect of L-carnitine on the level of both TG and LDL-C was shown following short-term supplementation (<18 weeks). Some included studies (19, 30, 47) in our umbrella review indicated that the lowering effect of L-carnitine on TC and LDL-C was achieved when doses of >2 gr/day L-carnitine were consumed. In sum, it seems that the effect of L-carnitine on TG and LDL-C depends on the health condition of individuals, so the patients with metabolic disorders and T2DM have had the most beneficial efficiency of this supplementation.

The results of our investigation indicate that l-carnitine supplementation did not exceed the minimally important difference (MID) in lipid profile (Except LDL-C) in comparison with the control group. The MID concept has been referred to as the minimal clinically important difference or the minimal clinically important improvement. The heterogeneity also in the study characteristics makes it difficult to reach any strong conclusions, particularly in

relation to clinical relevance. Therefore, the findings should be considered in the context of these limitations.

Based on pioneering meta-analyses investigating the effect of L-carnitine supplementation on lipid profiles the evidences are contradictory. The main reasons for these discrepancy between the results might partly be due to difference between the included participants, duration of the study, the dosage and types of L-carnitine supplements.

To assimilate the wide number of current evidences available on L-carnitine consumption and dyslipidemia, we done this umbrella review of existing meta-analyses to capture the breadth of outcomes. We found suggestive evidence that L-carnitine may be considered as lipid-modulating agent solo or in concomitant with other lipid lowering drugs. Considerable information from previous studies the L-carnitine was well tolerated without any serious adverse events. However, some trials have reported muscle cramps, asthenia, diarrhea, flu syndrome and headache following high dose (5 g/day or more) of L-carnitine supplementation (19, 23, 30, 48).

The present umbrella meta-analysis study has several strengths. First, this is the first umbrella review to assess the effect of L-carnitine supplementation on lipid profiles with up-to-date literature search strategy from a large number of databases. Second, our umbrella review was registered in the PROSPERO. There were some limitations in our study. First, significant between-studies heterogeneity observed. Second, participants of included studies were from people with different health statuses that leads to indirectness. However, we performed subgroup analysis to present a comprehensive view on the anti-hyperlipidemia effectiveness of L-carnitine.

Conclusion

In conclusion, although the results indicated that L-carnitine supplementation resulted in a clear improvement in lipid profile in terms of reduction in TC, LDL-C and TG, and significant increasement in HDL-C levels, nevertheless, further large scale RCTs are needed in order to receive a definite conclusion.

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

NR and VM designed research. ZK, MK, and HA conducted research. VM performed statistical analysis. ZK, MK, PD, and EM wrote paper. VM and NR had primary responsibility for final content. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1214734/full#supplementary-material

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A cross-sectional study on the association between dietary inflammatory index and hyperuricemia based on NHANES 2005–2018

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Background: Hyperuricemia is a common condition that can lead to gout and other related diseases. It has been suggested that Inflammatory factors play important role in the development and progression of hyperuricemia. The dietary inflammatory index (DII) enables the assessment of the inflammatory potential of an individual's diet. This study aimed to investigate the association between DII and hyperuricemia.

Methods: This study was performed based on a cross-sectional dataset from the National Health and Nutrition Examination Survey (NHANES) 2005–2018. Participants aged 18 years and above with dietary intake and serum uric acid level information were included. DII scores were calculated using dietary intake data, based on which participants were categorized into tertiles. Multivariable logistic regression analysis was adopted to investigate the association between DII and hyperuricemia.

Results: Among a total of 31,781 participants in the analysis, 5,491 had hyperuricemia. After adjusting confounding factors, the odds of hyperuricemia are significantly higher in the second (OR 1.17, 95% CI 1.07–1.29) and third tertiles (OR 1.31, 95% CI 1.19–1.44) relative to the first one.

Conclusion: This study suggested that diet with higher inflammatory potential, as measured by DII, is associated with increased hyperuricemia risk. These findings indicated that dietary modification may be a potential approach for hyperuricemia's prevention and control.

KEYWORDS

dietary inflammatory index, hyperuricemia, adults, the United States, NHANES

1. Introduction

Hyperuricemia is defined as the overproduction or under-excretion of uric acid, and it tops the list of global disease burdens associated with gout and a wide spectrum of other diseases, affecting patients of all ages and sexes (1, 2). It is an independent risk factor for various systemic diseases, including gout, cardiovascular diseases, hypertension, chronic kidney disease, and

many others (3). Up to 2016, the global prevalence of hyperuricemia has hit 21% (4). Its prevalence in U.S. ranged from 14.6 to 20% (5) and showed a tendency to affect younger people (38.6 ± 11.8 years), even non-obese adults (6, 7).

Unhealthy eating habit is closely related to hyperuricemia since its influence on inflammation (8). For instance, Western dietary patterns characterized by high caloric content and significant fat levels have been linked to an increased presence of inflammatory markers in the body (9). Correspondingly, anti-inflammatory diets could reduce this level, and dietary management targeting at asymptomatic hyperuricemia may be of great benefit (10).

Since inflammation is an essential factor for hyperuricemia, measuring inflammation level might be useful for the prediction of and protection against hyperuricemia. The Dietary Inflammatory Index (DII) was firstly proposed by Shivappa et al. in 2009 based on published literatures (11) and was updated in 2014 (12). It specifically aimed to measure dietary inflammation potential. DII, as is closely related to the expression of blood inflammatory markers, has been widely used in the investigation of the association between inflammation caused by diet and the onset and progression of diverse ailments (13).

Up to now, there are only two studies on the association between DII and hyperuricemia. One was a cross-sectional study in China, whose results showed that higher DII scores were associated with higher hyperuricemia risk after covariates adjustment (14). The other was a case–control study in Korea, which reported that higher pro-inflammatory dietary intake was significantly associated with the risk of hyperuricemia only in males (6). However, both studies focused on Asian population, and can only provide limited reference for U.S. population due to the differences between Eastern and Western dietary habits (15). This study aimed to explore such a relationship in U.S. population.

2. Materials and methods

2.1. Study population

The National Health and Nutrition Examination Survey (NHANES) is a sweeping cross-sectional study administered by the National Center for Health Statistics. Its objective is to compile crucial data about individuals' health conditions by conducting a range of interviews, physical assessments, and laboratory examinations. To ensure an accurate representation of the overall U.S. population, a sophisticated multistage sampling technique was employed for this particular survey. In order to evaluate the dietary patterns of NHANES participants, two comprehensive interviews were conducted to obtain dietary-recall information. Specifically, the first interview was implemented face-to-face by highly trained dietary interviewers in NHANES Mobile Examination Center (MEC), while the second interview was conducted over phone three to ten days after the MEC interview (the next week of MEC interview). During the dietary-recall interviews, U.S. Department of Agriculture Automated Multiple-Pass Method dietary interview approach was used to collect information on food intake. Participants utilized measuring guides during in-person interviews while a food model booklet was used during the telephone interviews to quantify food. The methodology and materials used for the survey underwent ethical review and were approved by the Ethics Review Board of the National Center for Health Statistics. Further, prior to participating in the survey, written informed consent was obtained from all individuals involved. Data from NHANES 2005–2018 cycle were selected in this study, including a total of 70,190 participants initially. Exclusion criteria were as follows: (a) participants without dietary data for DII calculation ($n = 9,549$), (b) participants with missing uric acid data ($n = 18,995$), (c) individuals under the age of 18 years old ($n = 6,267$), (d) pregnant individuals ($n = 642$), and (e) individuals with missing estimated glomerular filtration rate (eGFR) or eGFR values less than 60 mL/min/1.73 m (2) ($n = 2,956$). Ultimately, 31,781 participants were included in the analysis, as depicted in Figure 1.

2.2. Calculation of DII

DII developed by Shivappa et al. was computed to measure the inflammatory potential of different dietary patterns (12). DII calculation was conducted by a standardized global database that contains daily dietary intake information of 11 regionally representative populations. Both the standard mean and standard deviation were provided for all DII food parameters from the world database. To ensure that the investigation is thorough and accurate, a scoring system was created. A score of "+1" is given to dietary components that increase the levels of CRP, TNF-a, IL-1b, and IL-6, or decrease the levels of IL-4 and IL-10. Conversely, a score of "−1" is assigned to dietary components that reduce the levels of CRP, TNF-a, IL-1b, and IL-6, or increase the levels of IL-4 and IL-10. This comprehensive approach allows for a more detailed examination of how dietary components affect these specific indicators. These values were weighted according to the study design. The z-score for each food parameter was calculated by subtracting the standard mean from the value of consumption reported by each individual and then dividing that result by the standard deviation. These z-scores were transformed into proportions (ranging from 0 to 1) to minimize the effect of positive skewing. To obtain a symmetrical distribution centered around zero with bounds between −1 and +1, each proportion was doubled, and then 1 was subtracted. This value was then multiplied by the corresponding inflammatory effect score for each food parameter. In this study, there were 28 parameters available in NHANES data that could be utilized to calculate DII, including energy, protein, carbohydrate, dietary fiber, total fatty acid, total saturated fatty acid, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), cholesterol, β-carotene, niacin, folate, magnesium, iron, zinc, selenium, caffeine, alcohol, n3 polyunsaturated fatty acid, n6 polyunsaturated fatty acid, and vitamins A, B1, B2, B6, B12, C, D, and E. An elevated DII score signifies the consumption of a diet that triggers inflammation, whereas a reduced score indicates the adoption of an anti-inflammatory diet.

2.3. Serum uric acid measurement

This investigation was primarily focused on hyperuricemia condition, which is characterized by elevated uric acid level in bloodstream. The measurement of serum uric acid level was carried out using the Beckman UniCel® DxC800 Synchron or Beckman Synchron LX20 (Beckman Coulter, Inc., Brea, CA, United States),

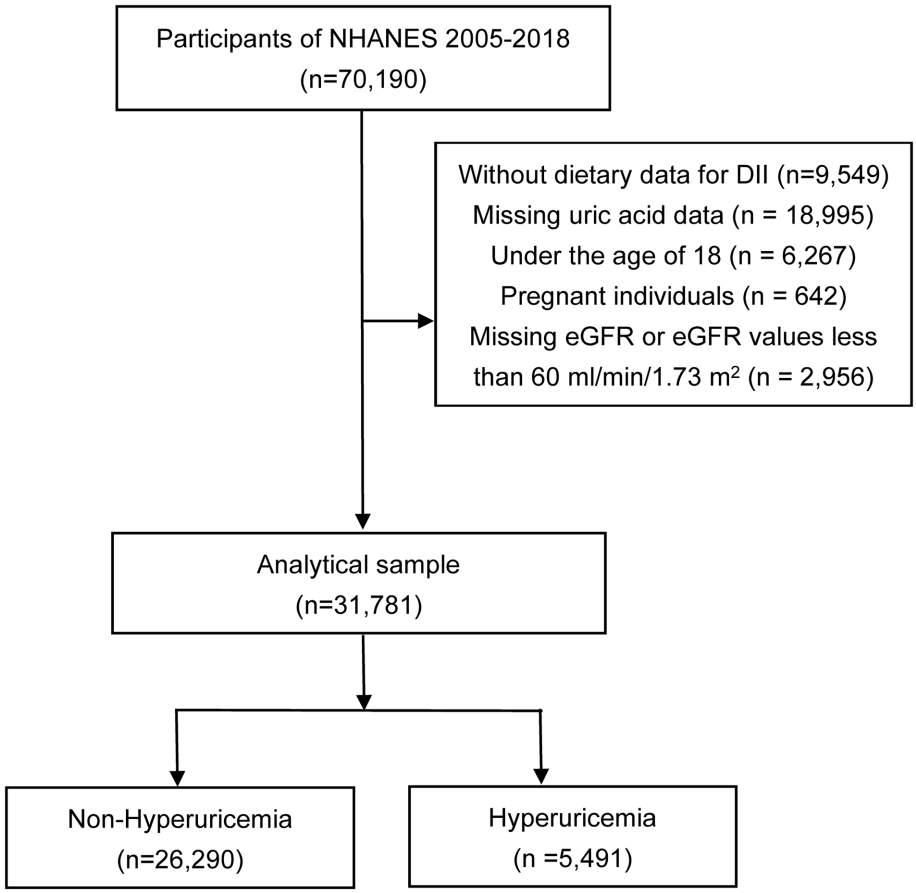

FIGURE 1
Flowchart of the study. NHANES, the National Health and Nutrition Examination Survey; DII, dietary inflammatory index; eGFR, estimated glomerular filtration rate.

which employs an oxidizing process to convert uric acid to allantoin and H2O2. Following established diagnostic standards, hyperuricemia was delineated as a serum uric acid threshold of 7.0 mg/dL or more in males and 6.0 mg/dL or more in females (16).

2.4. Covariates

In this study, demographic, lifestyle variables, physical measurements, laboratory tests, and self-reported health status were assessed in a computer-assisted personal interview. Demographic information includes age, sex, race/ethnicity, and educational level; health status contains smoking, drinking, physical activity, and disease history (hypertension, diabetes and hyperlipidemia); physical health examination involves height, body mass, and blood pressure measurements; and laboratory tests covers uric acid, serum glucose level.

The smoking status was divided into three distinct groups for this study. The first group consisted of individuals who had never smoked, meaning that they had smoked less than 100 cigarettes in their entire lifetime. The second group comprised individuals who were former smokers, having smoked more than 100 cigarettes in their lifetime and subsequently quit smoking at the time of the survey. Finally, the third group consisted of current smokers, who had smoked more than 100 cigarettes in their lifetime and continued to smoke at least every few days. Current drinking was classified into three categories: heavy drinking (≥3 drinks per day for females; ≥4 drinks per day for males; binge drinking on 5 or more days per month), moderate drinking (≥2 drinks per day for females; ≥3 drinks per day for males; binge drinking ≥2 days per month), and mild drinking (others). To determine the individual's metabolic equivalent of task (MET)/week, a calculation was performed by multiplying the total number of minutes spent on various activities during the week by the metabolic equivalents estimated by the Compendium of Physical Activities. This approach allowed for an accurate assessment of the intensity and frequency of the individual's physical activity over the course of the week. By utilizing the Compendium of Physical Activities, which provides standardized estimates of metabolic equivalents for various activities, the calculation was based on scientifically informed data and avoided potential inaccuracies and bias. The level of physical activity was determined in terms of hours of activity per week (MET/week), and results were divided into three groups: low (<600 METs/week), moderate (600–1,199 METs/week), and vigorous (≥1,200 METs/week). The eGFR was determined through the application of the Chronic Kidney Disease Epidemiology Collaboration creatinine equation. This formula calculates eGFR values based on specific parameters for both male and female. For male, the equation is as follows: eGFR = (140-age) × body weight (kg) × 1.23/creatinine

(mmol/L). Similarly, for female, the equation is: eGFR = (140-age) × body weight (kg) × 1.03/creatinine (mmol/L). Hypertension was defined as blood pressure ≥ 140/90 mmHg, a diagnosis of hypertension, or a prescription of antihypertensive drugs in health questionnaire. Diabetes was diagnosed when patients met one or more of the following criteria: (1) a medical diagnosis of diabetes as recorded by the patient's healthcare provider ("doctor told you have diabetes"), (2) glycohemoglobin A1c (HbA1c) level of greater than 6.5%, (3) a fasting blood glucose level of equal to or greater than 7.0 mmol/L, (4) a random blood glucose level of equal to or greater than 11.1 mmol/L, or (5) a two-hour blood glucose level of equal to or greater than 11.1 mmol/L following an oral glucose tolerance test (OGTT). Hyperlipidemia was defined by TG levels equal to or greater than 150 mg/dL, hypercholesterolemia, or medication that lowers lipid levels. Individuals who met any of the following criteria were considered to have hypercholesterolemia: (1) TC levels equal to or greater than 200 mg/dL, (2) LDL-C levels equal to or greater than 130 mg/dL, or (3) HDL-C levels less than 40 mg/dL for males and less than 50 mg/dL for females.

2.5. Statistical analysis

We initiated our analysis by comparing the baseline data. Based on sex, participants were divided into two groups according to the presence of hyperuricemia for baseline characteristic analysis. Given that different components of the DII might exhibit varying impacts on inflammation and could demonstrate exponential growth or decline, we presented the distribution of different DII components among different groups in the form of medians and interquartile ranges. Mean ± standard deviation was used to report continuous variables, while percentages were used to represent categorical variables. For variables with a normal distribution, analysis was conducted using Student's t-test or chi-squared test. When variables exhibited skewed distribution, non-parametric tests or Fisher's exact probability test were employed for analysis. Furthermore, a multivariable logistic regression model was utilized in both the overall population and sex-stratified subgroups to estimate odds ratios and 95% confidence intervals. The DII score was included in the model as an independent variable, both in continuous and tertile forms (T1 (−5.28 to 0.79, $n = 10{,}594$), T2 (0.79 to 2.60, $n = 10{,}593$), T3 (2.60 to 5.79, $n = 10{,}594$)), to explore potential correlations with hyperuricemia. The transformation of DII into tertile variables aims to assess whether DII exhibits correlations with hyperuricemia across different variable type states. Additionally, linear trend tests were conducted to assess linear relationships, and a Generalized Additive Model (GAM) along with smoothed curve fitting and a two-part logistic regression model were employed to explore nonlinear correlations. Subgroup and interaction analyses were performed for covariates such as sex, age, race/ethnicity, hypertension, diabetes, and BMI, while controlling for potential confounding factors. All statistical analyses were conducted using R (version 3.5.3) and EmpowerStats,[1] with statistical significance defined as $p < 0.05$.

1 http://www.EmpowerStats.com

3. Results

3.1. Baseline characteristics

Table 1 displays the baseline characteristics based on sex for the hyperuricemia and non-hyperuricemia groups. The male group comprises 15,828 individuals, while the female group consists of 15,953 individuals. Among males, age ($p = 0.067$), METs/week ($p = 0.355$), and diabetes ($p = 0.837$) showed no statistically significant differences between the hyperuricemia and non-hyperuricemia groups. However, there were statistically significant differences between the groups in terms of race/ethnicity, education level, smoking, alcohol consumption, BMI, hypertension, hyperlipidemia, and eGFR. Among males, those with lower education levels, higher BMI, higher blood pressure, lower eGFR, history of smoking, moderate-to-heavy alcohol consumption, and higher lipid levels were more prone to hyperuricemia. Furthermore, among the female population, statistically significant differences were observed among all variables.

Additionally, Table 2 presents the scores for each component of the DII based on sex-specific grouping for the hyperuricemia and non-hyperuricemia groups. From Table 2, it can be observed that, for both males and females, there are no statistically significant differences in cholesterol intake and caffeine intake between the groups. Among males, there are no statistically significant differences in vitamin B6 and niacin intake between the hyperuricemia and non-hyperuricemia groups. Among females, there are no statistically significant differences in β-Carotene intake between the groups. However, for the other components of the DII, statistically significant differences are observed between the hyperuricemia and non-hyperuricemia groups for both males and females.

3.2. Association between DII score and hyperuicemia

The logistic regression modeling results shown in Table 3 indicates the association between DII score and hyperuricemia. After adjusting for all covariates (age, sex, BMI, race/ethnicity, educational level, smoking, drinking, MET, eGFR, diabetes, hypertension, and hyperlipidemia), a positive association was established between these two (OR 1.06, 95% CI 1.04–1.09). The second and third tertile groups showed higher odds than the first tertile (T2: OR 1.17, 95% CI 1.07–1.29; T3: OR 1.31, 95% CI 1.19–1.44), which was also confirmed by the trend test ($p<0.05$). At the same time, we also conducted sex based stratified analysis and found that the relationship between DII and hyperuricemia remained unchanged among different sexes. Additionally, we used a GAM and a smooth curve fit to evaluate the correlation between them and evaluated their nonlinearity using a two part logistic regression model. When DII was treated as a continuous variable, a positive correlation was observed between DII and uric acid (Figure 2A). When DII was treated as a categorical variable with three tertiles, the relationship between DII and uric acid remained unchanged (Figure 2B). The results of a two part logistic regression model also show that there is no nonlinear relationship between DII and uric acid (Supplementary Table S1).

The forest plot illustrates interactions between DII and hyperuricemia concerning age and diabetes ($p < 0.05$). Borderline

TABLE 1 Baseline characteristics of subjects.

Characteristics	Male				Female			
	Total *n*=15828	Non-Hyperuricemia *n*=12548	Hyperuricemia *n*=3280	*p*-value	Total *n*=15953	Non-Hyperuricemia *n*=13742	Hyperuricemia *n*=2211	*p*-value
Age (years)	45.9 ± 17.9	46.1 ± 18.1	45.4 ± 17.3	0.067	46.0 ± 17.5	45.0 ± 17.5	52.0 ± 16.8	<0.001
Race/ethnicity (%)				<0.001				<0.001
Non-Hispanic White	41.94	41.73	42.74		39.83	39.69	40.71	
Non-Hispanic Black	20.92	20.29	23.32		21.38	20.16	28.95	
Mexican American	17.10	18.09	13.32		17.18	18.00	12.03	
Others	20.04	19.89	20.61		21.61	22.14	18.32	
Education level (%)				<0.001				0.043
Less than high school	26.08	26.74	23.55		23.49	23.69	22.31	
High school	22.70	22.63	22.97		20.32	20.01	22.22	
More than high school	51.22	50.63	53.48		56.19	56.30	55.48	
BMI (kg/m^2)	28.55 ± 6.17	27.79 ± 5.65	31.47 ± 7.14	<0.001	29.40 ± 7.65	28.56 ± 7.12	34.65 ± 8.71	<0.001
SBP (mmHg)	124.21 ± 16.15	123.78 ± 16.03	125.87 ± 16.52	<0.001	120.54 ± 18.73	119.58 ± 18.47	126.49 ± 19.24	<0.001
DBP (mmHg)	71.58 ± 12.13	71.01 ± 11.95	73.79 ± 12.60	<0.001	69.28 ± 11.29	69.07 ± 11.11	70.61 ± 12.28	<0.001
Smoking, *n* (%)				<0.001				<0.001
Never	47.34	46.82	49.29		65.07	66.00	59.50	
Former	27.65	27.21	29.33		17.19	16.28	22.66	
Now	25.01	25.96	21.39		17.74	17.72	17.84	
Drinking (%)				<0.001				<0.001
Never	8.44	8.60	7.81		20.18	20.23	19.84	
Former	15.18	15.66	13.33		14.28	13.72	17.60	
Mild	38.06	38.66	35.78		27.98	28.25	26.32	
Moderate	12.42	12.11	13.57		19.89	20.06	18.84	
Heavy	25.91	24.96	29.51		17.69	17.73	17.40	
METs/week (%)				0.355				0.015
Low	19.97	20.06	19.61		27.51	27.14	29.99	
Moderate	11.84	11.63	12.63		16.40	16.26	17.32	
Vigorous	68.20	68.31	67.77		56.09	56.60	52.69	
eGFR (ml/min/1.73 m^2)	98.23 ± 19.26	99.18 ± 19.12	94.58 ± 19.33	<0.001	101.22 ± 20.45	102.82 ± 20.08	91.27 ± 19.93	<0.001
UA (mg/dl)	5.99 ± 1.26	5.52 ± 0.88	7.79 ± 0.77	<0.001	4.72 ± 1.16	4.39 ± 0.83	6.77 ± 0.77	<0.001
Serum glucose (mg/dl)	102.94 ± 38.40	103.17 ± 40.35	102.10 ± 29.79	<0.001	99.11 ± 35.64	97.99 ± 35.69	106.09 ± 34.50	<0.001
Diabetes (%)	16.53	16.50	16.65	0.837	15.14	12.95	28.81	<0.001
Hypertension (%)	37.98	35.47	47.59	<0.001	36.28	32.31	60.92	<0.001
Hyperlipidemia (%)	66.96	64.70	75.61	<0.001	70.11	67.75	84.80	<0.001
DII	1.15 ± 1.88	1.10 ± 1.89	1.35 ± 1.84	<0.001	1.87 ± 1.80	1.84 ± 1.81	2.08 ± 1.73	<0.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MET, metabolic equivalent of task; eGFR, estimated glomerular filtration rate; UA, uric acid; DII, dietary inflammatory index.

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TABLE 2 Comparison of each component of DII scores between individuals with hyperuricemia and individuals without hyperuricemia in different sexes.

DII components	Male				Female			
	Total *n* = 15,828	Non-Hyperuricemia *n* = 12,548	Hyperuricemia *n* = 3,280	*p*-value	Total *n* = 15,953	Non-Hyperuricemia *n* = 13,742	Hyperuricemia *n* = 2,211	*p*-value
Energy	0.103 (−0.120–0.179)	0.108 (−0.117–0.180)	0.078 (−0.130–0.179)	<0.001	−0.125 (−0.176–0.066)	−0.122 (−0.175–0.069)	−0.144 (−0.177–0.036)	<0.001
Protein	0.010 (−0.016–0.021)	0.010 (−0.015–0.021)	0.008 (−0.017–0.021)	0.002	−0.016 (−0.021–0.007)	−0.015 (−0.021–0.008)	−0.016 (−0.021–0.005)	0.034
Carbohydrate	−0.001 (−0.091–0.095)	0.006 (−0.089–0.096)	−0.027 (−0.094–0.091)	<0.001	−0.085 (−0.097–0.009)	−0.084 (−0.097–0.014)	−0.092 (−0.097--0.026)	<0.001
Dietary fiber	0.287 (−0.453–0.608)	0.249 (−0.484–0.595)	0.425 (−0.296–0.633)	<0.001	0.490 (−0.054–0.636)	0.478 (−0.075–0.634)	0.553 (0.118–0.646)	<0.001
Total fatty acid	0.154 (−0.153–0.294)	0.165 (−0.146–0.295)	0.121 (−0.180–0.290)	<0.001	−0.094 (−0.256–0.190)	−0.091 (−0.255–0.194)	−0.113 (−0.265–0.164)	0.001
Total saturated fatty acid	−0.057 (−0.314–0.312)	−0.042 (−0.309–0.321)	−0.126 (−0.331–0.269)	<0.001	−0.271 (−0.354–0.016)	−0.268 (−0.354–0.025)	−0.290 (−0.358--0.041)	<0.001
MUFA	−0.004 (−0.009–0.007)	−0.004 (−0.009–0.006)	−0.003 (−0.009–0.007)	<0.001	0.005 (−0.005–0.009)	0.005 (−0.005–0.009)	0.005 (−0.004–0.009)	0.003
PUFA	−0.247 (−0.337–0.153)	−0.252 (−0.337–0.146)	−0.228 (−0.337–0.184)	0.012	−0.016 (−0.318–0.276)	−0.022 (−0.319–0.273)	0.026 (−0.315–0.288)	0.008
Cholesterol	−0.006 (−0.108–0.110)	−0.002 (−0.107–0.110)	−0.014 (−0.108–0.110)	0.096	−0.101 (−0.110–0.070)	−0.101 (−0.110–0.070)	−0.101 (−0.110–0.070)	0.472
Vitamin A	0.264 (0.098–0.335)	0.257 (0.084–0.332)	0.290 (0.154–0.343)	<0.001	0.285 (0.156–0.341)	0.283 (0.153–0.340)	0.296 (0.181–0.347)	<0.001
Vitamin B1	0.006 (−0.062–0.058)	0.002 (−0.065–0.056)	0.020 (−0.046–0.065)	<0.001	0.050 (−0.000–0.078)	0.049 (−0.002–0.077)	0.058 (0.011–0.081)	<0.001
Vitamin B2	−0.026 (−0.060–0.017)	−0.028 (−0.061–0.015)	−0.016 (−0.054–0.025)	<0.001	0.007 (−0.034–0.037)	0.005 (−0.035–0.036)	0.015 (−0.023–0.041)	<0.001
Vitamin B6	−0.205 (−0.348–0.024)	−0.206 (−0.349–0.023)	−0.200 (−0.345–0.034)	0.239	−0.004 (−0.226–0.175)	−0.008 (−0.228–0.172)	0.016 (−0.204–0.191)	<0.001
Vitamin B12	−0.020 (−0.070–0.064)	−0.016 (−0.069–0.067)	−0.029 (−0.074–0.051)	<0.001	−0.057 (−0.084–0.001)	--0.056 (−0.084–0.003)	−0.062 (−0.086--0.006)	<0.001
Vitamin C	0.362 (−0.065–0.413)	0.357 (−0.087–0.413)	0.379 (0.024–0.415)	<0.001	0.374 (0.076–0.414)	0.370 (0.057–0.413)	0.390 (0.180–0.415)	<0.001
Vitamin D	00.359 (−0.055–0.435)	0.352 (−0.086–0.434)	0.394 (0.070–0.438)	<0.001	0.394 (0.150–0.438)	0.394 (0.135–0.437)	0.410 (0.232–0.438)	<0.001
Vitamin E	0.271 (−0.375–0.416)	0.255 (−0.385–0.416)	0.317 (−0.322–0.418)	<0.001	0.389 (−0.089–0.419)	0.387 (−0.101–0.418)	0.402 (0.012–0.419)	<0.001
β-Carotene	0.537 (0.388–0.557)	0.537 (0.380–0.557)	0.540 (0.414–0.559)	0.004	0.536 (0.340–0.558)	0.536 (0.342–0.558)	0.537 (0.322–0.558)	0.697
Niacin	−0.016 (−0.164–0.110)	−0.016 (−0.165–0.110)	−0.016 (−0.161–0.110)	0.749	0.110 (−0.000–0.177)	0.109 (−0.001–0.176)	0.117 (0.004–0.181)	0.016
Folate	0.172 (0.011–0.189)	0.169 (−0.009–0.189)	0.180 (0.074–0.189)	<0.001	0.185 (0.130–0.190)	0.185 (0.125–0.190)	0.187 (0.151–0.190)	<0.001
Magnesium	0.014 (−0.253–0.231)	0.006 (−0.261–0.224)	0.050 (−0.224–0.259)	<0.001	0.184 (−0.036–0.321)	0.179 (−0.041–0.318)	0.222 (0.014–0.340)	<0.001
Iron	0.011 (−0.018–0.031)	0.013 (−0.017–0.031)	0.003 (−0.022–0.029)	<0.001	−0.013 (−0.027–0.015)	−0.013 (−0.027–0.017)	−0.018 (−0.028–0.008)	<0.001
Zinc	−0.184 (−0.312–0.190)	−0.199 (−0.312–0.181)	−0.114 (−0.311–0.223)	<0.001	0.155 (−0.192–0.292)	0.148 (−0.200–0.291)	0.193 (−0.134–0.299)	<0.001
Selenium	−0.184 (−0.191--0.098)	−0.185 (−0.191--0.101)	−0.182 (−0.191--0.089)	0.012	−0.110 (−0.184–0.035)	−0.112 (−0.184–0.032)	−0.096 (−0.181–0.044)	0.003
Caffeine	0.084 (0.083–0.085)	0.084 (0.083–0.085)	0.084 (0.083–0.085)	0.508	0.084 (0.084–0.085)	0.084 (0.084–0.085)	0.084 (0.084–0.085)	0.574
Alcohol	0.278 (−0.001–0.278)	0.278 (0.136–0.278)	0.278 (−0.278–0.278)	<0.001	0.278 (0.278–0.278)	0.278 (0.278–0.278)	0.278 (0.278–0.278)	<0.001
n3 Polyunsaturated fatty acid	0.287 (0.273–0.294)	0.287 (0.273–0.294)	0.286 (0.271–0.294)	0.003	0.290 (0.279–0.295)	0.290 (0.279–0.295)	0.290 (0.278–0.295)	0.024
n6 Polyunsaturated fatty acid	−0.102 (−0.153--0.010)	−0.103 (−0.153--0.012)	−0.096 (−0.152--0.000)	0.003	−0.050 (−0.130–0.036)	−0.052 (−0.130–0.035)	−0.041 (−0.127–0.044)	0.002

Data are presented as the median and interquartile ranges (Q1-Q3). DII, dietary inflammatory index; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

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TABLE 3 Odd ratios and 95% confidence intervals for hyperuricemia according to DII.

Characteristics	Model 1	Model 2	Model 3
Total (n = 31,781)			
Continuous	1.05 (1.03, 1.06)	1.05 (1.04, 1.07)	1.06 (1.04, 1.09)
DII Tertile			
T1 (−5.28, 0.79)	Reference	Reference	Reference
T2 (0.79, 2.60)	1.15 (1.07, 1.23)	1.14 (1.06, 1.23)	1.17 (1.07, 1.29)
T3 (2.60, 5.79)	1.21 (1.12, 1.30)	1.26 (1.17, 1.36)	1.31 (1.19, 1.44)
P for trend	<0.001	<0.001	<0.001
Male (n = 15,828)			
Continuous	1.07 (1.05, 1.10)	1.06 (1.04, 1.08)	1.07 (1.05, 1.10)
DII Tertile			
T1 (−5.28, 0.79)	Reference	Reference	Reference
T2 (0.79, 2.60)	1.24 (1.13, 1.36)	1.18 (1.07, 1.29)	1.27 (1.13, 1.42)
T3 (2.60, 5.79)	1.34 (1.21, 1.47)	1.27 (1.15, 1.40)	1.37 (1.21, 1.56)
P for trend	<0.001	<0.001	0.007
Female (n = 15,953)			
Continuous	1.08 (1.05, 1.11)	1.06 (1.03, 1.09)	1.05 (1.02, 1.09)
DII Tertile			
T1 (−5.28, 0.79)	Reference	Reference	Reference
T2 (0.79, 2.60)	1.16 (1.03, 1.32)	1.11 (0.97, 1.26)	1.03 (0.88, 1.22)
T3 (2.60, 5.79)	1.38 (1.23, 1.55)	1.28 (1.13, 1.44)	1.23 (1.05, 1.44)
P for trend	<0.001	<0.001	<0.001

Model 1: Non-adjusted. Model 2: Adjusted for age, sex, and BMI. Model 3: Adjusted for age, sex, BMI, race/ethnicity, educational level, smoking, drinking, MET, eGFR, diabetes, hypertension, and hyperlipidemia. The sex variables were not adjusted in the stratified analysis of sex. DII, dietary inflammatory index; BMI, body mass index; MET, metabolic equivalent of task; eGFR, estimated glomerular filtration rate.

significance levels for interactions in hypertension and BMI are observed at a p value of 0.05. However, interactions between DII and hyperuricemia are not significant for sex and race/ethnicity ($p > 0.05$) (Figure 3).

4. Discussion

In this cross-sectional study using NHANES data, we found that DII, which is an indicative index for pro-inflammatory diet, was in significant positive association with hyperuricemia after adjusting for multiple covariates. Compared with the lowest tertile subgroup of the DII, the highest tertile subgroup of the DII increased the risk of hyperuricemia by 31% for all participants, including 37 and 23% for male and female, respectively. Likewise, this positive correlation has also been validated in the GAM and through smooth curve fitting.

The results between the DII components and hyperuricemia showed that both male and female, the hyperuricemia group had higher intakes of dietary fiber, PUFA, vitamin A, vitamin B1, vitamin B2, vitamin B12, vitamin C, vitamin D, vitamin E, folate, magnesium, zinc, selenium, and n6 polyunsaturated fatty acids, as compared with the non-hyperuricemia group. In addition, female participants had higher levels of vitamin B6 and niacin intake in the hyperuricemia group than in the non-hyperuricemia group; and the male hyperuricemia group had higher levels of beta-carotene intake than the non-hyperuricemia group. Moreover, the differences between the cholesterol and caffein were not significant in either male or female. Hyperuricemia group had a slightly higher intake of dietary fiber than the other. The possible mechanism behind might be that the viscosity and bulk of dietary fiber interfere with the absorption of purines or adenine in the digestive system (17), or enhance intestinal motility and potentially binding uric acid in the intestine to facilitate its excretion (18). Additionally, In addition, our study found significant differences in certain vitamins in DII between the hyperuricemia and non-hyperuricemia groups, regardless of sex differences, which these vitamins are associated with hyperuricemia in various ways. For example, vitamin C mediates serum uric acid level by effectively preventing the impairment of renal epithelial function caused by uric acid crystals (19). Vitamin B12, as summarized by Zhang et al., its combination with folic acid could inactivate xanthine oxidoreductase, interfere with the conversion of purines to uric acid, and also reduce homocysteinemia level that may induce significant DNA damage and release purine nucleotides, ultimately reducing uric acid (20). Vitamin D affects the secretion and transport of uric acid by influencing serum parathyroid hormone level. Vitamin E acts by inhibiting xanthine oxidase activity and reducing uric acid formation (21). In addition, an experimental study reported that vitamin E also promotes uric acid excretion in deoxycorticosterone-salt-treated rats (22). In the end, a significant difference was also identified in alcohol intake between two groups. In addition, in this study we observed that cholesterol and caffeine intake did not differ significantly between the hyperuricemia and non-hyperuricemia groups of male and female, which may be related to the prevalence of caffeine consumption as well as to the Western dietary pattern in the US, which is characterized by a high-calorie and fat diet and is usually associated with higher levels of inflammatory markers in the body (9, 23, 24). Most Americans follow this pattern of dietary consumption, eliminating or diminishing its role between the sexes.

In addition, most people tend to consume food in several dietary patterns or their combinations. Current assessment only focused on the effect of single nutrient, which may lead to one-sided outcomes due to its neglect on synergistic effects of nutritional patterns (25). Further regression analysis showed that there is a positive association between DII and hyperuricemia risk after adjusting for potential confounders, with similar associations for male and female participants. A study among a Chinese population indicated a highly positive correlation between DII score and serum uric acid levels, regardless of sex (14). While, a Korean study found that only female with higher DII scores had a higher risk of HUA (26). The discrepancy could be partially due to the differences in the eating habits of different populations. The Korean diet is characterized by containing large amounts of fermented foods and seafood (27). In contrast to the

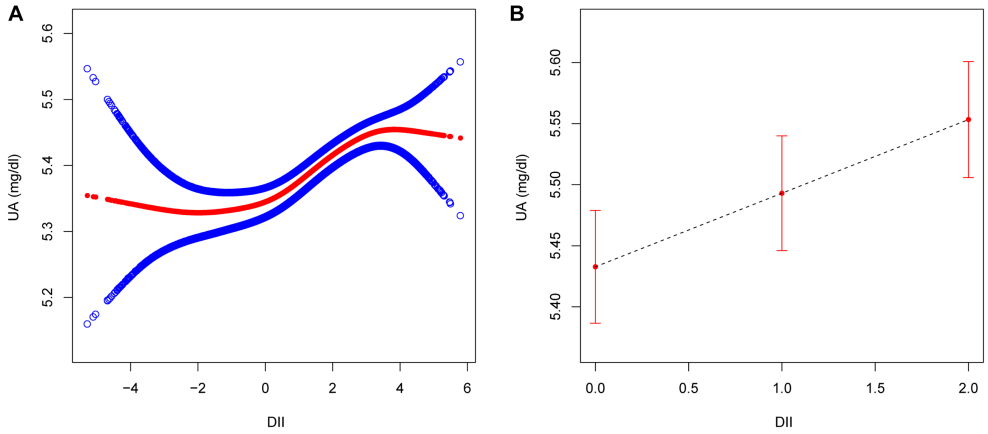

FIGURE 2
The association between DII ((**A**) as continuous variable; **(B)** as categorical variable) and UA. Age, sex, BMI, race/ethnicity, educational level, smoking, drinking, MET, eGFR, diabetes, hypertension, and hyperlipidemia were adjusted. DII, dietary inflammatory index; UA, uric acid; BMI, body mass index; MET, metabolic equivalent of task; eGFR, estimated glomerular filtration rate.

Characteristics	OR (95% CI)	P-value	P-interaction*
Sex			0.262
Male	1.07 (1.05, 1.10)	<0.001	
Female	1.05 (1.01, 1.08)	0.011	
Age (years)			0.019
<30	1.01 (0.97, 1.06)	0.659	
30-44	1.05 (1.01, 1.09)	0.019	
45-59	1.06 (1.02, 1.11)	0.004	
≥60	1.11 (1.07, 1.16)	<0.001	
Race/ethnicity			0.893
White	1.07 (1.04, 1.10)	<0.001	
Non-white	1.07 (1.04, 1.10)	<0.001	
Hypertension			0.053
Yes	1.09 (1.05, 1.12)	<0.001	
No	1.04 (1.01, 1.07)	0.004	
Diabetes			0.015
Yes	1.12 (1.07, 1.18)	<0.001	
No	1.05 (1.03, 1.08)	<0.001	
BMI(kg/m^2)			0.053
<25	1.03 (0.98, 1.08)	0.279	
≥25	1.08 (1.06, 1.11)	<0.001	

FIGURE 3
Stratified analyses between DII and hyperuricemia. OR values are based on different population stratifications, estimated using a multivariable logistic regression model to assess the odds ratios and 95% confidence intervals between DII and hyperuricemia. The first column of *p*-values represents the significance of OR values after stratification by different population groups. The second column of P-values pertains to the significance of models considering interaction terms between DII and various variables (sex, age, race/ethnicity, hypertension, diabetes and BMI). *Each stratification adjusted for all the factors (age, sex, BMI, race/ethnicity, educational level, smoking, drinking, MET, eGFR, diabetes, hypertension, and hyperlipidemia) except the stratification factor itself. OR, odd ratio; CI, confidence interval; DII, dietary inflammatory index; BMI, body mass index; MET, metabolic equivalent of task; eGFR, estimated glomerular filtration rate.

dietary habits in Korea, Western diets are characterized by high fat intake, which contain processed meats and red meats (24). According to the DII concept (12), a pro-inflammatory diet contains mainly of red and processed meats, fried foods, high-sugar foods and refined grains, which are associated with high uric acid levels (28). An anti-inflammatory diet includes more vegetables, fruits, soy products, whole grains and nuts. Therefore, a healthy diet might be able to reduce the risk of hyperuricemia.

Previous studies have confirmed that serum uric acid level is determined by the production-excretion balance of uric acid, in which

excretion via kidney plays a major role (29). In addition, it has been shown that intestine, other than kidney, is an important potential organ for the excretion of uric acid, and it works primarily through the action of intestinal flora and uric acid transporters (30). Thus the potential mechanism by which DII influence hyperuricemia are as follows. DII is closely associated with the development of hyperuricemia through direct effects of host purine metabolism or indirect effect of gut microbiota. On one hand, diets that promote inflammation can have adverse effects on an individual's health by inducing oxidative stress and disrupting the immune system. Such diets can significantly increase the levels of inflammatory cytokines, exacerbating the problem. Additionally, they could alter the gastrointestinal microbial ecosystem, leading to chronic inflammation and other related health issues (31). Chronic inflammation is one of the main mechanism causing renal injury, which may ultimately impede uric acid excretion (32). On the other hand, anti-inflammatory diets in DII downregulate adenosine deaminase and xanthine oxidase activities, and improve intestinal barrier function and restores intestinal microbiota metabolism (33). Therefore, it might be a new therapeutic strategy targeting at metabolic balance of purines along with the regulation of intestinal excretion of uric acid, to prevent and alleviate hyperuricemia in the future.

In the final forest plot, our study indicates that there is an interaction between age and diabetes concerning the relationship between DII and hyperuricemia (p value for interaction <0.05). Additionally, blood pressure and BMI might also have a certain influence on the association between DII and hyperuricemia (borderline significant p value for interaction). Previous study pointed out that the population of participants who were ≥ 60 years old had the highest hyperuricemia risk (34). Possible explanations for this discrepancy could be attributed to different leptins levels in different age groups. It has been shown that leptin plays a critical role in inflammatory and immune responses (35), and is an important independent variable of uric acid values across all age groups (36). Also, a study by Francesco et al. showed that fasting leptin was higher in elderly subjects than in younger subjects, even after adjusting for the covariate of fat mass (37). Based on these studies, it is reasonable to deduce that the age-based DII-hyperuricemia relationship might be influenced by leptin levels. Additionally, we found that DII and hyperuricemia had a stronger association in patients with diabetes, which was quite expectable and was consistent with other studies which have confirmed a linear and positive correlation between serum uric acid levels in diabetes mellitus and serum insulin levels (38). A longitudinal study had reported that patients with diabetes at baseline were related to an increased hyperuricemia risk (39). Diabetes may regulate the association between DII and hyperuricemia by insulin resistance. Insulin resistance is a prevalent medical condition that could potentially cause an upsurge in the activity of hexose monophosphate shunt. This could lead to an augment in purine biosynthesis and turnover, ultimately resulting in an increase in the level of uric acid (40). Moreover, we found that as DII scores increase, the risk of hyperuricemia in individuals with a BMI > 25 also increases. This is consistent with a previous analysis of a representative sample of U.S. adults, which indicated that obesity plays a mediating role in the relationship between diet and hyperuricemia, with an indirect effect proportion of BMI as high as 53.34% (41). In the meanwhile, some studies have reached conclusions that are inconsistent with ours. A Korean case–control study evaluating a pro-inflammatory diet and the risk of hyperuricemia found that participants with a BMI <25 had a higher risk of developing hyperuricemia (26, 42). Additionally, research suggests that not only overweight but also being underweight is associated with higher levels of inflammation. This could explain why low BMI is also correlated with an increased risk of hyperuricemia (43, 44). It is worth emphasizing that these studies are currently based on studies conducted in single-country populations, leading to limitations in the extrapolation of findings. Meanwhile, similar to our findings, a survey from the U.S. indicated participants with hypertension had an elevated DII compared with those without hypertension (45). Although the underlying mechanisms are not clear, it is possible that the underlying hypertension risk or improved dietary pattern of these participants played a partial role in the correlation between DII and HUA risk (46). Hence, further investigation is needed to elucidate the question of whether hypertension might play a role in the relationship between DII and hyperuricemia. In contrast, a study showed a positive association between DII score and hyperuricemia that did not vary by hypertension status. The discrepancy could be partially due to the criteria for inclusion and exclusion, and sample size of the study participants. In our study, to minimize the potential adverse effects of renal impairment on nutritional intake, we only included individuals with an eGFR of ≥60 mL/min/1.73 m (2). In terms of sample size, we incorporated a larger number of samples (n = 31,781 VS n = 19,004), which might provide a more representative reflection of the characteristics of a larger population.

There are some advantages and limitations of this study. This is the first study to confirm the association between DII and hyperuricemia using data from a large-scale U.S. adults based on NHANES. However, present results should be interpreted with caution as cross-sectional observational studies cannot demonstrate causation and directionality. Secondly, although confounding factors have been extensively adjusted, other elements cannot be completely ruled out. Besides this, a possible limitation is the self-reporting of some conditions by the study participants. Information about self-reported may be recall biased or interview subjects diagnosed with hyperuricemia might change their diet pattern. Hyperuricemia represents a major global public health burden, thus further longitudinal studies should be conducted to provide stronger evidence for the relationship between DII and hyperuricemia.

5. Conclusion

In conclusion, our study based on an existing database highlighted the association between a diet with higher inflammatory potential measured by DII and increased risk of developing hyperuricemia. These findings suggest that adjusting dietary habits to reduce inflammation may be an effective prevention and control strategy for hyperuricemia. This study provides valuable evidence for healthcare professionals and individuals to consider when making dietary choices to maintain optimal health. Future research aiming to perform interventions based on dietary modifications may yield promising results for combating hyperuricemia and other related health conditions.

Data availability statement

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

Ethics statement

All data came from NHANES, which was approved by National Centre for Health Statistics Institutional Ethics Review Board, and all the subjects agreed on the survey and signed written consent. 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

HW: writing-most of manuscript, data curation and processing. SQ: writing-part of the manuscript and data curation. FL: writing-part of the manuscript. HZ: software, writing—review and editing. LZ: methodology, writing—review and editing, and supervision. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1218166/full#supplementary-material

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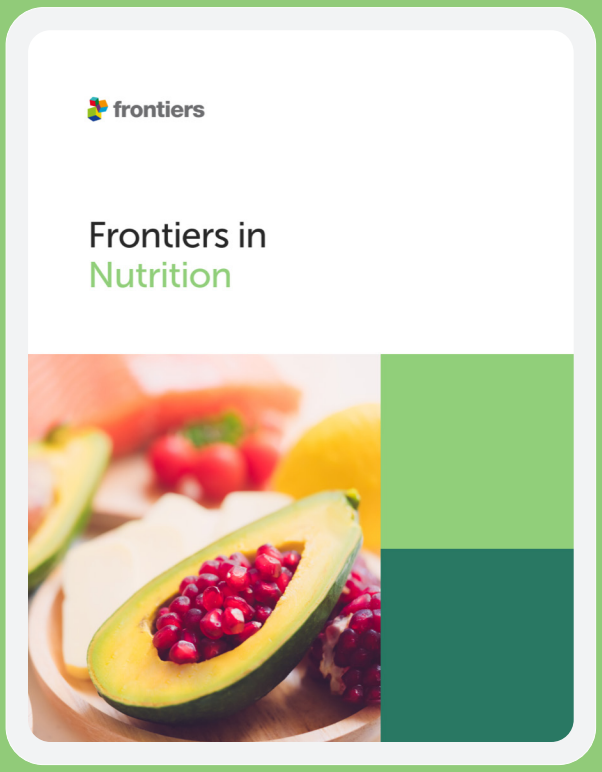

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