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Dietary and supplement strategies for the prevention and treatment of metabolic syndrome

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Dietary and supplement strategies for the prevention and treatment of metabolic syndrome

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The association between dietary intake of flavonoids and its subclasses and the risk of metabolic syndrome

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Background: The healthiest way to prevent metabolic syndrome (MetS) is through behavioral and nutritional adjustments. We examined the relationship between total flavonoids intake, flavonoid subclasses, and clinically manifest MetS.

Methods: A cross-sectional analysis was conducted among 28,719 individuals from the National Health and Nutrition Examination Survey (NHANES) and Food and Nutrient Database for Dietary Studies (FNDDS) 2007–2011 and 2017–2018. Two 24-h reviews were conducted to determine flavonoids intake and subclasses. The link between flavonoids intake and MetS was investigated using a multivariate logistic regression model.

Results: Q2 and Q3 of total flavonoids intake were associated with 20 and 19% lower risk of incident MetS after adjusting age and sex. Anthocyanidins and flavanones intake in Q2 and Q3 substantially reduced the MetS risk compared to Q1. MetS risk decreased steadily as the total intake of flavonoids increased to 237.67 mg/d. Flavanones and anthocyanidins also displayed V-shaped relationship curves (34.37 and 23.13 mg/d).

Conclusion: MetS was adversely linked with total flavonoids intake, flavanones, and anthocyanidins. Moreover, the most effective doses of total flavonoids, flavanones, and anthocyanidins were 237.67, 34.37, and 23.13 mg/d, respectively, potentially preventing MetS.

KEYWORDS

metabolic syndrome, flavonoids intake, flavanones, anthocyanidins, NHANES

Introduction

Metabolic syndrome (MetS) is characterized by a cluster of metabolic abnormalities, such as impaired glucose metabolism, high blood pressure, low high-density lipoprotein cholesterol (HDL-c) levels, and dyslipidemia (1). Subjects with MetS mainly present with abdominal obesity, hyperglycemia, hypertension, and dyslipidemia (2). It was also closely associated with a higher cardiovascular disease (CVD) risk, type 2 diabetes (T2D) risk, and overall mortality (3, 4). MetS results from complicated risk factors interaction among genetic, metabolic, diet, lifestyle, and environmental factors (5). For instance, smoking, alcohol drinking, and an unbalanced diet contributed significantly to the development of MetS (5). Studies have demonstrated that some dietary life and nutrients played a protective role against MetS development (6, 7). For instance, Mediterranean diet (MedDiet) interventions could improve MetS (8, 9). MedDiet is characterized by a high concentration of polyphenols, which are prospective candidates for ameliorating the chronic low-grade inflammation and oxidative stress of MetS patients (10).

Previous research examined the relationship between dietary polyphenols and MetS, CVD, T2D, cancer, and all-cause mortality (11–17). For instance, high total polyphenols, flavonoids, and phenolic acid intake had 50, 51, and 45% lower odds of MetS, respectively, when compared to low total polyphenols, flavonoids, and phenolic acid intake. In contrast, larger intakes of total polyphenols, flavonoids, and phenolic acids were related to a reduced risk for elevated systolic blood pressure (SBP) and HDL-c, which were independent cardiovascular risk factors (16). However, no relationship was found between total polyphenols intake and other MetS components (18). A French prospective cohort study reported that individuals with polyphenols intake, including anthocyanins, dihydro flavonols, catechins, flavonols, hydroxybenzoic acids, lignans, and stilbenes, had a low risk of T2D, which is independent of major potential confounders (11). Furthermore, a decreased risk of gestational diabetes mellitus (GDM) is related to a large intake of fruit polyphenols. However, no clear association exists between total vegetable polyphenol intake and GDM risk (15). Moreover, a high intake of stilbenes, lignans, hydroxy benzaldehydes, hydroxy coumarins, and tyrosols was associated with a lower gastric cancer risk (12). Additionally, peonidin, naringenin, and catechin intakes were negatively correlated with cancer mortality (17). However, total polyphenols intake was not significantly associated with cancer mortality risk reported in Japanese adults (13). Importantly, individuals' biological aging or the discrepancy between the biological and chronological age of a subject (Δage) was inversely associated with the polyphenol antioxidant content (PAC) score (14). Therefore, a polyphenol-rich diet helps decelerate biological aging, which benefits the long-term risk of MetS, cardiovascular disease, and cancer.

Previous studies have highlighted dietary flavonoids intake as a potential protective factor against developing extra body fat (19). Studies have evidenced that flavonoids are strong antioxidants and metal chelators to decrease energy intake, increase energy expenditure and fat oxidation, influence macronutrient absorption and uptake, and inhibit adipogenesis (20–25). According to a previous study, long-term use of flavonoids may improve cardiovascular disease by restoring the body's natural antioxidant defenses and lowering the risk of low-density lipoprotein cholesterol (LDL) oxidation (26). In addition, flavonoid compounds extracted from apple peel can reduce blood pressure and control body fat (27, 28). Although a previous cross-sectional study including 9,108 Chinese individuals investigated the relationship between flavonoid and copper intake and MetS, there is no proof that any flavonoid compound can ameliorate MetS symptoms (5). A Tehran Lipid and Glucose Study based on Iran's population reported that only flavonols and flavones showed protective effects against MetS. Simultaneously, no significant association was found between the intake of other flavonoid subclasses and MetS risk (29). Due to the intricacy of MetS and the variety of flavonoids, additional research is required to assess the relationship between flavonoid consumption and MetS risk. Furthermore, because each flavonoid component has unique active activity, it was necessary to confirm whether consuming the various flavonoid subclasses was linked with lowering MetS (18).

Consequently, this study aimed to demonstrate the possible association of total flavonoids and subclasses of flavonoid intake with MetS in the USA using the NHANES data system from 2007 to 2010 and 2017 to 2018. We hypothesized that flavonoids intake would be positively associated with a reduced risk of MetS. Additionally, the impact of an individual's clinical characteristics and lifestyle on the MetS reduction brought on by using flavonoids was discussed. This study also explored the most effective dose of each flavonoid subtype for reducing the MetS rate.

Methods

Study population

The NHANES was a cross-sectional design that employed stratified, multistage probability sampling of the U.S. population to examine health and nutritional status through interviews and laboratory examinations (30). This survey was approved by the Ethics Review Board of the NCHS (available on the web at: https://www.cdc.gov/nchs/nhanes/). This study employed three cycles of NHANES 2007–2010 and 2017–2018 data to extract flavonoids intake information, and 28,719 participants were included (Table 1). Participants were disqualified if they could not provide dietary information within 24 h. Moreover, pregnant women and cancer patients receiving medical therapy or radiotherapy were excluded. Demographic, health-related lifestyle, and chronic disease information were also gathered from the participants.

Assessment of flavonoid intakes

The information on total flavonoids and their subclasses intake was extracted from the United States Department of Food and Nutrient Database for Dietary Studies (FNDDS) linked to the NHANES database. These flavonoid compounds' levels were determined by averaging the results of two 24-h interviews (31). The USDA food code for each survey cycle was used to assign the flavonoid compounds (version 4.1 for 2007–2008 and version 5.0 for 2009–2010) (32). USDA Database provided values for 29 kinds of flavonoids, six flavonoid subclasses (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and isoflavones), and total flavonoids for all food codes linked to NHANES 2007–2010 and 2017–2018 (32). These values can be used to estimate flavonoids consumption in the U.S. population.

Definition of metabolic syndrome

Metabolic syndrome (MetS) is defined according to the criteria and definition published in the Lancet statement on metabolic syndrome in 2005 guidelines (33, 34). The following requirements were described in detail: (1) waist circumference was $\geq$88 cm for women and $\geq$102 cm for men, (2) hypertriglyceridemia (triglycerides $\geq$ 1.7 mmol/L), (3) low HDL cholesterol (HDL $<$ 1.03 mmol/L in men or HDL $<$ 1.29 mmol/L in women), (4) elevated blood pressure (SBP $\geq$ 130 mm Hg, DBP $\geq$ 85 mm Hg, or both) or antihypertensive drug treatment for hypertension, and (5) elevated

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TABLE 1 Characteristics of NHANES participants by tertiles of total flavonoid intake.

	Total population (n = 28,719)	Total flavonoid intake quintiles			
		Q1 (n = 8,937)	Q2 (n = 9,265)	Q3 (n = 10,517)	P-value
Total flavonoid intake, mean (SE) (mg/d)	220.03 (7.37)	18.57 (0.34)	80.00 (0.93)	579.24 (13.69)	<0.0001
Socio-economic characteristics					
Female, *n* (%)	14,690 (51.15)	4,553 (50.95)	4,656 (50.25)	3,851 (52.10)	0.21
Male, *n* (%)	14,029 (48.85)	4,384 (49.05)	4,609 (49.75)	5,409 (47.90)	
Age, years, mean (SE)	37.26 (0.29)	33.62 (0.39)	37.17 (0.39)[a*]	40.44 (0.41)[b*]	<0.0001
Ethnicity					
White, *n* (%)	1,8191 (63.34)	5,656 (63.29)	5,716 (61.69)[a*]	6,820 (64.85)[b*]	<0.0001
Black, *n* (%)	3,464 (12.06)	1,219 (13.64)	1,106 (11.94)[a*]	1,140 (10.84)[b*]	
Mexican, *n* (%)	2,970 (10.34)	990 (11.08)	1,061 (11.45)[a*]	919 (8.74)[b*]	
Others, *n* (%)	4,092 (14.25)	1,072 (12.00)	1,383 (14.93)[a*]	1,639 (15.58)[b*]	
Healthy behavior factors					
Smoke status					
Current smokers, *n* (%)	4,431 (15.43)	1,635 (18.30)	1,141 (12.32)[a*]	1,54 (15.73)[b*]	<0.0001
No current smokers, *n* (%)	24,288 (84.57)	7,302 (81.70)	8,124 (87.68)[a*]	8,863 (84.27)[b*]	
Drinking					
No drink user, *n* (%)	6,979 (24.30)	2,308 (25.83)	2,339 (25.25)[a*]	2,330 (22.15)[b*]	<0.0001
Former drink user, *n* (%)	2,257 (7.86)	798 (8.93)	671 (7.24)[a*]	788 (7.49)[b*]	
Mild drink user, *n* (%)	8,587 (29.90)	2,166 (24.24)	2,867 (30.94)[a*]	3,555 (33.80)[b*]	
Moderate drink user, *n* (%)	4,474 (15.58)	1,423 (15.92)	1,390 (15.00)[a*]	1663 (15.81)[b*]	
Heavy drink user, *n* (%)	6,422 (22.36)	2,241 (25.07)	1,998 (21.57)[a*]	2,181 (20.74)[b*]	
Physical activity level					
Never, *n* (%)	10,399 (36.21)	3,695 (41.34)	3,327 (35.91)[a*]	3,377 (32.11)[b*]	<0.0001
Low, *n* (%)	5,775 (20.11)	1,589 (17.78)	1,885 (20.35)[a*]	2,301 (21.88)[b*]	
Intermediate, *n* (%)	6,304 (21.95)	1,723 (19.28)	2,129 (22.98)[a*]	2,453 (23.32)[b*]	
High, *n* (%)	6,241 (21.73)	1,930 (21.60)	1,924 (20.77)[a*]	2,386 (22.69)[b*]	
Dietary intake					
kCal/day, kCal, mean (SE)	2,032.07 (8.20)	1,846.08 (11.69)	2,091.19 (12.00)[a*]	2,138.07 (12.75)[b*]	<0.0001
Carbohydrates/day, g/100 kCal, mean (SE)	249.00 (0.95)	222.01 (1.69)	257.74 (1.44)[a*]	264.23 (1.54)[b*]	<0.0001
Protein/day, g/100 kCal, mean (SE)	78.01 (0.40)	71.10 (0.57)	80.17 (0.54)[a*]	81.99 (0.51)[b*]	<0.0001
Fiber/day, g/100 kCal, mean (SE)	15.85 (0.15)	12.32 (0.13)	17.25 (0.14)[a*]	17.61 (0.19)[b*]	<0.0001
Total fat, mean (SE)	78.49 (0.39)	73.27 (0.54)	79.01 (0.60)[a*]	82.47 (0.60)[b*]	<0.0001
Medications, *n* (%)					
Anti.Diabetic					
No	26,829 (93.42)	8,344 (93.37)	8,687 (93.76)	9,798 (93.16)[b*]	0.6
Yes	1,890 (6.58)	593 (6.63)	578 (6.24)	719 (6.84)[b*]	
Anti.Hypertensive					
No	27,533 (95.87)	8,628 (96.54)	8,866 (95.69)	10,041 (95.47)[b*]	0.08
Yes	1,186 (4.13)	309 (3.46)	399 (4.31)	476 (4.53)[b*]	

(Continued)

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TABLE 1 (Continued)

	Total population ($n = 28,719$)	Total flavonoid intake quintiles			
		Q1 ($n = 8,937$)	Q2 ($n = 9,265$)	Q3 ($n = 10,517$)	*P*-value
Chronic disease factors					
BMI, kg/m^2, *n* (%)					
BMI < 25	12,677 (44.14)	4,027 (45.06)	4,260 (45.98)	4,390 (41.74)	0.001
BMI ≥ 25	16,042 (55.86)	4,910 (54.94)	5,005 (54.02)	6,127 (58.26)	
Hypertension, *n* (%)					
No Hypertension	20,816 (72.48)	6,638 (74.28)	6,755 (72.91)	7,421 (70.56)[b*]	<0.0001
Hypertension	7,903 (27.52)	2,299 (25.72)	2,510 (27.09)	3,096 (29.44)[b*]	
Diabetes, *n* (%)					
No diabetes	23,972 (83.47)	7,529 (84.25)	7,759 (83.75)	8,683 (82.56)	0.11
Diabetes	4,747 (16.53)	1,408 (15.75)	1,506 (16.25)	1,834 (17.44)	
Chronic kidney disease					
No CKD	20,867 (72.66)	6,212 (69.51)	6,523 (70.40)	8,132 (77.32)[b*]	<0.0001
CKD	7,852 (27.34)	2,725 (30.49)	2,742 (29.60)	2,385 (22.68)[b*]	
COPD					
No COPD	27,645 (96.26)	8,593 (96.15)	8,931 (96.39)	10,122 (96.24)	0.82
COPD	1,074 (3.74)	344 (3.85)	334 (3.61)	395 (3.76)	
CVD					
No CVD	26,855 (93.51)	8,375 (93.71)	8,707 (93.98)	9,773 (92.93)	0.03
CVD	1,864 (6.49)	562 (6.29)	558 (6.02)	744 (7.07)	
MetS					
No MetS	22,694 (79.02)	6,995 (78.27)	7,417 (80.05)[a*]	8,280 (78.73)	0.15
MetS	6,025 (20.98)	1,942 (21.73)	1,848 (19.95)[a*]	2,237 (21.27)	

Values are means (SE) for continuous variables and *n* (%) for categorical variables; SE, standard error; NHANES, National Health and Nutrition Examination Survey; BMI, body mass index; CKD, chronic kidney disease; MET, metabolic equivalent; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; MetS, metabolic syndrome. The total flavonoid value was the sum of 29 kinds of flavonoids in six subclasses of classes, which include anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and isoflavones. Data were generated using χ^2 tests for the categorical variables, and *T*-tests were used for continuous variables. [a*]indicated that the *p*-value for the Q2 vs. Q1 comparison was <0.05, while [b*]indicated that the *p*-value for the Q3 vs. Q1 comparison was <0.05.

fasting plasma glucose (FPG) (FPG ≥ 5.6 mmol/L, or diagnosed as type 2 diabetes).

Covariates

Using standard questionnaires, NHANES supplied demographic and lifestyle information about people in this study. In this study, gender, age, ethnicity, and body mass index (BMI) were demographic variables. Furthermore, smoking, drinking, physical activity, and fiber or protein consumption were lifestyle variables. Smoking was classified as no current (those who had never smoked more than 100 cigarettes in their lifetime or had smoked at least 100 cigarettes but did not currently smoke) or current (a minimum of 100 cigarettes had been smoked, or had smoked some days). Alcohol consumers were categorized as nondrinkers, previous drinkers, light, moderate, and heavy. Physical activity was evaluated by self-report and measured in weekly metabolic equivalent (MET) minutes. MET was calculated into trisection [Q1 (low), Q2 (intermediate), and Q3 (high)], and participants were categorized as never, low, intermediate, and high levels of physical activity. The ethnicity categories were White, Black, Mexican, and other ethnicity. Hypertension, diabetes, chronic kidney disease, and COPD were defined. Medication information, including anti-diabetes and anti-hypertension drugs, was defined as "No" and "Yes".

Statistical analyses

We used NHANES-recommended weights to balance for planned oversampling and ensure the analysis accuracy. The continuous variables were expressed as means ± standard errors (SE). Categorical variables were expressed as counts and percentages. Moreover, missing data were imputed using the forest R package. Individuals were separated into three groups

based on total flavonoids and flavonoid subclasses' consumption values (tertiles: Q1, Q2, and Q3). Significant differences between MetS subjects and control subjects were identified using χ^2 tests for categorical variables and ANOVA analysis for continuous variables. To evaluate the adjusted odds ratios (OR) and 95% confidence intervals (CIs) between flavonoids intake and MetS risk, a multivariate logistic regression analysis model was used. A model-adjusted risk ratio was calculated to compare the risk of MetS and flavonoids intake (Q2 and Q3) with the lowest (Q1) category. Then, we also constructed several adjusted models to modify various characteristics: model 1 (adjusted for age), model 2 (adjusted for age, sex, and ethnicity), model 3 (Model 2 plus smoke status, drinking status, and physical activity), and model 4 (Model 2 plus BMI, hypertension, diabetes, COPD, and medications). Then, a restricted cubic spline (RCS) was employed to explore the non-linear associations between the risk of MetS and the total flavonoids and its subclasses intake (35). A stratified analysis was performed to explore the heterogeneity of the effect of flavonoids intake on MetS risk. Moreover, an interaction model was used to evaluate the interaction between flavonoids intake and other variables. In this study, *p*-values of <0.05 were considered statistically significant. R software (version 4.1.2), Rstudio software, and the nhanesR package were used for all analyses.

Results

Basic characteristics of this study population

In 2007–2010 and 2017–2018, for all included individuals, the mean (SE) total flavonoids intake in the first tertile was 18.57 (0.34) mg, in the second tertile was 80.00 (0.93) mg, and in the third tertile was 579.24 (13.69) mg. The demographic, lifestyle characteristics, dietary intake components, and diseases of the included individuals are illustrated in Table 1. This population of 28,719 individuals from the USA was followed for a mean age of 37.26 years, and 14,029 (48.85%) were men. According to the criteria, 6,025 people (20.98%) were identified as MetS participants. Compared to participants in Q1 of total flavonoids intake, those in Q3 were more likely to be older, have a lower BMI, and be more physically active. Participants who consumed more flavonoids also tended to consume more total fat, protein, carbs, and fiber. Furthermore, these individuals take more anti-hypertension drugs (Table 1). These participants were less likely to be smokers and heavy drink users with CKD and COPD. However, these participants had a higher risk of diabetes, hypertension, and CVD (Table 1).

Overall associations between total flavonoids and six subclassess intake and MetS

We classified the total flavonoids intake by tertiles to investigate the association between flavonoids and MetS. The mean (SE) values of total flavonoids, six subclasses of flavonoids, and sole flavonoids are listed in Supplementary material 1. A multiple logistic regression model indicated that the Q2 and Q3 of total flavonoids intake were associated with a 20 and 19% lower risk of incident MetS after adjusting age and sex (model 1: second vs. first tertile, OR = 0.80 (95% CI: 0.71–0.90); Q3 vs. Q1, OR = 0.81 (95% CI: 0.72–0.91), P trend = 0.001) (Table 2). Total flavonoids intake is still strongly inversely associated with the MetS risk after adjusting for age, sex, lifestyles, and other nutrient intakes (Model 2: second vs. first tertile, OR = 0.82 (95% CI: 0.73–0.92); Q3 vs. Q1, OR = 0.83 (95% CI: 0.74–0.93), P trend = 0.003; model 3: Q2 vs. Q1, OR = 0.87 (95% CI: 0.76–0.98); Q3 vs. Q1, OR = 0.85 (95% CI: 0.75–0.97), P trend = 0.018). However, there were no statistically significant differences between the flavonols and flavones intake and the MetS risk. There were significant differences between the flavan_3_ols intake and the MetS risk only in model 1 (adjusting for sex and age) and model 2 (adjusting for sex, age, and lifestyles). The analysis showed that Q2 of flavanones intake reduced the risk of MetS compared to Q1 in model 1 (OR = 0.80, 95%CI: 0.71–0.89), model 2 (OR = 0.80, 95% CI: 0.71–0.89), and model 3 (OR = 0.84, 95% CI: 0.79–0.95). Furthermore, Q3 of flavanones intake had more strongly reduced the MetS risk than Q1 (model 1: OR = 0.73, 95% CI: 0.66–0.80), model 2 (OR = 0.73, 95% CI: 0.66–0.81), and model 3 (OR = 0.75, 95% CI: 0.67–0.84). Individuals who consumed anthocyanidins also experienced a considerable reduction in their MetS risk, as indicated in Table 2.

Stratified analyses

Results presented that Q3 of total flavonoids intake was significantly related to a lower risk of MetS in participants of age <20 years (OR = 0.64, 95% CI: 0.50–0.82) compared to Q1, but Q2 of total flavonoids intake was significantly related to a lower risk of MetS in participants of age >60 years (OR = 0.76, 95% CI: 0.58–1.01). Moreover, compared to Q1, Q2, and Q3, total flavonoids intake was significantly related to MetS lower risk in Mexican participants (Q2: OR = 0.78, 95% CI: 0.66–0.92; Q3: OR = 0.79, 95% CI: 0.66–0.95). A negative correlation between total flavonoids intake and MetS prevalence was also found in participants with a BMI of <25 kg/m^2. Interaction analysis revealed that different degrees of physical activity had distinct effects on the extent to which total flavonoids consumption affected MetS risk ($P < 0.0001$; Table 3). Compared to Q1, MetS risk was reduced in Q3 of the isoflavones intake (Supplementary material 2), while no significant difference was found in Q2 of the isoflavones intake. Moreover, no interaction existed in the effect of these covariates with isoflavones intake on MetS risk. Additionally, compared to Q1, anthocyanidins and flavanones intake in Q2 and Q3 could significantly lower MetS risk (Supplementary material 3, 4).

Overall dose–response associations between flavonoids intake and MetS

We employed an RCS to analyze the dose–response relationship of flavonoid consumption (Figure 1). The median total intake of total flavonoids, flavanones, isoflavones, and anthocyanidins was set as the reference point to illustrate the association between

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TABLE 2 Odd ratios (ORs) and 95% confidence intervals (CIs) of MetS risk by tertiles of flavonoid intake.

	Total flavonoid intake quintiles			*P* for trend
No. events	Q1 ($n = 8,937$)	Q2 ($n = 9,265$)	Q3 ($n = 10,517$)	
Total flavonoid				
Mean (SE) (mg/d)	18.57 (0.34)	80.00 (0.93)	579.24 (13.69)	<0.0001
OR (95%CI)				
Model 1	Ref	0.80 (0.71,0.90)	0.81 (0.72,0.91)	0.001
Model 2	Ref	0.82 (0.73,0.92)	0.83 (0.74,0.93)	0.003
Model 3	Ref	0.87 (0.76,0.98)	0.85 (0.75,0.97)	0.018
Flavonols				
No. events	8,136	9,026	11,557	
Mean (SE) (mg/d)	4.29 (0.03)	11.65 (0.04)	26.85 (0.33)	<0.0001
OR (95%CI)				
Model 1	Ref	0.83 (0.74,0.93)	0.95 (0.85,1.07)	0.722
Model 2	Ref	0.82 (0.73,0.92)	0.95 (0.84,1.07)	0.696
Model 3	Ref	0.87 (0.77,0.98)	1.07 (0.97,1.21)	0.145
Flavan_3_ols				
No. events	9,121	9,268	10,327	
Mean (SE) (mg/d)	4.12 (0.05)	39.23 (0.88)	351.61 (9.93)	<0.0001
OR (95%CI)				
Model 1	Ref	0.82 (0.74,0.92)	0.88 (0.798,0.97)	0.02
Model 2	Ref	0.84 (0.75,0.94)	0.89 (0.81,0.98)	0.041
Model 3	Ref	0.89 (0.80,1.00)	0.91 (0.81,1.01)	0.077
Flavones				
No. events	8,570	9,101	11,048	
Mean (SE) (mg/d)	0.10 (0.00)	0.51 (0.00)	1.54 (0.04)	<0.0001
OR (95%CI)				
Model 1	Ref	0.91 (0.81,1.01)	0.96 (0.84,1.09)	0.064
Model 2	Ref	0.87 (0.78,0.97)	0.8793 (0.78,0.99)	0.059
Model 3	Ref	0.89 (0.79,0.98)	0.8855 (0.78,0.99)	0.582
Flavanones				
No. events	9,922	9,943	8,854	
Mean (SE) (mg/d)	0.06 (0.00)	7.34 (0.15)	36.55 (0.50)	<0.0001
OR (95%CI)				
Model 1	Ref	0.80 (0.71,0.89)	0.73 (0.66,0.80)	<0.001
Model 2	Ref	0.80 (0.71,0.89)	0.73 (0.66,0.81)	<0.001
Model 3	Ref	0.84 (0.75,0.95)	0.7503 (0.67,0.84)	<0.001
Isoflavones				
No. events	10,870	8,570	9,279	
Mean (SE) (mg/d)	0.00 (0.00)	0.07 (0.00)	5.47 (0.25)	<0.0001
OR (95%CI)				
Model 1	Ref	1.05 (0.94,1.17)	0.69 (0.62,0.76)	<0.001

(Continued)

TABLE 2 (Continued)

No. events	Total flavonoid intake quintiles			*P* for trend
	Q1 (n = 8,937)	Q2 (n = 9,265)	Q3 (n = 10,517)	
Model 2	Ref	1.04 (0.92,1.17)	0.69 (0.62,0.76)	<0.001
Model 3	Ref	1.09 (0.96,1.23)	0.70 (0.63,0.79)	<0.001
Anthocyanidins				
No. events	9,696	9,371	9,652	
Mean (SE) (mg/d)	0.25 (0.01)	6.51 (0.08)	36.08 (0.94)	<0.0001
OR (95%CI)				
Model 1	Ref	0.81 (0.73,0.90)	0.61 (0.55,0.68)	<0.001
Model 2	Ref	0.82 (0.74,0.91)	0.62 (0.55,0.70)	<0.001
Model 3	Ref	0.84 (0.75,0.94)	0.64 (0.57,0.73)	<0.001

OR, odd ratios; CI, confidence interval. Data are presented as OR and 95% CIs. ORs obtained from average marginal predictions in the logistic analysis model. Model 1 adjusted for age and sex; Model 2 adjusted for age, sex, BMI, smoking status, physical activity, and alcohol consumption; Model 3 adjusted for all covariates in Model 2 plus intakes of energy, protein, carbohydrate, fiber, and total fat. *P*-value based on orthogonal polynomial contrasts of the adjusted prevalence estimates by category of flavonoid (total or subclass) intake.

flavonoids intake and MetS reduction. Figure 1A displays a non-linear between flavonoid intake and the risk of MetS after adjusting for age, sex, ethnicity, lifestyle, and other nutrient intakes. MetS prevalence decreased steadily following the increase in total intake of flavonoids until the total flavonoids' intake reached 237.67 mg/day. Then, MetS risk began to increase as the total flavonoids intake evaluate further. Flavanones (Figure 1B) and anthocyanidins (Figure 1D) also displayed V-shaped relationship curves. At 34.37 mg/day (flavanones) and 23.13 mg/day (anthocyanidins) points, MetS risk was the lowest, respectively. In contrast to these flavonoids, when isoflavones intake increased, MetS risk consistently dropped, and the downward trend then slightly increased (Figure 1C). In addition, there was a slight difference between men and women in the dose–response relationship of total flavonoids intake and flavanones intake. As shown in Supplementary Figure 1, the change point of total flavonoids intake was 222 and 237 mg/day for women and men, respectively, and the change point of flavanones intake was 31.09 and 39.09 mg/day for women and men, respectively.

Discussion

This study first investigated the relationship between total flavonoids and their subclasses intake and the MetS risk in the USA population based on NHANES 2007–2010 and 2017–2018. The results demonstrated that higher total flavonoids intake was significantly related to a lower MetS risk, with 13 and 15% reductions in Q3 and Q2 vs. Q1, respectively. Moreover, this reverse effect of total flavonoids on MetS risk was considerably pronounced in men, younger, Mexican, obese, and CVD individuals. The flavanones, isoflavones, and anthocyanidins intake are also inversely related to MetS risk in any population. Furthermore, the dose–response effect demonstrated a U-shaped curve between the total flavonoids intake (change point at 237.67 mg/day), flavanones (change point at 34.37 mg/day), anthocyanidins (change point at 23.13 mg/day), and MetS risk.

Currently, MetS is an urgent public health problem. Previous epidemiological surveys predicted that the prevalence rate of MetS ranges from 20 to 45%. Additionally, over 85% of adults will be overweight or obese by 2030 (36). Therefore, it is critical to explore effective intervention manner for MetS. Flavonoid-enriched diet and moderate physical activity could be the most effective and less costly manner to improve MetS. A previous study investigated the relationship between flavonoids intake and MetS risk. For instance, higher dietary flavonoid intakes were negatively associated with MetS among Polish individuals (37), Iranian adults (29, 38), and Chinese urban adults (5). In this study, total flavonoids intake, flavanones, isoflavones, and anthocyanidins can also reduce the metabolic syndrome risk, even after adjusting for sex, age, and other factors, which was different from a previous study performed in the Iran population (29). Flavonoids have been associated with other health benefits, including reduced T2D and CVD risk, certain cancers, and neurodegenerative disorders. For example, the Health Professionals Follow-Up Study (1986–2006) reported that higher consumption of anthocyanins and anthocyanin-rich fruit was associated with a lower risk of T2D in the USA population (39). Total flavonoids, anthocyanidins, flavan-3-ols, and flavanones intake were also inversely associated with high CVD and atrial fibrillation (AF) risk (19, 40), whereas intakes of flavones and flavonols were not. Moreover, a high intake of flavones (OR 0.62), flavanones (0.64), and anthocyanins were associated with lower odds of subjective cognitive decline (SCD) (41).

Flavonoids are a large, diverse group of bioactive polyphenolic compounds mainly sourced from fruits, vegetables, cereals, and tea (19, 42). There are six subclasses of flavonoids: anthocyanidins (fruits, particularly berries), flavan-3-ols (tea), flavanones (citrus fruits and juices), flavones (tea, peppers, and celery), flavonols (tea, onions, and potatoes), and isoflavones (soy products) (43–45). An investigation of the main food sources of total polyphenol intake and subclasses between 2008–2009 and 2017–2018 in the Brazilian population showed that coffee was the most significant food source for hydroxycinnamic acids and phenolic acids, contributing with 59.4 and 54.1% to the daily total polyphenols intake (46).

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TABLE 3 Stratification analysis of the association between total flavonoid intake and MetS.

Covariates	Total population ($n = 28,719$)				*p* for trend	*p* for interaction
Age						0.56
≤20, *n*	7,804	ref	0.72 (0.57,0.92)	0.64 (0.50,0.82)	<0.001	
21–39, *n*	6,813	ref	0.94 (0.77,1.13)	0.87 (0.66,1.14)	0.3	
40-59, *n*	7,847	ref	0.96 (0.74,1.24)	0.93 (0.74,1.18)	0.54	
≥60, *n*	6,255	ref	0.76 (0.58,1.01)	0.84 (0.66,1.07)	0.22	
Sex						0.73
Female, *n*	14,690	ref	0.92 (0.80,1.05)	0.99 (0.87,1.13)	0.94	
Male, *n*	14,029	ref	0.86 (0.73,1.00)	0.90 (0.75,1.08)	0.27	
Ethnicity						0.19
White, *n*	18,191	ref	0.98 (0.83,1.14)	1.03 (0.88,1.21)	0.63	
Black, n	3,465	ref	0.82 (0.67,1.00)	0.91 (0.73,1.12)	0.37	
Mexican, n	2,970	ref	0.78 (0.66,0.92)	0.79 (0.66,0.95)	0.02	
Other, n	4,093	ref	0.72 (0.56,0.92)	0.77 (0.61,0.96)	0.05	
BMI (kg/m2)						0.11
BMI ≥ 25, *n*	12,677	ref	0.98 (0.83,1.15)	0.97 (0.84,1.12)	0.7	
BMI ≤ 25, *n*	16,042	ref	0.74 (0.56,0.97)	0.70 (0.55,0.91)	0.01	
Smoke						0.41
No, *n*	24,288	ref	0.90 (0.79,1.03)	0.92 (0.80,1.07)	0.32	
Yes, *n*	4,431	ref	0.90 (0.72,1.12)	1.09 (0.86,1.38)	0.43	
Alcohol drinking						
Never, *n*	6,979	ref	0.75 (0.62,0.89)	0.87 (0.69,1.10)	0.3	0.65
Former, *n*	2,257	ref	0.83 (0.63,1.09)	1.00 (0.72,1.39)	0.97	
Mild, *n*	8,587	ref	1.01 (0.76,1.33)	0.94 (0.69,1.26)	0.61	
Moderate, *n*	4,474	ref	0.93 (0.64,1.35)	1.13 (0.83,1.54)	0.35	
Heavy, *n*	6,422	ref	0.92 (0.74,1.15)	0.93 (0.72,1.18)	0.54	
Physical activity						
No, *n*	10,399	ref	0.76 (0.65,0.89)	1.16 (1.00,1.35)	0.04	<0.001
Low, *n*	5,775	ref	0.93 (0.73,1.18)	0.80 (0.62,1.03)	0.07	
Intermediate, *n*	6,304	ref	1.08 (0.86,1.35)	0.82 (0.64,1.05)	0.07	
High, *n*	6,241	ref	0.87 (0.69,1.09)	0.94 (0.71,1.24)	0.73	
Hypertension						0.08
No, *n*	20,816	ref	0.79 (0.69,0.91)	0.87 (0.74,1.04)	0.16	
Yes, *n*	7,903	ref	0.99 (0.83,1.19)	0.92 (0.77,1.09)	0.29	
Diabetic						0.28
No, *n*	23,972	ref	0.88 (0.75,1.02)	0.92 (0.80,1.06)	0.32	
Yes, *n*	4,747	ref	0.88 (0.71,1.09)	0.86 (0.72,1.04)	0.14	
CVD						0.07
No, *n*	26,855	ref	0.91 (0.80,1.03)	0.96 (0.84,1.10)	0.62	
Yes, *n*	1,864	ref	0.71 (0.54,0.94)	0.72 (0.52,0.98)	0.05	
CKD						0.002
No, *n*	20,867	ref	0.96 (0.84,1.10)	0.92 (0.80,1.05)	0.21	
Yes, *n*	7,852	ref	0.77 (0.63,0.94)	1.04 (0.84,1.29)	0.6	
COPD						0.03
No, *n*	27,645	ref	0.91 (0.80,1.02)	0.94 (0.82,1.07)	0.41	
Yes, *n*	1,074	ref	0.58 (0.38,0.89)	1.07 (0.68,1.69)	0.66	

OR, odd ratios; CI, confidence interval. Data are presented as OR and 95% CIs. ORs were obtained by the logistic analysis with adjusted intakes of energy, protein, carbohydrate, fiber, and total fat. *P*-value based on orthogonal polynomial contrasts of the adjusted prevalence estimates by category of flavonoid (total or subclass) intake. BMI, body mass index; CKD, chronic kidney disease; MET, metabolic equivalent; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; MetS, metabolic syndrome.

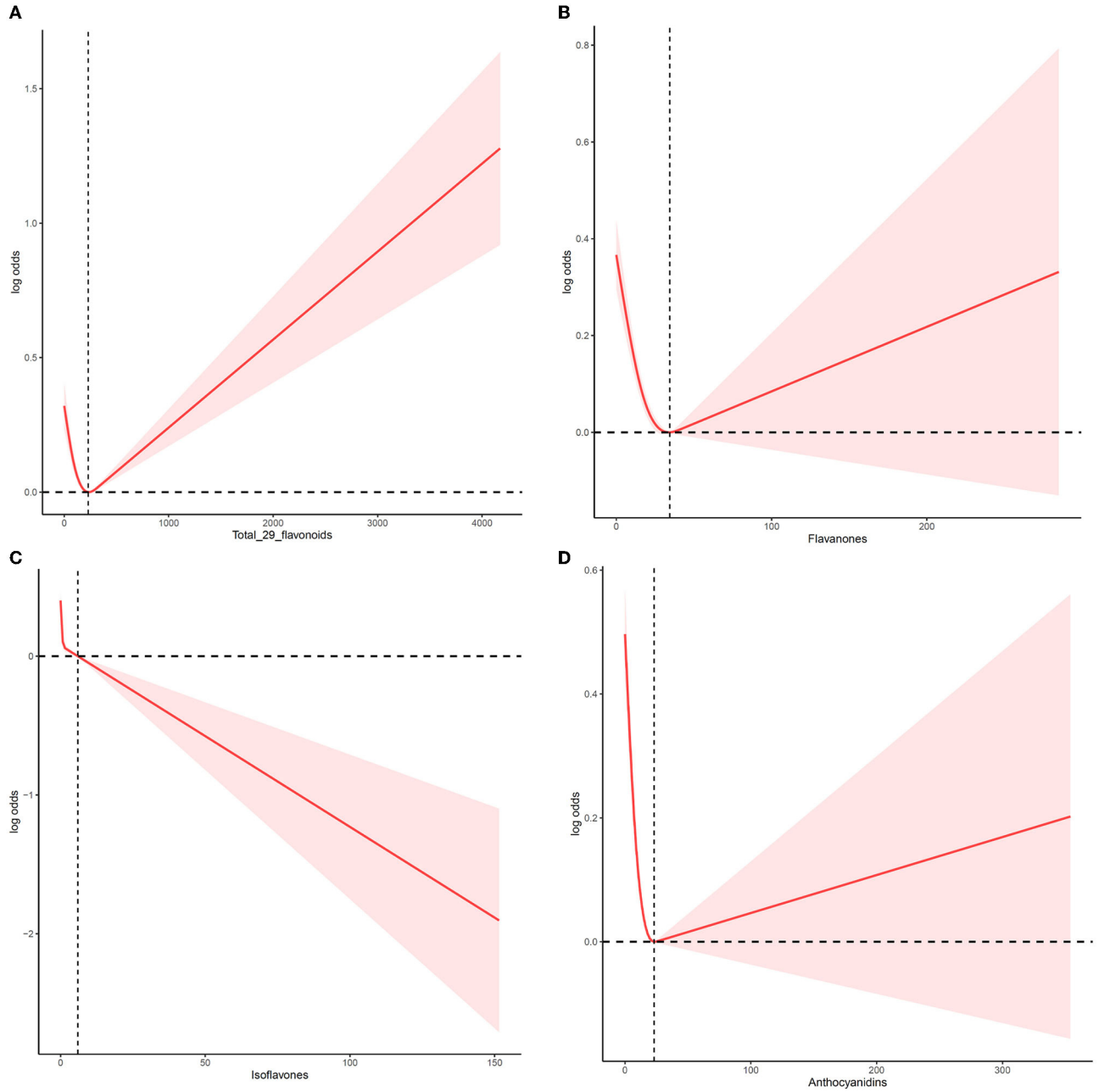

FIGURE 1
The non-linear trend between the intake of flavonoids and the risk of MetS using a restricted cubic spline. Data are presented as log (odds ratios) (y-axis) and level of flavonoids (mg/d) after adjusted for age, sex, ethnicity, drinking, smoking, physical activity, and other nutrient intake (fiber, fat, protein, and carbohydrate). **(A)** Total flavonoids, **(B)** flavanones, **(C)** isoflavones, and **(D)** anthocyanidins.

Tea, coffee, and fruits are the main sources of flavonoids. Among the population of the USA, flavan-3-ols, primarily derived from tea (94%), comprised 80% of flavonoids intake (47). It has been found that consumption of certain foods, such as blueberries, strawberries, apples, orange juice, grapefruit juice, bananas, onions, tea, and peaches, could independently predict the development of SCD in the future (41). However, future multiple-center longitudinal studies must confirm the causal relationship between each flavonoid intake and metabolic syndrome and other diseases.

Indeed, earlier research specified that flavonoids improved metabolic syndrome through their antioxidant and anti-inflammatory properties to repair endothelial function and enhance nitric oxide (NO) bioavailability (48, 49). For example, naringenin, one flavanone compound, can downregulate the levels of triglyceride (TG) and phospholipid and increase the gene expression of PPAR-α, CPT-1, and uncoupling protein (UCP)-2 to reduce blood lipid (50, 51). It could also activate the peroxisome proliferator-activated receptor (PPAR) and adiponectin expression and decrease the liver X receptors (LXR)-α

level (52, 53) to treat adiposity and atherosclerosis. Moreover, naringenin showed strong anti-inflammation activity by inhibiting NF-κB activation, the levels of myeloperoxidase (MPO), N-acetyl-β-D-glucosaminidase (NAG), TNF-α, interleukin (IL)-1β, IL-6, and IL-12 (54), and involved into the NO-cGMP-PKG-KATP signaling pathway (36). Therefore, naringenin can potentially improve MetS, which aligns with our findings that flavanones may lower the risk of MetS. Isoflavones, like genistein and puerarin, may influence insulin release and lipids metabolism by blocking adipocyte-specific proteins and controlling PPAR-γ levels (55–57). Additionally, consuming anthocyanins, such as pelargonidin, cyanidin, delphinidin, peonidin, and malvidin, can help treat the pathology of MetS and disorders linked to MetS by reducing body weight, insulin resistance, inflammation, and oxidative stress injury (36). Recent studies have found that dietary lifestyle can affect the structure of the gut microbiome and its metabolites, thereby influencing the development of MetS. By altering the host gut flora, resveratrol, for instance, could decrease body weight and body fat to improve glucose homeostasis and obesity (58). Future randomized clinical trials should be designed to confirm these potential mechanisms in multiple districts.

Although flavonoid intake effectively attenuated MetS, RCS curves showed complex non-linear relationships between flavonoids intake and MetS risk rather than monotonic increasing or decreasing relationships. Additionally, the RCS curves of flavonoids consumption showed slight differences for men and women, indicating that the prevalence of MetS and the amount of flavonoids intake varied by gender. Indeed, men had a significantly higher intake of flavanones (citrus) and flavonols (mixed dishes and beer) than women. Women had a significantly higher intake of anthocyanidins (berries) compared with men (47). Moreover, our previous study found that a diet of 7,8-dihydroxyflavone (7,8-DHF) could protect the function of the female hypothalamic–pituitary–ovarian (HPO) axis and activate tissue-specific ERα to maintain body metabolic homeostasis (59). Additionally, intake rates of flavonoids varied by geographic region, dietary preferences, sociodemographic characteristics, and lifestyle choices. Previous studies reported that the mean flavonoids intake was 34.68 mg/day in Chinese (5), whereas the mean flavonoids intake was 189.7 mg/day in 1999–2002 (60) in the U.S. population. Future large-scale and multi-center clinical trials should be conducted to establish safe doses and create an individual's healthcare program for the potential health implications of attuning the risk of MetS.

The strength of this study was that it was the first large sample, population-based, cross-sectional study that reported the effect of flavonoid intake and its subclasses on MetS based on NHANES from 2007–2010 to 2017–2018. The effects of total flavonoids, flavanones, isoflavones, and anthocyanidins consumption on MetS were evaluated, providing diet recommendations for people in the USA. However, this study has several limitations. First, this study is a cross-sectional investigation that could only present relationships between flavonoids and MetS. Second, flavonoid data was obtained by the 24-h recall, whereas MetS might have already occurred before the interview. Therefore, a bias in the effects of flavonoids intake on MetS is unavoidable. Third, the participants included in this study were all Americans. Consequently, the effects of flavonoids intake on MetS observed in this study may be unsuitable for Asians or other populations.

Conclusion

Higher flavonoid intakes could reduce MetS risk in the USA population. In addition, flavanones, isoflavones, and anthocyanidins are the most effective flavonoid subclasses in attenuating MetS, while other flavonoid subclasses showed a relatively small effect. Total flavonoids, flavanones, isoflavones, and anthocyanidins demonstrated non-linear relationships with MetS risk. The most effective doses of total flavonoids, flavanones, and anthocyanidins were 237.67, 34.37, and 23.13 mg/day, respectively. Further large-scale randomized controlled trials should be performed to establish causality between flavonoids intake and MetS risk and the safe doses of flavonoids in different populations. Regarding the perspective of public health, our findings may provide fresh insight into MetS risk based on flavonoids intake and build future tailored dietary recommendations as a preventative tool against metabolic syndrome based on the most effectively calculated amounts.

Data availability statement

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

Author contributions

ZZ and WX: conceptualization and writing—review and editing. WG and XD: methodology. WG and CX: software. GM and CX: validation. ZZ: formal analysis. XX: data curation. GM and WX: writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

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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.2023.1195107/full#supplementary-material

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Endothelial dysfunction, platelet hyperactivity, hypertension, and the metabolic syndrome: molecular insights and combating strategies

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Metabolic syndrome (MetS) is a multifaceted condition that increases the possibility of developing atherosclerotic cardiovascular disease. MetS includes obesity, hypertension, dyslipidemia, hyperglycemia, endothelial dysfunction, and platelet hyperactivity. There is a concerning rise in the occurrence and frequency of MetS globally. The rising incidence and severity of MetS need a proactive, multipronged strategy for identifying and treating those affected. For many MetS patients, achieving recommended goals for healthy fat intake, blood pressure control, and blood glucose management may require a combination of medicine therapy, lifestyles, nutraceuticals, and others. However, it is essential to note that lifestyle modification should be the first-line therapy for MetS. In addition, MetS requires pharmacological, nutraceutical, or other interventions. This review aimed to bring together the etiology, molecular mechanisms, and dietary strategies to combat hypertension, endothelial dysfunction, and platelet dysfunction in individuals with MetS.

KEYWORDS

metabolic syndrome, atherosclerotic cardiovascular disease, hypertension, endothelial dysfunction, platelet hyperactivity

1. Introduction

Metabolic syndrome (MetS) is a medical condition characterized by a combination of metabolic abnormalities, including insulin resistance, hyperglycemia, hyperlipidemia, hypertension, and obesity. This condition has a negative impact on the vascular wall due to events involving endothelial dysfunction, platelet hyperactivity, oxidative stress, and low-grade inflammation. The activation of these events leads to increased vasoconstriction and atherosclerosis, ultimately promoting a pro-thrombotic state (1). Clinical studies have shown that endothelial dysfunction, hyperlipidemia, oxidative stress, and platelet hyperactivity are important factors in the development of atherosclerotic vascular problems (2). There is still a need for further improvement in our understanding of the molecular mechanisms underlying endothelial dysfunction, platelet hyperactivity, high blood pressure, and vascular damage. The challenge of comprehending the role of the vascular endothelium in the development of hypertension persists. A well-functioning endothelium is responsible for producing vasodilators

that play a crucial role in maintaining healthy blood vessel function and vascularity. Endothelial dysfunction is a condition that is marked by a decrease in endothelial-dependent vasodilation and an increase in endothelial inflammatory activity (3). Endothelial dysfunction increases the likelihood of the blood vessels being in a more contracted state. This is caused by an imbalance between factors that relax the endothelium, such as nitric oxide (NO), prostacyclin (PGI_2), and down regulation of endothelioum-dependent hyperpolarization (EDH) and factors that cause contraction such as thromboxane A_2 (TxA_2) and upregulation of endothelin-1 (ET-1). Endothelial dysfunction promotes pro-inflammatory and oxidative stress pathways through the generation of reactive oxygen species (ROS) in endothelial mitochondria. This, in turn, drives vascular growth and remodeling. The endothelium undergoes a significant transformation in metabolic syndrome, shifting to a dysfunctional state. This transformation involves the host immune system and the production of reactive oxygen species (ROS). The progression of diseases associated with metabolic syndrome occurs through a range of dynamic changes within the vasculature (4). Although there is a general consensus that endothelial dysfunction is a reliable indicator of the advancement of atherosclerosis and potential cardiovascular events, additional research is needed to fully understand its impact on hypertension and platelet hyperactivity. The components of MetS, whether alone or in combination, are significant risk factors for the development of cardiovascular diseases (CVDs). As a result, the implications for future healthcare costs and management are both relevant and challenging. Although underlying metabolic or genetic predispositions are essential, the condition typically affects individuals who consume high-caloric foods with sedentary lifestyles (5); modifying lifestyles, such as physical exercise and diet, can address many causative factors contributing to MetS. Diet modification represents an essential strategy for MetS prevention, as the increasing evidence indicates a robust inverse association between CVD risks, MetS, and the consumption of plant-based foods and their bioactive compounds.

This review highlights the prevalence, molecular mechanisms and combating approaches of underlying risk conditions by managing endothelial dysfunction, hypertension, and platelet hyperactivity/prothrombotic state with bioactive compounds like polyphenols, small molecules like miRNA, and gene therapy. The study also highlights the potential effects of lifestyle measures and pharmacological interventions on endothelial function in patients diagnosed with MetS.

1.1. Prevalence of MetS

MetS has a varying prevalence across the globe and is frequently associated with central obesity, diabetes, and CVD. Prevalence rates differ significantly based on age, gender, racial or ethnic background, and specific diagnostic criteria. MetS affects around 25% of the European population and at least 20% of the American people. MetS is less prevalent in South and Southeast Asia. However, its incidence has gradually increased and is expected to reach the levels observed in Western countries. With a 95% confidence range of 28 to 33%, 30% of Indian adults have MetS. According to research, one-third of people in low- and middle-income nations in the Middle East, Southeast Asia, and Latin America showed MetS symptoms (6). According to the study, India's rapid economic development and urbanization may explain its rising MetS rate, and it may affect lifestyle and nutrition (7). According to study, MetS was more common in urban (32%; 95% CI: 29–36%) than tribal (28%; 21–36%) settings and it may contribute to urban MetS due to unfavorable lifestyle choices, higher socioeconomic class, lesser physical exercise, increased stress, and excessive salt and red meat consumption (8). MetS affects both genders and ethnic groups differently. The prevalence of MS was higher in females (35%; 95% CI: 31–38%) than in males (26%; 95% CI: 22–29%). Similar discoveries have also been made in the Eastern Mediterranean, South East Asia, and Western Pacific regions (9). This conclusion is attributed to factors such as menopause, polycystic ovarian syndrome, and the use of hormonal contraceptives (10). Additional factors contribute to the differences between men and women, such as the increased risk women face due to certain characteristics. These characteristics include higher body weight, larger waist circumference, and lower HDL cholesterol levels (11). MetS is more prevalent in Hispanic women by 26% compared to Hispanic men and in African-American women by 57% compared to African-American men. Insulin resistance is a predominant symptom of MetS among Hispanics, while African-Americans are more likely to experience hypertension, and dyslipidemia is more common among Whites (5). Miller and colleagues conducted a more recent analysis of the same database and discovered that 10.1% of adolescents in the United States had MetS (12). They demonstrated that MetS was more prevalent among men and Hispanics than women. The incidence of MetS rises significantly in tandem with an individual's Body Mass Index (BMI). The prevalence of MetS is 9.8% among men in urban North India while 42.0% among women in urban Iran (13). The growth is evident irrespective of the parameters considered, and it indicates a shift from a conventional, older lifestyle to a contemporary, Western one. As developing countries grew economically more robust, they experienced a demographic transition. This transition led to a decrease in the prevalence of infectious diseases and an increase in the majority of lifestyle-related diseases. During this period, there was a shift in the prevalent types of conditions. Infectious diseases became less common, while lifestyle-related diseases became more prevalent. This change occurred alongside what is known as an epidemiological transition. Disruption of these processes can result in increased BMI, generalized and abdominal obesity, hypertension, dyslipidemia, type 2 diabetes, and CVD (13).

1.2. Etiology of MetS

Despite decades of investigation, the precise cause of MetS remains unclear. A poor diet, insufficient exercise, and the consequent weight gain are the primary causes of the condition in most instances (14). Other contributing variables include endothelial dysfunction, elevated blood pressure, and hyperactive platelets. As aforementioned, the main objective behind establishing the MetS was to recognize characteristics linked with a high susceptibility to CVD (15). The term "syndrome" suggests that the underlying cause of MetS is not readily identifiable, and it was not initially expected that a singular, comprehensive pathological etiology would exist for MetS (16). MetS is closely concomitant to a "Westernized lifestyle" that includes

sedentary behavior and easy access to high-fat meals. Obesity in children is a risk factor for adult MetS (17). In addition, many features of MetS, including its potential significance, are more widespread among low-income people. Even though not everyone with MetS has them, hereditary variables for both the syndrome's components and body fat and muscle are now well established (18). Approximately 30–40% of the reported variation in body mass index (BMI) and 70–80% in fat distribution is attributable to hereditary factors. In 2007, the first single nucleotide polymorphism (SNP) linking high BMI to the fat mass and obesity-related (FTO) gene was found (19). The FTO gene affects mood and metabolism, potentially contributing to obesity. Since then, researchers have used SNPs to identify about 40 genetic variants connected to body mass index, fat distribution, obesity risk, and MetS (20). Although common allelic alterations only account for 2% of the variation in obesity, those with a higher number of risk alleles (>10) acquire more body weight than those with a smaller number (21). Recent evidence suggests that environmental factors must interact with obesity's genetic basis. Lifestyle variables that increase intra-abdominal fat and metabolic risk include being overweight, eating high-fat, smoking, physically inactive, and drinking excessively (22).

1.3. Pathophysiology of MetS

The MetS has a harmful influence on many bodily systems. Endothelial dysfunction, vascular resistance, hypertension, and arterial wall inflammation are more likely to occur in patients with pre-existing microvascular damage, such as that caused by insulin resistance (23). Endothelial dysfunction can disrupt homeostasis, increasing the risk of atherosclerotic disease and hypertension. Renal impairment, elevated vascular resistance and stiffness, and structural cardiac disorders like left ventricular hypertrophy and stroke are all brought on by the adverse effects of hypertension on the body's normal functioning (24).

Ischemic heart disease is a problem that may develop as a result of the cumulative effects of MetS, such as endothelial dysfunction and hypertension. Endothelial dysfunction caused by elevated plasminogen activator type 1 and adipokines may lead to thrombosis. For instance, coronary artery disease is a possible outcome of hypertension and other conditions related to MetS. Hypertension can lead to coronary artery disease (CAD) due to increased vascular resistance. Dyslipidemia can cause symptomatic ischemic heart disease in some patients and is associated with MetS. This disorder has the potential to accelerate the advancement of atherosclerosis (25).

1.4. Clinical-based definition of MetS

Although MetS has several components and clinical implications, there is currently no universally accepted pathogenic mechanism or firmly established diagnostic criteria for this condition. Furthermore, it is unclear whether this entity represents a distinct medical condition or acts as a substitute for a combination of risk factors that contribute to an individual's vulnerability to a particular ailment. The prevalence of MetS is on the rise among children and young adults. The emerging aspect of the disease is significant because it will have future consequences for the global health burden (26). The Adult Treatment Panel III report (ATP III) from the National Cholesterol Education Programme highlighted the significance of MetS as a multifaceted risk factor for CVD (27). The report indicated that this condition necessitates further therapeutic attention. MetS can be diagnosed using the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) definition. The diagnostic criteria for MetS consist of five parameters. These parameters include a waist circumference that exceeds 40 inches in men or 35 inches in women, blood pressure that surpasses 130/85 mmHg, fasting triglyceride levels that exceed 150 mg/dL, fasting high-density lipoprotein (HDL) cholesterol levels below 40 mg/dL in men or 50 mg/dL in women, and fasting blood sugar levels that exceed 100 mg/dL (28).

1.4.1. Insulin resistance

Insulin resistance plays a crucial role in developing and advancing CVD. It is a MetS component and is commonly linked to obesity and dyslipidemia. In addition, it has been linked to chronic low-grade inflammation. Insulin resistance is linked to several negative consequences, such as endothelial dysfunction, reduced cardiac diastolic relaxation, impaired vascular relaxation, decreased coronary blood flow, and increased susceptibility to ischemia.

The pathophysiology of MetS and its components, including elevated triglycerides, blood pressure, glucose, waist circumference, and low high-density lipoprotein (HDL) cholesterol, are closely linked to insulin resistance and abdominal obesity. MetS Consequently, this section aims to explore how insulin resistance and abdominal obesity may contribute to the pathophysiology of MetS (29). To explain the pathophysiology of MetS, the concept of insulin resistance has emerged as the most popular and unifying framework. The definition of insulin resistance has always centered on glucose (30). Lack of insulin action leading to fasting hyperinsulinemia to maintain euglycemia indicates insulin resistance. However, postprandial hyperinsulinemia may exist before fasting hyperinsulinemia develops (31). Insulin influences various biological activities, including amino acid absorption, protein synthesis and degradation, and triglyceride breakdown in adipose tissue.

Furthermore, it impacts the functioning of lipoprotein lipase and the release of VLDL cholesterol. The hormone insulin plays a crucial role in promoting the uptake of glucose in adipose and muscle tissue, as well as in synthesizing glycogen in muscle and secreting triglycerides (32). Therefore, an individual's insulin sensitivity or resistance is frequently assessed by evaluating their reaction to glucose or insulin stimulation, whether administered orally or intravenously (33). One of the key reasons contributing to the development of insulin resistance is the presence of an excess of circulating fatty acids.

As illustrated in Figure 1, MetS is associated with an elevation in oxidative stress, which is the underlying cause of various complications such as atherosclerosis, hypertension, myocardial infarction, and inflammation. Elevated levels of oxidative stress have been identified as a detrimental factor contributing to insulin resistance development. This phenomenon occurs due to an imbalance between the body's antioxidant defence mechanisms and the production of reactive oxygen species (ROS). β-cells that have low levels of antioxidants are especially susceptible to chronic oxidative stress. This stress can cause a decrease in the production of inflammatory cytokines like TNF-α and interleukins (such as IL-6, IL-10, and IL-1β), ultimately leading to the death of these cells.

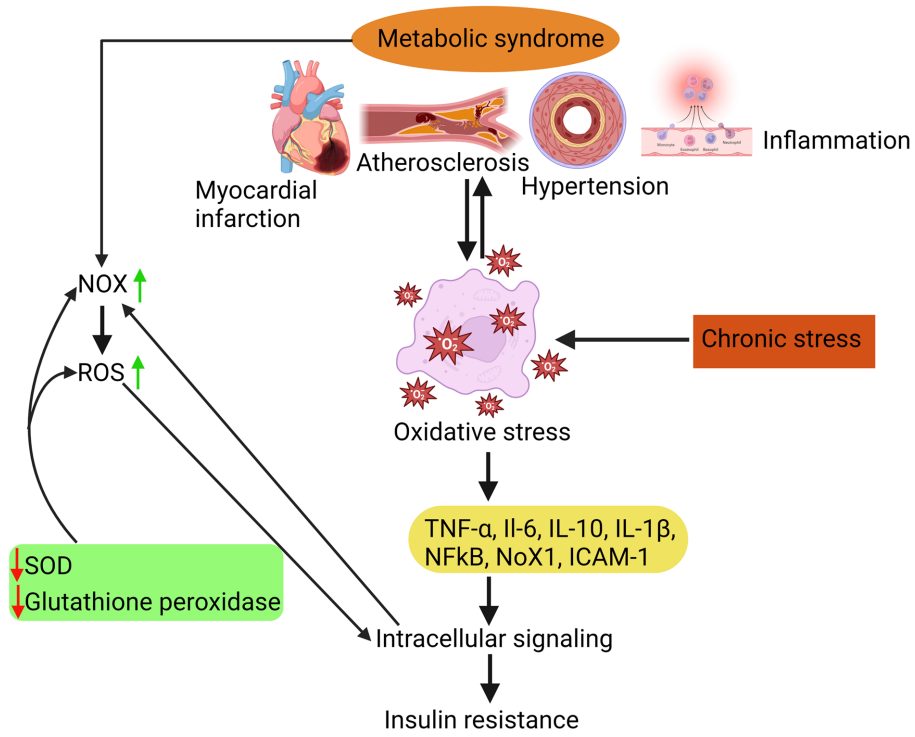


FIGURE 1
Insulin resistance due to oxidative stress is attributed to a disparity between the body's antioxidant system and the generation of ROS, which in turn triggers inflammatory responses, atherosclerosis, hypertension, and myocardial infarction.

1.5. Risk factors of MetS

MetS is a group of risk factors linked to developing atherosclerotic CVD and type 2 diabetes mellitus. Metabolic risk factors are the leading cause of noncommunicable diseases (NCDs) (34). Additionally, MetS refers to a group of metabolic anomalies linked to visceral fat accumulation. The disorders above encompass insulin resistance, hypertension, dyslipidemia (characterized by low levels of high-density lipoprotein cholesterol and hypertriglyceridemia), and central obesity (35), as shown in Figure 2. The primary causes of these metabolic risk factors include a poor diet, inactivity, an unhealthy lifestyle, smoking, alcohol consumption, etc. Hypertension, obesity, hyperglycemia, and hyperlipidemia are metabolic risk factors that increase NCD susceptibility (36).

1.6. Rationale

MetS is associated with insulin resistance, abdominal obesity, hyperlipidemia, hypertension, platelet hyperactivity, and other CVD risk factors. Individuals with MetS may require pharmacological and nutraceutical modifications to alleviate the unfavorable effects that lead to endothelial dysfunction, platelet hyperactivity, and hypertension. The proper functioning of the endothelium is crucial for maintaining homeostasis, and any disturbance in its function can lead to atherosclerosis, hypertension, and diabetes. Endothelium dysfunction is characterized by impaired endothelial cell-dependent vasodilation, inflammation, apoptosis, and cell proliferation. It is essential to comprehend and address endothelial dysfunction to prevent vascular complications.

2. MetS and cardiovascular disease

Each component of the MetS has been documented as a risk factor for CVDs. Moreover, the cumulative effect of multiple risk factors goes beyond simply adding up individual risks, further impacting the overall risk (37). In recent research, it has been found that MetS, which was defined by the World Health Organisation (WHO) in 1999, is strongly associated with an increased risk of coronary heart disease (CHD). These individuals were at a higher risk of experiencing myocardial infarction and cerebrovascular disease. The disorder was also found to be associated with a significant increase in cardiovascular mortality (38). Having diabetes significantly increases the likelihood of developing CVD in individuals with MetS. Many individuals with type 2 diabetes, particularly those who also have other risk factors for cardiovascular disease, face a substantial risk of experiencing severe cardiovascular events (39). In a study involving a large group of individuals with type 2 diabetes, insulin resistance (IR) was found to be a significant factor in predicting both the prevalence of CVD at the beginning of the study and the occurrence of new cases of CVD during the follow-up period. There was no evidence to suggest a direct relationship between smoking, variables related to insulin resistance (IR), such as body mass index (BMI), and the subject in question. The researchers utilized data from the Kuopio Ischaemic Heart Disease Risk Factor study to examine the relationship between MetS and cardiovascular mortality (40). Even in the absence of CVD and

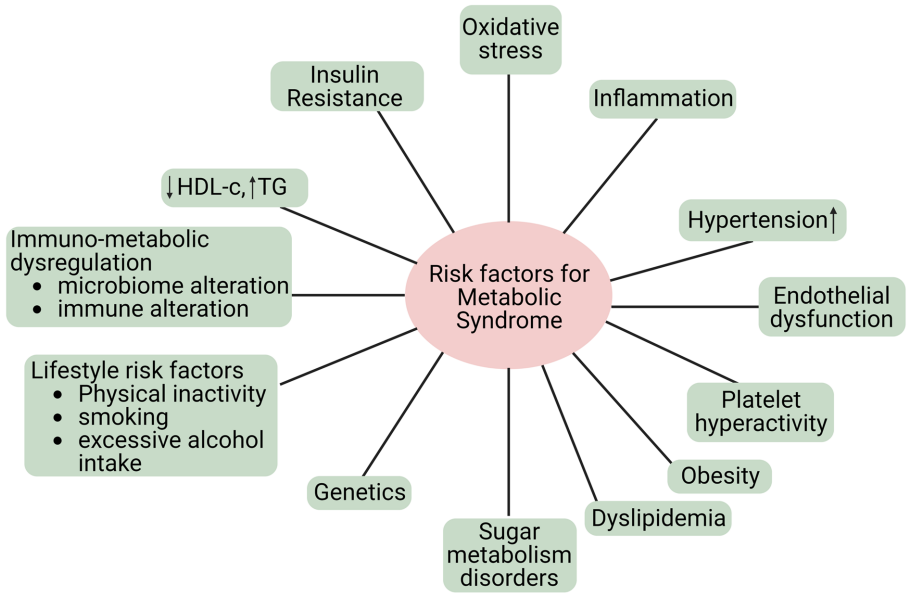


FIGURE 2
Risk factors of MetS.

diabetes at the beginning of the study, MetS was found to be linked to an increased risk of cardiovascular disease and cardiovascular mortality in males. Among individuals with MetS, the relative risks for mortality due to CHD and CVD were approximately four and three and a half, respectively (41).

3. MetS and diabetes

The worldwide prevalence of MetS and type 2 diabetes is increasing. Diabetes and MetS are associated with numerous health problems, such as hypertension, stroke, breast, prostate, and colon cancers, limb amputation, blindness, and renal and gallstone disease (42). Consequentially, the burden of chronic diseases on healthcare systems has been burdensome for Western nations and may be fatal for countries with limited resources (43). There is currently no cure for diabetes. Consequently, primary prevention via dietary and lifestyle modifications is crucial (44).

4. Management of MetS risk factors

The metabolic risk variables in the syndrome's definition include atherogenic dyslipidemia, elevated blood pressure, high plasma glucose, a prothrombotic condition, and a pro-inflammatory state (45). If the underlying risk factors can be adequately addressed, the severity of all metabolic risk factors will be reduced. The most successful treatment for those with MetS should focus on promoting modest weight reduction and regular involvement in physically active leisure activities (46). The therapy must target the multi-pathological process involved with MetS, with each disorder component being recognized and aggressively treated (47). If lifestyle changes are insufficient, a multimodal treatment strategy will be needed to achieve the requisite blood pressure, lipid profile, and blood glucose control targets. In addition, clinical risk factors such as dyslipidemia, hypertension, and hyperglycemia should be addressed more aggressively. According to the professional agreement, appropriate LDL cholesterol or blood pressure objectives have yet to be determined in treating MetS (48).

Reduction in blood pressure has been demonstrated to reduce the risk of severe CVD in several clinical studies, including those involving people with type-2 diabetes (49). However, the most effective drug has yet to be fully resolved (50). Some studies have shown that angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers are better first-line therapy for patients with MetS, especially when type-2 diabetes is present.

A prothrombotic state is indicated by elevated levels of fibrinogen, plasminogen activator inhibitor-1, and other coagulation components. Low-dose aspirin or other antiplatelet drugs are available to diabetic patients as a potential therapeutic option for managing the increased risk of arterial thrombosis (51). It is generally recommended that individuals with CVD take these medications, unless otherwise specified. Although clinical trials have not demonstrated the effectiveness of these drugs in individuals with type-2 diabetes who do not have CVD, they are still widely recommended (52). Furthermore, aspirin prophylaxis is a viable treatment option for individuals diagnosed with MetS who are at a relatively high risk of experiencing CVD-related events (53).

5. Role of MetS on endothelial dysfunction

The role of endothelial dysfunction in the development of atherosclerosis is significant. Prospective studies have indicated that endothelial dysfunction is a predictive factor for the occurrence of vascular events, regardless of the presence or absence of preexisting vascular disease. The involvement of endothelial dysfunction in

developing T2DM has been observed. Due to the detrimental effects of all constituent elements of MetS on the endothelium, it is plausible that endothelial dysfunction is more prevalent among individuals with MetS. This dysfunction, in turn, may play a role in the increased vulnerability to vascular disease and T2DM observed within this particular cohort. There is ample documentation indicating that endothelial dysfunction, which is a response to cardiovascular risk factors, typically precedes the development of atherosclerosis (54). The treatment of cardiovascular risk factors leads to an improvement in endothelial function. Its ability to independently predict cardiac events is highly valuable. In addition to the elevated blood pressure readings obtained from the sphygmomanometer, patients with hypertension often experience other issues such as endothelial dysfunction, increased peripheral vascular resistance, and arterial stiffness. These problems can be detected in patients with hypertension. Metabolic syndrome is a condition characterized by the presence of abdominal obesity, insulin resistance, hypertension, dyslipidemia, and glucose intolerance. It is often considered a prediabetic condition. Metabolic syndrome frequently involves endothelial dysfunction (55). A research study discovered that within a sample of 100 male and female volunteers who were not diagnosed with diabetes and had no prior history of cardiovascular disease, individuals who met the ATP-III criteria for MetS exhibited a higher degree of endothelial dysfunction than those who did not. This finding is particularly significant because it was observed in individuals within an age range that is typically not associated with vascular disease. The level of endothelial dysfunction was strongly associated with the amount of metabolic components of the ATP-III criterion present in each participant. In a multivariate logistic regression model, it was found that the presence or absence of MS was the only independent predictor of endothelial dysfunction (55).The occurrence of endothelial dysfunction represents an initial pathogenic occurrence in the development of MetS. Increased cardiovascular morbidity and death are associated with this impairment (56). Endothelial coronary, peripheral, or cerebral vasculature dysfunction predicts vascular events. Endothelial dysfunction is linked to the pathophysiology of CVDs (57). The state of endothelial dysfunction is distinguished by a transition toward a pro-inflammatory state to diminished vasodilation and prothrombotic requirements, as well as a reduced ability to endure oxidative stress. This change may also be seen as a condition where thrombotic events are more prone to occur. These events create vascular inflammation, partly mediated by activated mononuclear cells' ROS (58).

5.1. Endothelial dysfunction

Vascular homeostasis relies on the endothelium, a monocellular lining that coats the inside arteries and separates the interstitial fluid from the blood (59). Nitric oxide (NO), natriuretic peptide type C (NPyC), and eicosanoids are all paracrine chemicals usually secreted by the endothelium. The variables mentioned above impact the proportion of vasodilation to vasoconstriction, the movement and growth of smooth muscle cells, the hindrance and encouragement of platelet adhesion and aggregation, and thrombogenesis and fibrinolysis (60). Endothelial dysfunction is a significant risk factor for CVD and a precursor to atherosclerosis (61). Diabetes, peripheral vascular disease, stroke, and heart diseases (62) are only some of the vascular and metabolic disorders associated with oxidative stress and endothelial dysfunction in humans.

5.1.1. Prevalence, prediction, and geographical status of endothelial dysfunction

MetS affects a significant portion of the population in different world regions. In the United States, it affects approximately 34.6% of the people, while in Europe, the prevalence ranges from 17.8 to 34.0%. In Asia, the prevalence ranges from 12.8 to 41.1% (63). The occurrence of endothelial dysfunction differed in different studies, ranging from 33 to 58%. The measurement of endothelial activation involves monitoring the levels of certain adhesion molecules in the blood, such as soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and E-selectin. Additionally, serum levels of von Willebrand factor (vWF), tissue plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1) are also monitored. Furthermore, the presence of circulating mature endothelial cells, endothelial progenitor cells, and endothelial microparticles is taken into account (64). However, it is important to note that not all of these indicators are exclusive to endothelium dysfunction. Levels of adhesion molecules in the blood increase during inflammatory situations. Additionally, levels of PAI-1 are associated with insulin resistance, a significant characteristic of MetS (65). It has been suggested that microalbuminuria is an indicator of generalized endothelial dysfunction (66). Indicators of endothelial dysfunction, such as sICAM-1, tPA antigen, and PAI-1 levels and activity, are higher in individuals with Metabolic Syndrome (MetS) (67). Additionally, research has shown that as the number of components of Metabolic Syndrome (MetS) increases, there is a corresponding increase in both plasma PAI-1 levels and activity. Patients with MetS also have higher levels of vasoconstrictor endothelin-1 in their blood (68).The prevalence of microalbuminuria is higher in patients with Metabolic Syndrome (MetS) (69). Additionally, the occurrence of microalbuminuria increases as the number of MetS components rises. The multivariate analysis found that the significant predictors of ED were the presence of CAD, diabetes, cigarette smoking, and the overall number of CVD risk variables (70). According to (71) ED was found in 67% of individuals with three CVD risk factors but did not have coronary artery disease (CAD). Among 137 patients with CAD, 44% had a notable endothelial dysfunction characterized by vasoconstriction greater than 20% (71). According to a study, even individuals with very low cardiovascular risk, such as healthy men and women, have been observed to experience endothelial dysfunction. Passing specific percentiles in males aged 50–55 and females aged 70–75 indicate an increased risk of CVD. Men appear to have a higher risk compared to women (72).

5.2. Oxidative stress and endothelial dysfunction

Many disorders, such as hypertension, atherosclerosis, dyslipidemia, diabetes mellitus, CVD, renal failure, and ischemia–reperfusion damage, are primarily caused by endothelial dysfunction and oxidative stress. Several clinical circumstances involve the oxidation of tetrahydrobiopterin (BH4) and mitochondrial electron transport, as well as the inactivation of nicotinamide-adenine dinucleotide phosphate

(NADPH) oxidase, xanthine oxidase (XO), cyclooxygenase, and uncoupled endothelial nitric oxide synthase (eNOS) (73). An imbalance that results in reduced NO production or increased ROS creation promotes endothelial dysfunction. Remodeling, platelet aggregation, vasodilation loss, inflammation, and smooth muscle cell proliferation may occur from this imbalance (74). Endothelial dysfunction is related to oxidative stress in cardiovascular disorders such as CAD and stroke (75). Renovascular hypertension, for example, is produced by constriction of the blood vessels, which activates the renin-angiotensin system and raises the synthesis of angiotensin II (Ang II), the system's main active peptide, thus increasing ROS (76).

5.3. Epigenetics in endothelial dysfunction

The importance of epigenetics in developing endothelial dysfunction and CVD is becoming more evident. It plays a crucial role in regulating various aspects of these conditions, from their underlying mechanisms to potential treatments. An epigenetic study was conducted on CVD due to its significance in inflammation and vascular involvement. The study revealed several alterations that affect the course of CVD. Furthermore, epigenetics play a role in influencing risk factors for CVD, including but not limited to age, hypertension, smoking, excessive alcohol consumption, and diabetes. Epigenetic modifications refer to all heritable changes in gene regulation that do not involve alterations to the DNA sequence (77). The principal epigenetic mechanisms observed in human cells encompass DNA methylation, posttranslational modifications of histones, and the involvement of non-coding RNA molecules, including microRNAs and long non-coding RNAs. Evidence suggests that epigenetic mechanisms, including DNA methylation, histone modification, and microRNAs, impact the endothelium's ability to respond to blood flow (78). There is mounting evidence that epigenetic processes such as NOS3 (eNOS) play a significant role in regulating crucial endothelial cell genes and are responsive to a wide range of intrinsic and environmental stimuli, including those involved in the etiology of CVD. The role of non-coding RNAs in histone protein modification and regulation in endothelial cell homeostasis and dysfunction is well established (79).

5.4. Molecular mechanisms

Two major pathophysiological causes of many diseases are endothelial dysfunction and oxidative stress. The endothelium also generates a variety of vasodilators and vasoconstrictors, including thromboxane A_2, NO, endothelin, and Ang II (80). Endothelial dysfunction is distinguished by reduced NO bioavailability and reduced vasodilation connected to a proinflammatory and prothrombotic condition. This results in a mild and reversible disorder in the endothelium that affects the whole body. The endothelium also generates a variety of vasodilators and vasoconstrictors, including thromboxane A2, endothelin, and Ang II (80). The condition of endothelial dysfunction is marked by impaired vasodilation and a state of prothrombotic and proinflammatory activity. According to the literature, endothelial dysfunction impacts physiological processes such as LDL oxidation, vascular smooth muscle cell proliferation and migration, cell permeability, leukocyte adhesion, and platelet activation (81). In addition, more mitochondria-produced ROS accumulate when inflammation causes oxidative stress. Oxidation of macromolecules is triggered by excess ROS, which in turn triggers cell death by releasing cytochrome c (Cyt-c) (82). In addition to promoting leukocyte adherence and altering endothelial signal transmission and redox-regulated transcription factors, oxidative stress also raises vascular permeability. Recent research has linked endothelial dysfunction to dysregulation of non-coding RNAs (miRNAs, lncRNA) and alterations in gene regulatory networks (82).

Endothelial dysfunction, a recognized phenomenon, exerts an influence on the permeability of the endothelial barrier, a pivotal component involved in the inflammatory response that plays a contributory role in the initiation of CVD. Blood arteries are composed of various components, including connective tissue, endothelial cells, fibroblasts, and vascular smooth muscle cells (VSMCs). The endothelium, a semipermeable layer, serves as a barrier between the bloodstream and the innermost lining of blood vessels. As per Rahimi's (83) findings, it has been observed that the endothelium possesses tightly specialized cell-to-cell junctions, which function as a selective barrier to impede the passage of macromolecules. Endothelial cells that have been stimulated and are in an activated state have the ability to generate various signalling molecules such as cytokines, chemokines, and growth factors. These molecules play a crucial role in enhancing the processes of endothelial cell proliferation, migration, and permeability (84). In accordance with the findings of Sun et al. (85), the introduction of endothelial cells with an inflammatory phenotype into the bloodstream's arterial vessels results in the initiation of an inflammatory response, thereby contributing to the advancement of CVD. Based on the proposed theory, it is postulated that the primary etiological factor underlying hypertension, ageing, atherosclerosis, stroke, venous thrombosis, obesity, heart disease, diabetes, and intimal hyperplasia is the inflammatory response exhibited by endothelial cells (85). The loss of nitric oxide (NO) bioactivity is a common characteristic observed in the majority of individuals with cardiovascular risk factors. Consequently, this phenomenon holds significant therapeutic relevance (86). There is a discernible correlation between MetS, endothelial dysfunction, and CVD. While the significance of endothelial dysfunction in the onset of atherosclerosis is widely recognized, its specific contribution to the pathogenesis of CVD has yet to be definitively determined (Figure 3).

In type 2 diabetes patients, elevated blood sugar levels cause the vascular endothelium to become glycosylated. It modifies the blood vessels by making them narrower and more susceptible to rupture due to sprouting neovasculature (87). This leads to endothelial dysfunction from augmented ROS production, inflammation, and eNOS deletion (88). As a result, type 2 diabetes patients exhibit an increased susceptibility to CVD, with a two to four times greater risk than non-diabetic counterparts.

5.5. Endothelial dysfunction and diabetes

The presence of endothelial dysfunction has been observed to be closely linked with the occurrence of type 2 diabetes in human subjects. Endothelial dysfunction has been found to exhibit associations with various disorders related to diabetes, such as obesity, sedentary lifestyle, and MetS (89). Diabetes is characterized by elevated concentrations of endothelium-derived adhesion molecules, specifically ICAM-1, ICAM-2, VCAM-1, and PECAM-1, which are

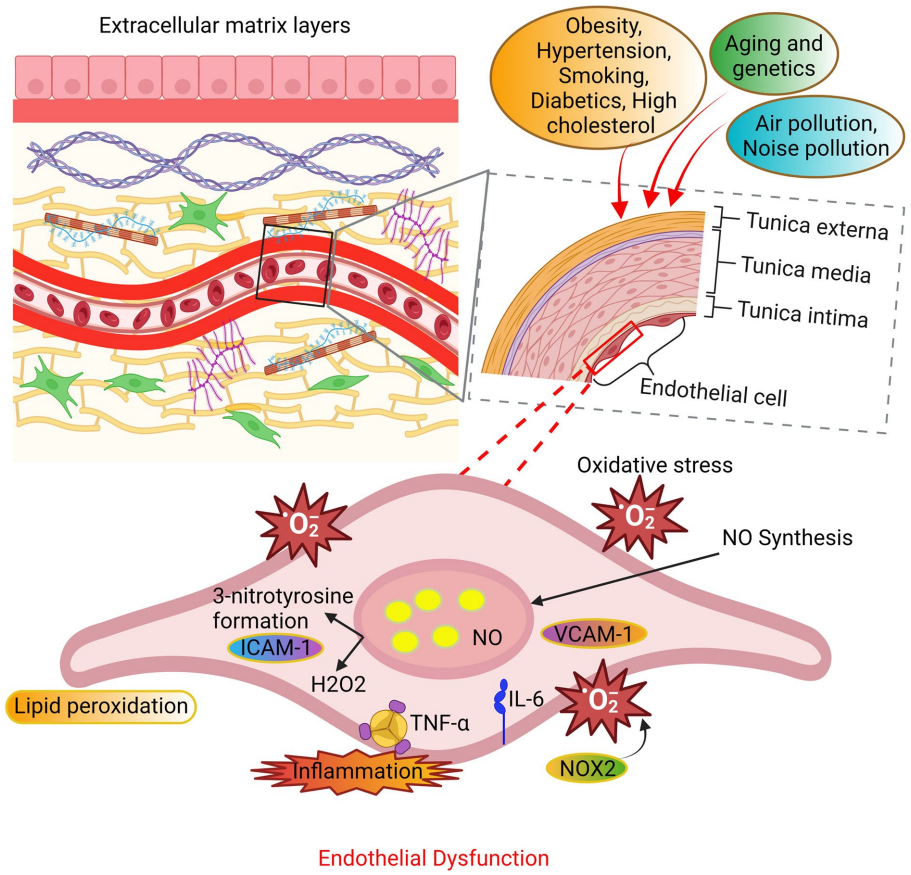


FIGURE 3
The mechanisms underlying endothelial dysfunction. The endothelial cells undergo oxidative stress due to alterations in their extracellular matrix layers caused by various factors such as aging, genetic predisposition, and environmental variables. The principal sources of ROS are NADPH oxidase and uncoupled nitric oxide synthase (NOS). The occurrence of endothelial function is attributed to an elevation in ROS beyond the innate antioxidant capabilities of the body. In addition, the production of substantial amounts of hydrogen peroxide (H_2O_2) contributes to the impairment of endothelial function and consequent cellular death.

members of the immunoglobulin-like molecule family located on the surface of endothelial cells. In order to facilitate robust adhesion and/or transendothelial migration, these molecules interact with counter-receptors present on leukocytes and plasminogen activator inhibitor-1 within the circulatory system, indicating the potential presence of an endothelial phenotype that is both pro-inflammatory and pro-thrombotic (90). Multiple studies have been conducted, which have consistently demonstrated a higher prevalence of endothelial dysfunction among individuals diagnosed with diabetes. It is widely acknowledged within the scientific community that elevated blood glucose levels and the presence of diabetes have a detrimental effect on the production and functioning of NO. The available evidence indicates that there is a notable impairment in endothelial-derived nitric oxide (NO)-mediated vasodilation in individuals with both insulin-dependent and non-insulin-dependent diabetes mellitus (91).

5.6. Endothelial dysfunction and cardiovascular disease

The maintenance of a healthy endothelium is of utmost importance in the regulation of various cardiovascular functions, including but not limited to blood flow and fibrinolysis, hemostasis, vascular tone, angiogenesis, monocyte/leukocyte adhesion, and platelet aggregation. The aforementioned functions play a crucial role in the regulation of cardiovascular homeostasis, as indicated by previous research (92). The regulatory role of the vascular endothelium in maintaining cardiovascular health is widely recognized in scientific literature. The role of the aberrant vascular endothelium in the development of cardiovascular pathologies, including atherosclerosis, ageing, hypertension, obesity, and diabetes, has been widely recognized (93). Endothelial dysfunction, which refers to an imbalance between various factors involved in relaxation and contraction, coagulation and anticoagulation, as well as inflammation and anti-inflammation, may play a substantial role in the pathogenesis of atherosclerosis and cardiovascular disease (94). The presence of endothelial dysfunction has been observed in individuals diagnosed with ischemic CAD who also exhibit signs of atherosclerosis. Atherosclerosis is a pathological state that is distinguished by a deviation from the typical physiological behavior of endothelial cells. Specifically, these cells exhibit impaired release of nitric oxide in response to serotonin (95). The occurrence of vasoconstriction within atherosclerotic regions has the potential to instigate the progression of coronary thrombosis. It has been observed that there is a correlation

between inflammation and CAD, wherein inflammation has been found to impede the production of endothelial nitric oxide. Patients diagnosed with CAD who experience myocardial ischemia are also at risk of mortality resulting from congestive heart failure (CHF) as a consequence of endothelial dysfunctions (96).

5.7. Treatment

Experimental, clinical, and translational evidence suggests that several medicines and therapeutic options may improve various aspects of endothelial dysfunction. Furthermore, most of these medications have shown encouraging cardioprotective benefits in preclinical and clinical research. Metformin reduced the risk of MetS and T2DM in overweight impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) patients, although lifestyle adjustments were more beneficial. Metformin did not reduce MetS prevalence. Metformin decreased EDV in MetS patients with normal glucose tolerance independent of glucose, LDL-C, HDL-C, body weight, or blood pressure. In another MetS study, metformin improved Flow-Mediated Dilation (FMD) and insulin resistance. MetS patients' NO levels also increased with metformin (97).

In the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) research, rosiglitazone reduced T2DM and improved normoglycemia in IFG patients. Vascular events remained stable, and heart failure risk increased. In MetS studies, rosiglitazone improved FMD. Endothelial function improved with lower hsCRP and higher adiponectin. Pioglitazone reduced hsCRP in MetS patients, however endothelial function was not examined (98).

After the bezafibrate infarction prevention (BIP) study, a subgroup analysis found that bezafibrate lowers myocardial infarction (MI) in CHD patients. Bezafibrate reduces T2DM in obese people and those with impaired fasting glucose (IFG). Fenofibrate lowered MetS. In MetS patients, fenofibrate raised flow-mediated dilation (FMD) and decreased sICAM-1 and sVCAM-1. Bezafibrate increased FMD. Statins and fibrates together improve FMD in mixed dyslipidemia patients. When MetS patients moved from atorvastatin to bezafibrate, endothelial function decreased (99).

Nicotinic acid (NA) may treat MetS-related mixed dyslipidemia. A Coronary Drug Project study shows that NA reduces non-fatal MI and all-cause mortality in CHD and MetS patients. MetS patients had higher FMD. In MetS patients, ezetimibe with atorvastatin at 10 mg daily reduced LDL-C and improved Endothelium-dependent vasodilation (EDV) compared to 40 mg alone. In another experiment, ezetimibe + simvastatin 10 mg/day prevented a reduction in FMD after a high-fat meal better than simvastatin 80 mg/day alone. Eicosapentaenoic acid lowered plasma sICAM-1 and sVCAM-1 in MetS patients. Orlistat improved T2DM in obese people with IGT. Orlistat and sibutramine improved FMD in obese people. Orlistat and sibutramine also lower MetS. It is unknown how these drugs affect endothelial function in MetS patients (100).

Estradiol increases NO generation and endothelial function. Estradiol interacting with HDL particles is unexpected. Other HDL-associated estradiol esters may boost HDL's atheroprotective activities, including macrophage cholesterol export. According to research, oral hormone therapy increases adhesion molecules, PAI-1, and tPA better than transdermal hormone therapy. The earlier intervention raised hsCRP more than the latter. Oral estradiol reduced E-selectin levels in postmenopausal women with MetS in trial w108. Transdermal estradiol had no effect. Estradiol increases NO and endothelial function. Estradiol seems to work through HDL particles. HDL-associated estradiol esters increase macrophage cholesterol efflux and other atheroprotective actions. According to, oral hormone treatment increases bloodstream adhesion molecules, PAI-1, and tPA more than transdermal hormone therapy,. The former increased hsCRP more than the latter. Oral estradiol lowered E-selectin levels in postmenopausal women with MetS, while transdermal did not (101).

5.7.1. Lipid lowering-statin

Subgroup analysis of primary and secondary preventative trials found statins reduce vascular events in MetS patients. Statins reduced MetS risk in multiple trials. Atorvastatin reduced MetS prevalence, increased FMD, and decreased sICAM-1 (102). Pravastatin did not affect sICAM-1, sVCAM-1, or E-selectin levels in MetS patients, whereas simvastatin lowered PAI-1 activity. Statins may affect endothelial function differently in MetS patients, according to data. No comparative studies have been done to assess whether statin improves endothelial function better. Statins, which work by inhibiting the enzyme hydroxymethylglutaryl coenzyme A reductase, are the standard treatment for lowering high cholesterol levels. However, the effects of statins are not limited to decreasing lipid levels; they also have extra cholesterol-dependent or pleiotropic effects (103). In addition, statins have been shown to have cardioprotective benefits by reducing oxidative stress, enhancing endothelial function and inflammation, stabilizing susceptible plaques, and clot formation (103).

5.7.2. Antihypertensive drugs

According to secondary prevention subgroup analyses, angiotensin-converting enzyme inhibitors (ACE-I) reduce vascular events in MetS patients. ACE inhibitors and angiotensin receptor blockers (ARBs) diminish the risk of T2DM in hypertensives. Diuretics and beta-blockers enhance T2DM risk. Calcium channel blockers (CCBs) are ineffective. In the placebo-controlled DREAM trial, Ramipril did not decrease T2DM or vascular disease in IFG patients. Ramipril-treated individuals had more normoglycemia relapses. A recent subgroup analysis of the Antihypertensive and Lipid-Lowering medication to Prevent Heart Attack Trial (ALLHAT) indicated that chlorthalidone medication increased the risk of T2DM but decreased the risk of vascular events compared to lisinopril. Compared to chlorthalidone, lisinopril increased systolic blood pressure throughout follow-up. MetS patients treated with amlodipine, chlorthalidone, or lisinopril had comparable risks of developing T2DM and vascular events. Irbesartan improved endothelial function in MetS in a study. Despite lowering blood pressure, irbesartan improved FMD and plasma PAI-1 levels. Statins or fibrates plus ACE-I or ARB improve FMD better than alone (104). ARBs, CCBs, and ACEIs have been shown to potentially improve endothelial function in individuals diagnosed with CVD (104). The aforementioned outcomes are accomplished through a range of mechanisms, including upregulating the expression of endothelial nitric oxide synthase (eNOS), enhancing the phosphorylation of eNOS at Ser1177, downregulating the expression of NADPH oxidase (NOX), increasing the levels of

tetrahydrobiopterin (BH4) in the vasculature through GCH1-dependent pathways, and restoring the coupling of eNOS to facilitate the availability of NO (105).

5.7.3. Antihyperglycemic drugs

Endothelial dysfunction has been observed to be closely linked with hyperglycemia. Hence, the amelioration of hyperglycemia may lead to enhanced endothelial function. This can be accomplished through the utilization of various pharmacological agents, such as DPP-4 inhibitors, SGLT2 inhibitors, insulin, glucagon-like peptide-1 (GLP-1) receptor agonists, and metformin (106).

6. Role of MetS on hypertension

While there are various distinguishing aspects of MetS, hypertension is the most difficult to treat and is likely the least connected to or reliant on the condition (107). Hypertension affects around 80% of people with MetS. However, the majority of MetS patients with hypertension are overweight or obese. The most significant modifiable risk factor for CVD and overall mortality is hypertension, as shown in Figure 4. Hypertension is defined as systolic blood pressure (BP) of ≥140 mmHg or higher and diastolic blood pressure (BP) of ≥90 mmHg or lower, affecting 31.1% of the world's adult population, or 1.38 billion people, in 2010 (108). Hypertension is rising internationally because of an aging population, increased exposure to lifestyle risk factors such as poor diets (high salt and low potassium consumption), and a lack of physical exercise. Globally, the prevalence of hypertension has fluctuated, although unevenly. Most hypertension has dropped somewhat in high-income countries during the previous two decades, while it has climbed dramatically in low- and middle-income nations.

6.1. Prevalence, prediction and geographical status of hypertension

Elevated blood pressure is a necessary criterion for diagnosing the syndrome. Research shows that hypertension is common in people with MetS (109). In the Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) study, high blood pressure was the most common component of MetS, affecting over 80% of patients. Individuals with MetS had higher blood pressure levels than those without the syndrome. Fatal events were more frequent in this population (110). Many hypertensive patients also have metabolic syndrome. In the Progetto Ipertensione Umbria Monitoraggio Ambulatoriale (PIUMA) study, 34% of hypertensive patients had MetS. Patients with MetS had more cardiovascular events than those without MetS (111). In a French study, MetS was increased with higher blood pressure values (112). In the PAMELA study, MetS-free participants were followed for 10 years. Individuals with certain types of hypertension were more likely to develop MetS (113). The Global Cardiometabolic Risk Profile in Patients with Hypertension Disease (GOOD) study found less than a third of hypertensive patients had acceptable blood pressure values. The study found that MetS was more common in participants with uncontrolled blood pressure than those with controlled blood pressure. The difference was significant (114).

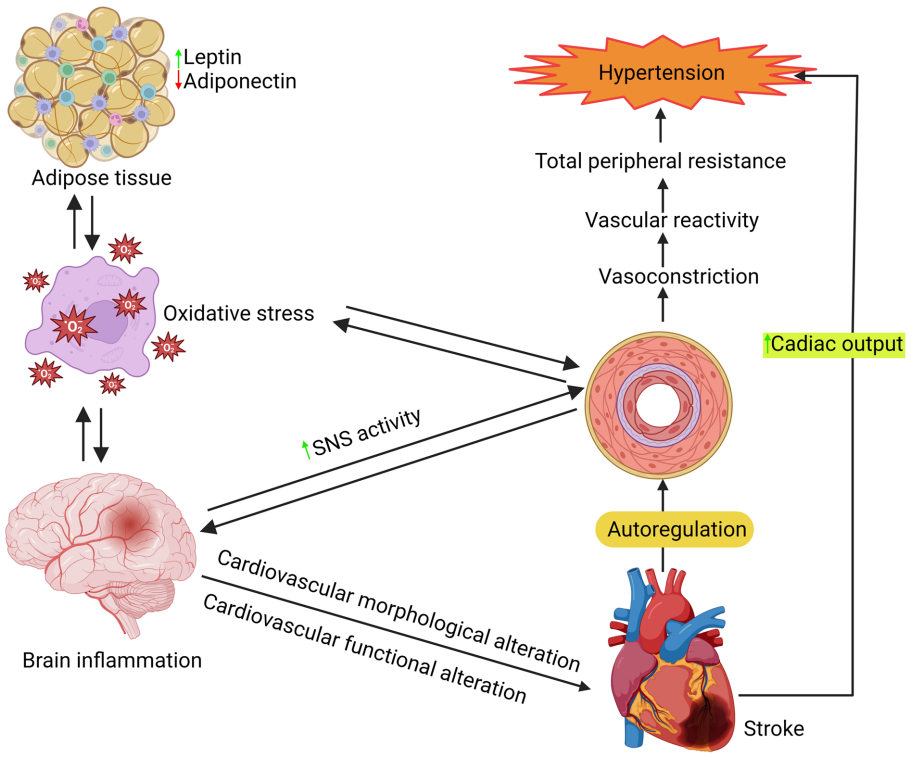


FIGURE 4
Illustration of a series of processes involved in the hypertension mechanism.

MetS in hypertensive patients increases end-organ damage. Hypertension and metabolic syndrome together lead to more cases of left ventricular hypertrophy, microalbuminuria, increased intima-media thickness, and hypertensive retinopathy (115). Hypertension has increased significantly over the past two decades in Southeast Asian and sub-Saharan African countries (116). The prevalence of hypertension and pre-hypertension among adults (18 years and older) in Uganda was determined through a cross-sectional survey representing the entire nation (117). The results showed an overall age- and sex-adjusted prevalence of 31.5% for hypertension and 38.8% for pre-hypertension. The majority of hypertension, adjusted for age and sex, was highest in the Central (34.3%), West (32.5%), and East (32.3%) regions. In comparison, it was lowest in the North (22.0%) and West Nile (24.1%) regions, based on geographical location (117). In the United States, hypertension is estimated to be prevalent when an individual has a systolic blood pressure of 140 mm Hg and a diastolic blood pressure of 90 mm Hg (118). The prevalence of hypertension in Southeast Asian urban populations was estimated at 33.82% based on pooled data. Among the total cases, 33.98% of hypertension was reported in the community, while 32.45% was reported among school adolescents. At present, at least 1 billion people worldwide suffer from hypertension. It is projected that this number will increase to 1.5 billion by the year 2025. A survey conducted in 2015 found that 25% of women and 20% of men suffer from hypertension. Less than 20% of people have well-controlled hypertension. Additionally, hypertension is associated with over 9 million deaths (116).

6.2. Oxidative stress and hypertension

There is a link between hypertension and increased oxidative stress in the vascular system; however, it is unclear whether oxidative stress is a cause or result of hypertension. It is well acknowledged that hypertension is the most critical risk factor for the development of CVD (119). A rising amount of evidence suggests that oxidative stress, which causes excessive ROS production, plays a significant role in the development of hypertension (120). ROS function as vasoconstriction mediators during vasomotor system modulation. Many factors, including angiotensin II, endothelin-1, and urotensin-II, trigger this vasoconstriction (121). In addition, the redox state influences the quantity of nitric oxide (NO) available for bioavailability in the body. NO is a potent vasodilator, and low levels of intracellular ROS play a crucial role in normal redox signaling that helps to maintain vascular function and integrity under physiological conditions (122). Higher levels of ROS contribute to vascular dysfunction and remodeling through oxidative damage when pathophysiological circumstances are present. Increased SOD and H_2O_2 generation, decreased nitric oxide synthesis, and impaired antioxidant bioavailability have all been associated with human hypertension (123).

6.3. Epigenetics and hypertension

It is now well-accepted that environmental and epigenetic, and genetic factors influence hypertension. Differential aetiologies of hypertension arise from complex interplays between genetic and environmental variables that modify biological pathways and lead to the disease (124). Hypertension significantly contributes to CVD pathologic remodeling, which may lead to severe complications such as heart failure, stroke, kidney failure, and cognitive impairment (125). Pulmonary vascular cells in people with progressive hypertension have a phenotype that is hyperproliferative, antiapoptotic, and inflammatory, leading to an increase in vasoconstriction and an aberrant remodeling of blood vessels. Gene mutations, epigenetic modifications, anomalies in sex hormones, and environmental variables all contribute to the development of hypertension (126). Altered DNA methylation patterns have been observed in patients with hypertension. Genomic DNA methylation has been associated with the onset and severity of hypertension (127). Individuals with chronic thromboembolic pulmonary hypertension exhibit differential methylation of multiple probes in pulmonary artery smooth muscle cells (PASMCs), which may be associated with PASMC remodeling. The epigenetic change in PASMC led to the activation of HIF-1, which caused a proliferative and antiapoptotic phenotype (hypoxia-inducible factor-1). It may be possible to differentiate between hypertension and pulmonary veno-occlusive diseases by analyzing dysregulated DNA methylation of specific genes (128).

6.4. Molecular mechanisms

It is crucial to comprehend the biology of hypertension to treat it and prevent any potential consequences effectively. Although there have been significant studies conducted on the pathophysiology and etiology of hypertension, it is still the case that over 95% of individuals with hypertension do not have a known cause. Vascular alterations, such as inflammation, remodeling, stiffness, calcification, and atherosclerosis, may play a role. Hypertension is a multifaceted condition that arises from the intricate interplay of genetic, physiological, and environmental factors. Hypertension's pathogenesis has been linked to several variables, such as inflammation, renin-angiotensin-aldosterone system overexpression, sympathetic nervous system activation, and aberrant G protein-coupled receptor signaling, as shown in Figure 4 (129–131). A study indicates that changes in T-cell activity, specifically in the immune system, are a factor in the onset of hypertension (132). These processes commonly result in increased bioavailability of ROS due to excess ROS formation, decreased nitric oxide (NO) levels, and impaired antioxidant capacity in the arteries, heart, brain, and kidneys (133, 134).

ROS may cause hypertension by activating redox-sensitive signaling pathways. Maintaining a balance between NO production and ROS formation is crucial in the vasculature. Any NO generation reduction and ROS production increase might reduce blood flow. The functions of superoxide anion and H_2O_2 as second messengers are highly controlled. ROS stimulates the activity of many physiological components, including mitogen-activated protein kinases (MAPK), tyrosine kinases, Rho kinases, and transcription factors (NF-kB, AP-1, and HIF-1) and increased concentration of intracellular free Ca^{2+} also inactivates the protein tyrosine phosphatases (PTP). Furthermore, it increases the expression and activity of genes associated with inflammation and cancer (135, 136).

The pathophysiologic pathways in MetS connected to several diseases have been investigated. The objective is to find better and more effective therapies (137). So far, several distinct pathophysiological ideas have been proposed about why MetS patients

have high blood pressure. The significant reasons are IR, obesity, activation of the sympathetic nervous system, and excessive salt consumption (138).IR raises heart rate and blood pressure via upregulating angiotensin II receptors and inhibiting nitric oxide synthesis (139). In addition, high levels of leptin, hypothalamic–pituitary–adrenal axis activity, obstructive sleep apnea, and baroreflex issues contribute to sympathetic nervous system activation in MetS (140). Finally, in obese patients, increased renal tubular reabsorption produces salt retention, which worsens high blood pressure. To summarize, the emergence of high blood pressure in patients with MetS is a multi-step process involving a variety of pathophysiological pathways (141).

6.5. Treatment

In cases where lifestyle modifications prove inadequate in achieving the desired blood pressure levels, pharmacological intervention may be deemed necessary. However, when hypertension continues after such therapy, antihypertensive medication is typically necessary, even for minor increases in blood pressure. The primary pharmacological agents utilized in the management of hypertension include diuretics, angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs), beta-blockers, and calcium channel blockers (CCBs) (142). Specific individuals may require multiple antihypertensive medications to attain their desired blood pressure level. In cases where blood pressure readings exceed the target by more than 20/10 mm Hg, newly diagnosed individuals may be promptly prescribed antihypertensive or a combination of hypertensive medications. To minimize adverse effects, taking a secondary medication with a complementary mode of action is advisable before taking the initial drug at the maximum recommended dose (143).

7. Role of MetS on platelet hyperactivity

Platelets serve an essential role in hemostasis and thrombosis. Therefore, they should stay dormant and only become active when vascular damage occurs. When activated, platelets produce and release a slew of prothrombotic substances from their granules. Coagulation factor V, fibrinogen, and vWF are examples of these molecules (144). In inactivated platelets, several surface glycoproteins were expressed differentially (GP). During this activation phase, the adhesion molecule P-selectin is translocated to the cell surface and stored in endothelial cell Weibel Palade bodies and platelet granules (145). In addition, the GPIIb-IIIa on the surface of activated platelets changes conformation and binds to fibrinogen during activation. Platelets triggered by thrombin had decreased levels of vWF binding sites on the GPIb-IX complex but increased levels of GPIV critical to thrombospondin, shown in Figure 5 (146).

MetS patients often have hyperactive platelets. Elevated levels of fibrinogen, PAI-1, thrombin, von Willebrand factor, factor VII, and other coagulation factors, as well as significant platelet aggregation, define it (147). Specific abnormalities include high levels of tissue factor, factor VIII, fibrinogen, and inhibitor of plasminogen activator type 1 activity (148). At baseline, patients with MetS showed a lower antiplatelet response to aspirin and higher platelet reactivity. Platelet hyperactivity may be exacerbated by increased systemic inflammation, obesity, dyslipidemia, and other metabolic diseases (149). Platelet hyperactivity may also be exacerbated by metabolic diseases such as obesity. Increases in platelet count and mean volume (150) are one example of an abnormality associated with platelet reactivity. They are a prognostic sign in several atherothrombotic diseases, including acute coronary syndrome, thrombosis, and stroke.

Furthermore, an increase in cytosolic calcium concentration stimulates platelet reactivity (151). Finally, high blood leptin levels cause increased platelet aggregability. The overall effect of these anomalies is increased platelet adhesion and activation (152).

7.1. Prevalence, prediction, and geographical status of platelet hyperactivity

Platelet hyperactivity is becoming increasingly common worldwide, with its frequency and prevalence rising. It is the most prevalent type, comprising approximately 90–95% of all cases worldwide. The activation of these events results in an increase in vasoconstriction and the promotion of thrombus formation, which can ultimately lead to the development of atherosclerosis (16). No significant differences were observed between healthy participants with hyperactive platelets and those without hyperactive platelets regarding age, race, body mass index, smoking status, or the prevalence of hypertension. Several studies on humans have demonstrated a preference for platelet hyperreactivity among females (153). Platelet hyperreactivity is a common occurrence that can be triggered by different stimuli, indicating that similar platelet activation pathways are involved in the process. Platelet reactivity varies significantly among individuals and is associated with factors such as female gender, high plasma fibrinogen levels, and genetic diversity (154).

7.2. Oxidative stress and platelet hyperactivity

Thrombotic events are a common cause of morbidity and mortality in the elderly population. Much evidence suggests that oxidative stress regulates various elements of thrombotic processes, including platelet activation (155). Platelet or vascular redox state, endogenous or exogenous antioxidants, and the production of reactive oxygen and nitrogen species are all potential factors in platelet-dependent thrombus development (156). The parameters and processes that govern the synthesis and metabolism of superoxide and nitric oxide may alter platelet activity and thrombus formation (157). Normal platelet activation may cause a shift in the redox state. Glutathione disulfide levels rise, and oxygen consumption increases with platelet aggregation. Conditions that cause oxidative stress but do not cause a florid aggregation response have the potential to be prothrombotic (158). Platelets have

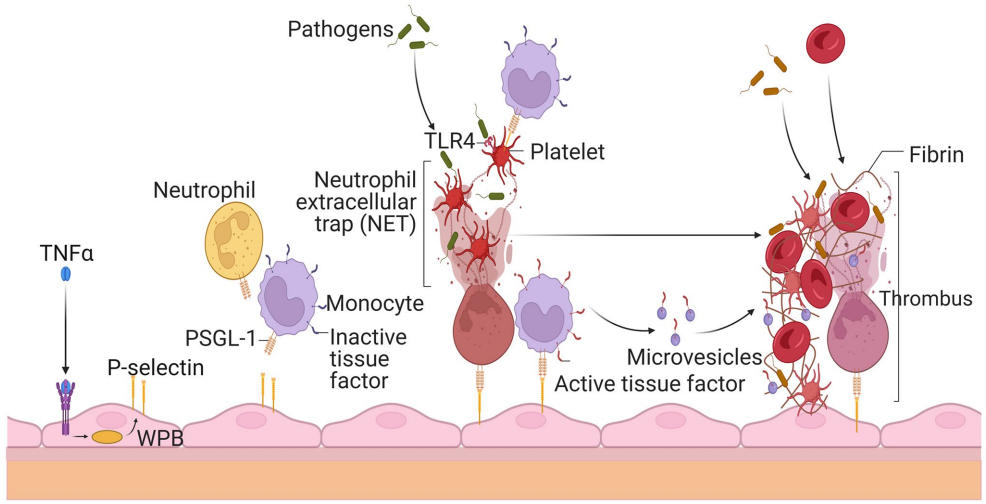


FIGURE 5
Thrombosis's rapid spread due to circulating white blood cells and platelets. Endothelial cells release P-selectin through the exocytosis of Weibel Palade bodies (WPB) when stimulated by tumor necrosis factor alpha during inflammation. P-selectin glycoprotein ligand-1 (PSGL-1) recruits leukocytes such as neutrophils and monocytes by P-selectin. Neutrophil extracellular traps (NET) ensnare invaders, stimulate thrombus development, and activate platelets after being released by neutrophils. Platelets' activation allows leukocytes to become activated and identify infections. Thrombus production is also encouraged by tissue factor (TF), which is released from the surface of monocytes and their microvesicles to initiate fibrin synthesis and red blood cell recruitment. The resultant thrombus aids in the entrapment of the infectious agent.

essential roles in a wide range of biological processes, including but not limited to hemostasis, thrombosis, inflammation, infection, immunobiology, cancer metastasis, wound repair, and angiogenesis (159). Even though a significant change in the redox state occurs during normal aggregation; as a result, oxidative stress may promote platelet hyperactivity by reducing the level of physiologically accessible nitric oxide. Therefore, the measurement of oxidative stress may aid in the early diagnosis of asymptomatic patients at risk of developing thrombosis (160).

7.3. Epigenetics in platelet hyperactivity

Epigenetic changes may influence the generation and release into the circulation of proteins involved in blood coagulation and fibrinolysis. They may also interact with platelet activities and their susceptibility to antiplatelet medications (161). Cancer and other multifactorial illnesses, including atherosclerosis, hypertension, MetS, and diabetes, have received considerable attention during the last decade because of the potential relevance of epigenetic mechanisms in explaining the remaining unknown heredity factors. However, new research suggests that microRNAs and DNA methylation may be more nuanced in controlling the hemostatic balance, particularly in developing a prothrombotic condition linked to CAD (162). Platelet miRNA is involved in the regulation of hemostasis. Most microRNAs in human platelets are produced by megakaryocytes, which have an efficient miRNA processing mechanism (163). During thrombopoiesis, megakaryocytes provide platelets with most of their miRNA and pre-miRNA pools. Platelets may produce fully mature miRNAs (such as Dicer, Ago2, and RISC) expressing the enzymes necessary for pre-miRNA digestion. Ablation of Dicer 1 alters mRNA expression patterns and reduces miRNA expression levels in megakaryocytes and platelets (164).

7.4. Molecular mechanisms

The process of platelet activation is a complex phenomenon that is dependent on a multitude of factors. In the context of vascular injury, it has been observed that platelets possess adhesion receptors on their surface, namely integrins α6β1, α2β1, GPIIb/IIIa, and the GPIb/V/IX complex. These receptors exhibit specific binding interactions with laminin, collagen, fibrinogen, and VWF, respectively. Notably, this binding process occurs in the presence of regulatory molecules such as small G-protein regulators (SGRs), SRC-family kinases (SFKs), and serine/threonine-protein kinases (STKs). Consequently, these molecular interactions induce discernible changes in the morphology of platelets (165). When collagen binds to the platelet collagen receptors, it triggers a cascade regulated by phospholipase C (PLC). The depletion of calcium stores is a result of the thick tubular system. Activating various enzymes, such as phospholipase A2 (PLA2) and glycoprotein kinases, elevates intracellular calcium levels. This increase in calcium is crucial for several cellular processes, including morphological changes, presentation of the procoagulant surface, secretion of platelet granular content, and activation of Phospholipase A2 (166). The synthesis of thromboxane A2 from arachidonic acid, which serves as a precursor to TBXA2, is facilitated by the activation of phospholipase A2 (PLA2) and subsequent conversion by cyclooxygenase 1 (COX-1) (167). The G protein-coupled receptors that the released agonists activate include the thrombin receptor (F2R), thromboxane A2 receptor (TBXA2R), and adenosine diphosphate (ADP) receptors (P2RY1 and P2RY12). Upon adenosine diphosphate (ADP) activation, the P2RY12 receptor interacts with Gi and inhibits adenylate cyclase activity (168). The interaction of clotting factors with activated platelets is another vital process. The production of thrombin is triggered by the exposure of tissue factors in the arterial wall, which in turn sets off the clotting cascade. It plays a crucial role as a platelet activator by interacting with

protease-activated platelet receptors (169). LAIR-1 and PECAM-1 are two proteins that are present in megakaryocytes. These proteins are crucial in keeping the platelets dormant within the bone marrow. In individuals with coronary heart disease, platelets are attracted to damaged blood vessels. Platelets assist in the process of blood clotting, thereby reducing bleeding. Contributing to the occlusion of sick arteries can worsen the condition, potentially leading to thrombosis (170).

7.5. Platelet hyperactivity and diabetes

Platelet hyperreactivity is a crucial determinant in developing thrombotic diseases such as heart attack and ischemic stroke, which may be fatal consequences of diabetes. Individuals with type 2 diabetes have higher amounts of the collagen receptor GPVI on the surface of their platelets (171). Elevated GPVI levels have been linked to cardiovascular events such as acute coronary syndrome, myocardial infarction, ischemic stroke, and transient ischemic episodes. Platelet activation is caused by the breakdown of atheromatous plaque or the de-endothelialization of the arterial wall. In order to conceal the exposed region, it is necessary to identify the sticky proteins through various receptors of platelet membrane glycoproteins (GPs) (172). When activated, a receptor triggers physiological reactions such as membrane phospholipid hydrolysis, intracellular calcium mobilization, and phosphorylation of vital intracellular proteins. The process of glycation of proteins on the surface of platelets has been reported to reduce membrane fluidity while boosting platelet adhesion. This, in turn, results in the incorporation of glycated proteins inside thrombi (173). Calcium mobilization from intracellular storage pools is associated with reduced membrane fluidity, which leads to increased intracellular calcium concentrations (174). Platelets in diabetic patients are hyperactive, with high adhesion, activation, and aggregation, which may be related to the dysregulation of various signaling pathways. Platelet activity was elevated in individuals with type 1 and type 2 diabetes in the early stages, which may increase their vulnerability to CVD (175). Platelet dysfunction in diabetics has the potential to be discovered before any visible damage to the vessel wall manifests itself. Diabetes increases platelet responsiveness to subthreshold stimuli, encouraging thrombotic events and the generation of new hyperreactive platelets (176).

7.6. Platelet hyperactivity and cardiovascular disease

Activated platelets are essential constituents of thrombi that obstruct arteries and contribute to plaque development in blood vessels during atherogenesis. Consequently, they are gaining prominence as novel participants, given that increased platelet aggregation constitutes a crucial risk factor for thrombosis, heart attacks, and strokes (177). The linkage between platelet activation and consequent thrombosis has been established in numerous CVD. The phenomenon of platelet hyperactivity encompasses many mechanisms, among which oxidative stress is included. Human platelets generate and discharge ROS, such as O^{2-}, H_2O_2, or OH-, in reaction to physiological agonists. These ROS molecules play a crucial role in augmenting the platelet activation response through various signaling pathways, including isoprostane formation, Ca^{2+} mobilization, and NO inactivation. In addition, the generation of ROS by platelets, the absorption of free radicals from the environment, and the depletion of antioxidants can lead to pro-oxidant, pro-inflammatory, and platelet hyperaggregability effects, which increase the risk of CVD (178).

7.7. Platelet hyperactivity and infertility

Platelets are essential for hemostasis, and abnormalities in their activation or hyperaggregability may play a role in the pathophysiology of conditions, including miscarriage and thrombosis with no known cause. Miscarriage is increasingly connected to platelet hyperaggregability, also known as sticky platelet syndrome (SPS) (179). Hyperandrogenism, repeated anovulation, and infertility are hallmarks of polycystic ovary syndrome (PCOS), one of the most common endocrine disorders affecting women of reproductive age. Increased risk of CVD insulin resistance, and impaired endothelial function are all associated with this condition (180). Patients with PCOS exhibit increased agonist-induced platelet aggregation, faster coagulation, and microparticles produced from platelets. Factor XII deficiency, dysfibrinogenemias related to thrombosis, protein C defects, protein S defects, antithrombin deficiency, heparin cofactor II deficiency, and fibrinolytic defects associated with thrombosis are all linked to infertility and pregnancy loss. Pregnancy increases the risk of uteroplacental thrombosis in a hypercoagulable state because of coagulation factors, regulators, and fibrinolytic system changes. Furthermore, placental perfusion may cause thrombosis due to its low pressure and turbulent flow (181).

7.8. Treatment

Several therapeutic targets for platelet hyperactivity have been reported in studies aimed at reducing vascular risk in patients. Platelet-derived microparticles (PMPs) have been demonstrated to play a role in the increased incidence of atherosclerotic plaque and arterial thrombosis formation in individuals (182, 183). It is indisputable that patients with CVD exhibit platelets with a hyperactive phenotype characterized by higher aggregation, activation, and adhesion (184). This may contribute to the emergence of atherothrombotic complications in such patients (185). Hence, identifying elevated concentrations of particular microparticles can be a significant prognosticator of vascular impairment and cardiovascular consequences. New treatments have been explored using pharmacological drugs with antiplatelet and anticoagulant characteristics to improve human platelet function (186). For a long time, people have known that aspirin, or acetylsalicylic acid and cyclooxygenase (COX) inhibitor, has beneficial effects against inflammation and blood clots (187).

Over previous decades, it has been demonstrated that vitamin K antagonists possess anticoagulant characteristics. Dicoumarol, warfarin, phenprocoumon, acenocoumarol, and rivaroxaban are among the most significant vitamin K antagonists presently employed (188). Compared to aspirin, rivaroxaban reduced the risk of recurrence in patients with venous thromboembolism without significantly increasing the risk of bleeding (189). Clopidogrel,

ticagrelor, prasugrel, and cangrelor are all examples of P2Y12 receptor antagonists, and each of these drugs has been shown to pharmacologically block adenosine diphosphate (ADP) receptors (186). Abciximab, tirofiban, and eptifibatide are examples of Glycoprotein IIb/IIIa inhibitors that have been shown to block fibrinogen adherence to activate platelets and, by extension, the formation of inter platelet bridges (190). The abovementioned medications treat and prevent atrial fibrillation and venous thromboembolism. Anticoagulants in question include unfractionated heparin and low molecular weight heparin. Fondaparinux, a parenteral anti-factor Xa medication, has lately emerged as a preferable alternative to unfractionated heparin (186).

8. Advanced/alternative treatment strategies

The primary goals of MetS treatment are to lower the risk of CVD and, ideally, to postpone the development of type 2 diabetes. Once type 2 diabetes has been established, medication may aid in maintaining health by reducing exposure to CVD risk factors (CVD). Therefore, the primary therapy for MetS is a lifestyle change that promotes cardiovascular wellness. Both nutritional supplements and physical exercise reduce MetS symptoms (191). Medicines, small molecules, and naturally occurring bioactive compounds are among the various materials that may be used in the treatment (192).

8.1. Pharmacotherapy

Because MetS is not yet a recognized disease and knowledge of common pathways as prospective therapy targets are still being researched, the current strategy is to address each problem individually. In addition to lowering the underlying risk factors, medication may be utilized to prevent CVD. Effective pharmacological therapies include statins for the treatment of dyslipidemia, antiplatelet medicines for the reduction of prothrombotic risk, and insulin sensitizers for the prevention of diabetes. There is no single pharmacological treatment for MetS, and patients with polypharmacy, limited adherence to current pharmacotherapy, and associated comorbidities find it challenging to continue taking multiple medications. Those who struggle to control their blood pressure are more likely to develop treatment-resistant hypertension. Antihypertensive drugs may now have unwanted side effects, an added complication. It has been suggested that aldosterone synthase, aldosterone receptors, and the ACE2/angiotensin 1-7/Mas receptor axis are all potential therapeutic targets (193).

Obesity is associated with type 2 diabetes, IR, and CVD, which may be avoided and treated using adiponectin, the principal peptide generated by adipocytes. Adiponectin levels are diminished by sickness. Endothelial, skeletal, cardiac, and adipocyte cells can produce this adipocytokine. Recently, the synthesis and release of higher-order adiponectin have been linked to several endoplasmic reticulums (ER)-associated proteins. These proteins include ER-resident protein 44 (ERp44), disulfide-bond A oxidase-like protein (DsbA-L), ER oxidoreductase 1- (Ero1-), and glucose-regulated protein 94 (GPR94). Adiponectin injections affect insulin sensitivity, atherosclerosis, inflammation, and weight in people and animals. Replacement treatment using human adiponectin may help identify therapeutic targets for preventing and treating metabolic diseases, including insulin resistance and type 2 diabetes (194).

Peroxisome proliferator-activated receptors (PPARs) regulate triglycerides (TGs), blood sugar, and abdominal adiposity. There are several kinds of PPARs. Fibrate and omega-3 fatty acid PPAR agonists lower TG. They raise HDL cholesterol via catabolizing TGs, especially when combined with fibrates (HDL-C). Glitazones, the major PPAR-agonist, decreases glucose but not TGs. Glitazones are antihyperglycemic and insulin-sensitizing. However, newer PPAR−/ agonists such as elafibranor reduce TG while increasing HDL-C. They may aid in the treatment of NAFLD caused by MetS. As a result, the PPAR system can potentially treat atherogenic dyslipidemias; however, side effects such as myopathy, gallstones, and drug–drug interactions (such as those caused by gemfibrozil) must be considered (195). The indirect antioxidant effects of statins, type 1 angiotensin II receptor antagonists, angiotensin-converting enzyme (ACE) inhibitors, and other cardiovascular medications are pleiotropic. Effects on vasodilation, antithrombosis, and antiproliferation are facilitated by endothelial progenitor cells. The therapeutic advantages in heart failure might be attributed to these defense systems. The pathophysiological mechanisms that statins attack are identical. Statins lessen the risk of cardiovascular events, including heart attacks and strokes, by lowering inflammation and atherothrombosis in the blood vessels. Statins enhance NO bioavailability by decreasing NADPH oxidase activity in a Rac1-dependent manner, reducing caveolin-1 activity, decreasing asymmetric dimethyl-l-arginine, improving activating eNOS phosphorylation, and upregulating eNOS mRNA (196). Nebivolol, a third-generation beta-blocker, was shown to stimulate eNOS activity in *ex vivo* tests, leading to the induction of vascular nitric oxide, which explains why individuals with essential hypertension had better NO bioavailability (197, 198).

Subsequent research has revealed that while first- and second-generation beta-blockers do not inhibit Nox2, nebivolol does exhibit inhibitory effects on Nox2 in hypertensive rats and isolated cells (199). Directly modifying the cytoplasmic membrane assembly of Nox2 and cytosolic subunits p47phox, p67phox, and rac1 may result in various potential outcomes, including reducing superoxide generation, prevention of eNOS uncoupling and nitric oxide breakdown through interaction with superoxide, and enhancement of endothelial function. Furthermore, it has been observed that Nebivolol can mitigate oxidative stress in individuals with hypertension through the reduction of nitric oxide's oxidative degradation (200).

8.2. Gene therapy

Gene therapy has progressed mainly to advances in delivery methods and vectors and the continual identification of novel mechanisms driving CVD (201). Clinical translation of cardiovascular applications lags substantially behind analogs for other conditions, such as metabolic diseases, blood disorders, and cancer. Differences in the intricacy and duration of the disease processes may account for this. In contrast to diseases resulting from a unique genetic mutation, most chronic cardiovascular conditions are multifactorial. The conditions mentioned above involve complex genetic and environmental interplays, rendering them less amenable to gene therapy (201). Attaining consistent

expression in the cardiac or susceptible vascular tissue via vector delivery has presented novel obstacles. Adenoviral-mediated methods to overexpress sarcoplasmic-endoplasmic reticulum Ca2+/ATPase (SERCA) have shown promise in preclinical and early-phase clinical trials. The SERCA pump is a regulatory mechanism that significantly impacts the management of myocardial calcium homeostasis and contractility. This mechanism has been linked to heart failure (202). The phase II clinical study, Calcium Up-regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID2), yielded no significant therapeutic effects. This finding is documented in academic literature. It was hypothesized that AAV1-neutralizing antibodies were blamed for the virus's failure to transduce SERCA. Disrupted redox signaling has not been the target of any gene therapy approach thus far. However, new targets from detailed molecular mechanistic analyses and improved gene therapy delivery technologies, such as using non-viral, nanoparticle-based DNA delivery mentioned below, provide renewed optimism.

It is well-established that vascular endothelial growth factor (VEGF) promotes angiogenesis. However, two additional benefits of improved redox balance are lowered NOX activity and eNOS coupling. VEGF may also stimulate NRF-2-mediated antioxidant mechanisms (184). The intramyocardial injection of adenoviral VEGF was safe and improved myocardial perfusion at 1 year in patients with severe CAD, as shown in phase I/II experiment. However, the precise distinction between increased redox signaling and direct activating pro-angiogenesis pathways remains to be seen (203).

8.3. miRNA therapy

MicroRNAs (miRNAs) are diminutive, non-coding RNA molecules that are crucial in regulating gene expression. MiRNAs exert significant control over translation through direct mRNA degradation and forming a protein complex called "RNA-induced silencing complex" (RISC), which can cleave miRNA targets. This underscores the critical role played by miRNAs in translation control. Both of these systems play a role in the regulation of translation (204). According to research findings, approximately 40 microRNAs have been observed to impact redox signaling, potentially in the onset of CVD or as a protective measure against it (205). In the last decade and a half, the discovery of AntagoMiRs, synthetic oligonucleotides with an anti-sense sequence explicitly targeting a particular miRNA, has been a significant breakthrough. Several side-chain modifications are also generated, making attaching to the target microRNA easier and providing protection from endogenous nucleases. These side-chain changes are also included. MicroRNAs, on the other hand, pose pharmacokinetic problems. They tend to amass in the kidneys and liver (206). Several nanoparticle compositions are now being developed to address these pharmacokinetic concerns. These formulations seek to increase antagoMir targeting to cardiovascular tissue while reducing cargo exposure to nuclease action. Currently, research on the cardiovascular system is only available in preclinical models.

In contrast, an antagoMiR that targets miR-34a has shown promise in a mouse model of heart attack by partly restoring myocardial function and boosting tissue survival rate. In addition, mir-34a increases oxidative damage in endothelial cells dependent on p66shc-sirtuin1. As a result, the benefits of antagoMiR-34a may be acquired by suppressing this apoptosis-promoting pathway (207).

8.4. Dietary strategies

Poor nutrition and lack of exercise are significant contributors to the worldwide rise in the prevalence of overweight and obesity. Until now, which dietary pattern is best for controlling MetS is unknown. Modifying one's lifestyle, especially diet, is the most important therapeutic strategy for this condition. Certain dietary modifications have been demonstrated to effectively reduce symptoms and improve several metrics associated with MetS (184). Scientific studies showed that the DASH (Dietary Approaches to Stop Hypertension) diet is the most effective strategy for preventing and treating MetS compared to low-fat and restricted diets. Health practitioners may suggest simplified diets without restrictive diets. Normalizing metabolic imbalances in people with MetS requires calorie restriction and increased activity (208). MetSAs part of treatment for MetS; nutritional recommendations should consider the overall eating pattern since focusing on just one nutrient can only go so far. Alternatives to restrictive diets that focus on calories or specific nutrients have been found in recent research (209, 210). Numerous studies have shown the potential health advantages of following the MedDiet, including the primary and secondary prevention of CVD, T2DM, and MetS (211, 212).

The "Mediterranean Diet" describes the food preferences, lifestyle choices, and cooking methods of people living around the Mediterranean Sea region (213). The various plant-based foods include greens, fruits, grains, beans, nuts, seeds, olive oil, and pulses. Sofrito is a seasoning that has been traditionally utilized in various dishes. It comprises olive oil, tomato, garlic, onion, and leek, rich in phenolic compounds and carotenoids, including naringenin, hydroxy-tyrosyl, lycopene, and β-carotene (214). The traditional Mediterranean diet is characterized by a high-fat content and low carbohydrate intake, with fat contributing 35 to 45% of daily caloric intake, protein contributing 15%, and carbohydrates contributing 40 to 45%. The principal source of fat is derived from olive oil and almonds. The lipid in question is primarily comprised of polyunsaturated and monounsaturated fatty acids. Olive oil is the primary source of monounsaturated fatty acids (MUFAs) in the Mediterranean diet (MedDiet). In addition, olive oil is rich in oleic acid, which has been associated in several studies with beneficial effects on IR, blood lipid profiles, and blood pressure (215).

Olive oil is beneficial because of the polyphenols it contains, which have anti-inflammatory and antioxidant properties. Fruits and vegetables are rich sources of various nutrients, including antioxidant vitamins such as vitamins C, E and beta-carotene and phytochemicals, folates, and minerals (216). Recent studies have demonstrated that the Mediterranean diet (MedDiet) can reduce mortality rates in individuals of various body sizes, including those who are overweight or obese, physically fit, or at an increased risk for CVD (217). Studies have shown a negative correlation between MedDiet adherence and mortality from CVD, cancer, and other degenerative diseases. The risk of acquiring significant complications of CVD is also reduced by following the MedDiet, both statistically and clinically (218). Changes in body composition, as shown by lower total and segmental fat, may be related to differences in metabolic profile and other health

outcomes associated with MedDiet treatment. The prevalence of both type 2 diabetes and CVD is falling, and research suggests that MedDiet may be contributing to this trend. Due to its many positive health effects, the MedDiet should be one of the first lines of defense in the fight against and management of MetS (219, 220).

There is a growing awareness of the significance of dietary factors in altering endothelial function. The primary focus of the research has been on n3 fatty acids, antioxidant vitamins (especially vitamins E and C), folic acid, and L-arginine (221). Studies have demonstrated that dietary factors significantly affect vascular reactivity. Studies have shown that fish oil and soy protein can improve endothelial function, which may help prevent CVD. These chemicals possess properties that protect the heart. Communities with a lower incidence of CVD have been given particular focus regarding their dietary habits. Research suggests that following a Mediterranean diet with a significant amount of vegetables, seafood, olive oil, and moderate wine consumption may positively impact endothelial function (222).

The risk of hypertension and its complications may be reduced by adopting dietary modifications that lower blood pressure. Nutritional therapies for hypertension prevention include reducing salt intake, limiting alcohol use, increasing potassium intake, and adopting a general healthy pattern like the DASH or Mediterranean diet (223). Recent research has shown that the DASH diet may effectively control hypertension. Consumption of fruits, vegetables, whole grains, low- or no-fat dairy, legumes, and nuts is preferred over red and processed meats and sugary drinks. The DASH diet is distinguished from other eating plans by its lower sodium intake (from 1,500 to 2,300 mg/day), higher fiber intake (>30 g/day), potassium, magnesium, and calcium content, and a lower intake of saturated fats (6% of energy) and dietary cholesterol (approximately 150 mg/d) (224). In epidemiological studies, improved adherence to the DASH diet has been related to a healthier cardiometabolic profile and a lower risk of CVD. The DASH diet's food content and distribution are likely responsible for many health advantages. The DASH diet emphasizes their output, increasing potassium, magnesium, and fiber intake. Evidence shows that these nutrients help check blood pressure, glucose levels, and insulin sensitivity. Blood insulin and glucose levels have been shown to decrease in correlation with the consumption of polyphenol- and antioxidant-rich fruits and vegetables (225).

Avoiding or limiting the consumption of animal products is a central tenet of "plant-based diets." In contrast, a focus on whole plant foods, including fruits, vegetables, nuts, legumes, and grains, is advocated. Consistent evidence links plant-based diets to a reduced risk of developing MetS and associated symptoms (226, 227). In addition, the risk of developing obesity, diabetes, and CVD, as well as the risk of dying prematurely, is lower among those who follow these dietary habits. In addition, the nutritional composition of plant-based diets promotes the consumption of a diverse range of plant-derived foods while discouraging the intake of red and processed meat, correlated with heightened susceptibility to diabetes, cardiovascular ailments, and certain forms of cancer. Furthermore, it has been established that the minerals and bioactive compounds in plant-based diets, such as vitamins C and E, beta-carotene, and polyphenols, exhibit antioxidant properties associated with reduced CVD and MetS (228, 229).

Several studies conducted in the last 20 years have demonstrated that the aqueous constituents present in tomatoes inhibit the aggregation of blood platelets, both *in vitro* and *in vivo*. The naturally-derived functional food component, Fruitflow®, a water-soluble tomato extract, has gained widespread global distribution and recognition (230). Fruitflow® is a dietary antiplatelet agent with a reversible mechanism of action and a milder antiplatelet effect than conventional antiplatelet medications such as aspirin, clopidogrel, and prasugrel (175). The antiplatelet tomato components present in Fruitflow® are known to protect the cardiovascular system by lowering platelet hyperactivity in response to potent platelet agonists such as adenosine diphosphate (ADP), collagen, arachidonic acid, thrombin, and inflammation. They may also aid in preventing endothelial dysfunction (231).

The findings of *in vitro* and *ex vivo* studies indicate that extracts derived from tomatoes and kiwifruits can mitigate platelet aggregation. The antiplatelet components found in tomato, strawberry, and kiwifruit have been observed to exhibit inhibitory effects on ACE (angiotensin-converting enzyme) and induce relaxation of the endothelium. These actions contribute to the protection of blood vessels (232). Recent studies have suggested that certain natural substances, such as olive oils, alperujo, ginseng, curcuminoids, and garlic, possess potential antiplatelet properties. Following the process of olive oil extraction, it has been observed that alperujo, the byproduct obtained, retains a significant amount of phenolic compounds. In their study, DeRoos et al. observed that applying alperujo extract at a concentration of 40 mg/L resulted in a notable reduction in platelet activation induced by ADP and TRAP in an *in vitro* setting. A recent study observed that the consumption of dietary vitamin-rich extra virgin olive oil for 1 year resulted in a significant reduction in ADP-induced blood platelet aggregation (233). The primary phenolic compound found in olive oil, known as hydroxytyrosol, has been observed to reduce platelet aggregation in humans (234) significantly. Ginseng has been historically employed to manage and mitigate cardiovascular disease (CVD) symptoms and its potential preventive properties. The inhibitory effects of ginsenosides on internal calcium mobilization and granule release have been observed, suggesting their potential as broad-spectrum antiplatelets (235). The platelet aggregation induced by ADP and arachidonic acid was significantly inhibited by curcuminoids derived from *Curcuma longa* plants (236). In a recent study, it has been observed that aged garlic extract can enhance the levels of cyclic nucleotides within the body. Moreover, it has been found to effectively inhibit the binding of fibrinogen, an essential protein involved in blood clotting. Additionally, the extract has been shown to mitigate platelet shape changes and modify their functional characteristics in response to collagen stimulation (237).

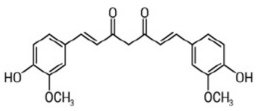
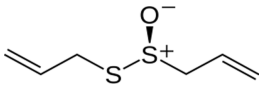
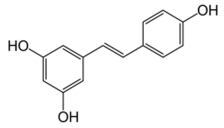
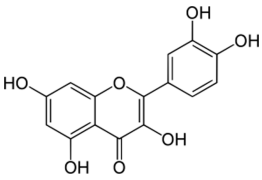
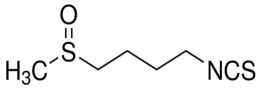
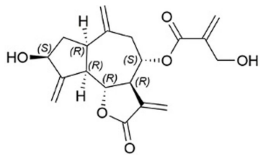
8.5. Natural bioactive compounds

Plant extracts, spices, herbs, and essential oils all include natural ingredients that have shown promise in treating MetS. However, these nutraceuticals are not suggested instead of standard pharmacotherapies for MetS since their advantages still need to be investigated (Table 1).

8.5.1. Curcumin

Curcumin is commonly found in Southeast Asian turmeric (*Curcuma longa*). Curcumin blocks NF-kB activation, resulting in lower levels of TNF-α and thrombotic plasminogen activator inhibitor

TABLE 1 List of Bioactive compounds and mechanisms in MetS.

Bioactive compounds	Category	Source	Structure	Mechanism and effects	Reference
Curcumin	Flavonoid polyphenol	*Curcuma longa* (Turmeric)		↑TNF-α, IL-6 ⊥ NF-kB	(238)
Allicin	Vegetable	*Allium sativum* (Garlic)		↑Nrf2 ↓SBP, Keap1, HIF-1α, and VEGF	(239)
Resveratrol	polyphenolic phytoalexin	3,5,4′-Trihydroxystilbene (Grapes, Apple)		↑Nrf2, ↓NF-kB	(240)
Quercetin	Flavonoid	2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one. (Berries, Grapes, Onion)		↑SOD, GPX, CAT ↓ROS, MDA, TNF-α, IL-1β, IL-10, Caspase-3, Bax/Bcl-2	(241)
Sulforaphane	Vegetable	1-Isothiocyanato-4-(methanesulfinyl) butane. (Broccoli, Cabbage)		⊥ Nrf2, NF-E2, NF-kB, MAPKs, P^{38}, JNK, ERK ↓TNF-α, IL-6, IL-1β, PGE2	(242)
Cynaropicrin (Cardoon)	Vegetable	*Cynara cardunculus* (Artichoke thistle)		↓TNF-α, IL-6, ROS, NO, ERK ↑Nrf-2	(243)

type 1 (238). Furthermore, curcumin stimulates peroxisome proliferator-activated receptor gamma (PPR-γ) in hepatic stellate cells and blocks the Wnt/−catenin pathway, contributing to obesity. As a result of curcumin's interference with leptin signaling, adiponectin expression is boosted. Obesity, IR, and inflammatory processes are all reduced in those with MetS (244). Research has shown that curcumin has the potential to enhance insulin sensitivity, inhibit the formation of fat cells, and lower high blood pressure, inflammation, and oxidative stress. As a result, it may help alleviate various aspects of MetS (245).

8.5.2. Allicin

The anti-inflammatory and antithrombotic qualities of garlic/Allicin (*Allium sativum*) make it useful in medicine. According to a study, fructose-fed rats whose diet included raw garlic had greater insulin sensitivity, suggesting that this food item could have similar benefits for people (246). Research shows that a placebo-controlled study reduces total cholesterol and triglyceride levels (247). According to research, aged garlic extract altered MetS patients' adiponectin levels and upregulated Nrf-2 express,sion, which raised adiponectin after 12 weeks (239). In addition, garlic's organosulfur derivatives reduce inflammation. The garlic organosulfur compounds were found to effectively reduce dyslipidemia and liver fat by boosting taurine levels and encouraging hepatic fatty acid-oxidation which helps in maintaining healthy glucose levels due to its ability to inhibit DPP-4 and hepatic gluconeogenesis (248). Garlic can cure MetS naturally because its thiol groups fight ROS-mediated inflammation.

8.5.3. Resveratrol

Resveratrol is a polyphenol found in grapes, nuts, and wine. It affects metabolism, oxidation, and aging via sirtuin pathway activation. It promotes lipolysis, reduces adipogenesis, and improves cellular energy balance. It inhibits antioxidant cyclooxygenase and Nrf-2 expression and downregulates NF-kB expression (240). Resveratrol enhances glucose tolerance, weight, insulin sensitivity, and BMI in MetS patients (249, 250). According to recent studies, resveratrol consumption has been linked to decreased risk of developing diabetes and its associated cardiovascular complications. Resveratrol has a protective effect due to its ability to regulate multiple signaling pathways. These pathways include those related to oxidative stress and inflammation, lipid metabolism, GLUT4 expression and translocation, and the SIRT1/AMPK signaling axis (251).

8.5.4. Quercetin

Quercetin, a plant-derived flavonoid, is found in onions, berries, and tea. Its metabolic activities include antioxidant and anti-inflammatory. Studies have shown that quercetin may help reduce the risk of developing osteoporosis, CVD, neuropathy, and certain types of cancer (252). Furthermore, it possesses various other advantages such as antiatherosclerotic, anti-inflammatory, anti-obesity,

anti-hyperlipidemic, anti-hypercholesterolemic, neuroprotective, anti-hypertensive, and anti-inflammatory properties (253). According to researchers, the metabolic effects of quercetin are thought to be influenced by the activation of transcription factors like PPAR-γ, AMPk, NF-kB, and SIRT1 (254). Quercetin inhibits obesity-causing adipokinesis and lipolysis through mitochondrial pathways. Obese Zucker fat rats were administered 2 mg/kg BW or 10 mg/kg BW of quercetin daily for 10 weeks. As a result, dyslipidemia, hypertension, and insulin resistance were reduced in these rats. The higher dose was the only one that effectively reduced body weight and inflammation in visceral adipose tissue. This was achieved by inhibiting the generation of tumor necrosis factor-alpha (255). Reduced adiponectin levels in the bloodstream have been linked to obesity, type 2 diabetes, and hypertension. Losing weight could lead to an improvement in these health conditions. The vasoprotective properties of quercetin have been linked to an increase in eNOS expression (255). Pfeuffer and coworkers discovered that in people with an apolipoprotein E genotype, quercetin reduces metabolic parameters, including waist circumference, postprandial blood glucose, and lipids, while increasing inflammatory markers like TNF-.α, IL-1β, IL-10, Caspase-3, Bax/Bcl-2 (241).

8.5.5. Sulforaphane

The anti-inflammatory and antioxidant characteristics of a phytochemical called sulforaphane (SFN), found in the Brassica family of plants like broccoli, may be used to treat MetS. It induces NF-E2, Nrf-2, and NF-kB expression and decreases inflammatory markers like TNF-α, IL-6, and IL-1β (242). In addition, experimental animal research has shown that sulforaphane may prevent the development of MetS hallmarks such as diabetes, hypertension, and hyperlipidemia (256). SFN interacts with the active cysteine residues of KEAP1, dissociating KEAP1 and the subsequent activation of NRF-2 (257). SFN acts as a cytoprotective factor in different types of cellular stress by triggering the oxidative stress response (OSR) and decreasing inflammation. Administering SFN can help control the NF-kB pathway, which may prevent diabetic neuropathy and high glucose-induced changes in animals (258). SFN protects against diabetic cardiomyopathy through increased NRF2 expression and correction of oxidative stress-induced modulation of the LKB1/AMPK pathway (259). Mice with nonalcoholic fatty liver disease were administered SFN, reducing their oxidative stress, endoplasmic reticulum stress, and mitochondrial dysfunction (260). SFN reduces the expression of adipogenic factors such as PPAR and CCAAT/enhancer-binding protein (C/EBP), which inhibits adipocyte formation and fat accumulation. The detection of AMPK activation upon SFN administration suggests that SFN may have potential anti-obesity and anti-diabetic benefits (261). Supplementation with SFN decreased fasting blood glucose and glycated hemoglobin (HbA1c) levels in obese individuals with dysregulated type 2 diabetes. The anti-diabetic properties of this substance are similar to those of metformin, considered the standard medication for treating diabetes (262).

8.5.6. Cardoon

The industrial potential of the cardoon plant is well acknowledged. It is an intriguing source of bioactive components, including cynaropicrin, phenolics, minerals, inulin, fiber, and sesquiterpene lactones, making it a functional food with great nutritional value. These bioactive compounds have anti-diabetic, anti-inflammatory, cardiotonic, antibacterial, anti-hemorrhoidal, antioxidant, anticancer, lipid-lowering, cytotoxic, and choleretic activities. The presence of vitamins, flavonoids, and polyphenols in *Cynara cardunculus L.* has been observed to contribute to its biological activity by scavenging free radicals and reducing oxidative stress. These properties have been associated with the plant's hypolipidemic, hepatoprotective, and antidiabetic effects, as well as it's capacity to increase Nrf-2 expression and reduce inflammatory markers such as TNF-α and interleukin-6 (243).

9. Conclusion

MetS is not classified as a disease *per se* but rather as a cluster of metabolic risk factors that have the potential to substantially elevate the likelihood of developing CVD. Despite several definitions for MetS, a singular purpose cannot be employed to assess potential hazards or outcomes in adolescents. Diagnosing the risk of MetS early on is imperative, as insulin resistance can lead to impaired glucose, lipid, and energy metabolism in various organs and tissues, resulting in the co-occurrence of multiple metabolic disorders. Alterations in migration, proliferation, and angiogenesis characterize the MetS. These modifications are caused by inflammation, hyperhomocysteinemia, oxidative stress, decreased angiogenic factors, and cellular senescence. Empirical data indicate a reciprocal association exists between hypertension, endothelial dysfunction, platelet hyperactivity, and MetS. The coexistence of hypertension, endothelial dysfunction, platelet hyperactivity, and MetS contributes to the advancement of organ damage. Thus, this population is critical to managing blood pressure, regulating the prothrombotic state, and controlling blood glucose.

A detailed understanding of the MetS mechanism will help develop effective prevention strategies and appropriate intervention tools. MetS and its components do not yet have specific therapy recommendations. Some patients are at moderate or high risk of developing atherosclerosis. The initial approach for managing the latter group involves implementing therapeutic lifestyle changes. In cases where the possibility of developing CVD is significant, it may be imperative to implement pharmacological measures to modify risk factors. Every person with MetS must go through a risk evaluation. Obesity, dyslipidemia, hypertension, insulin resistance, and hyperglycemia may all be successfully managed with dietary and physical activity changes. These treatments show the highest promise for managing MetS. A pharmacological approach targeting many MetS components and extensive gene and miRNA Therapy management may be necessary for high-risk people. MetS is often associated with gut microbiota dysbiosis, inducing a low-grade inflammatory response and insulin resistance via different mechanisms. This review did not discuss the gut microbiota as a potential target for treating MetS.

Although their success varies, lifestyle adjustments and other non-pharmaceutical methods are sometimes recognized as the first line of therapy. Improved insulin resistance, blood pressure, plasma lipid and lipoprotein metabolism, and general health may result from a diet high in nutrients and total calories, as well as from regular

physical exercise and healthy body weight. Pharmaceutical therapy is only somewhat beneficial. In contrast to when lifestyle measures are used alone, it is connected with more significant adverse results when used in conjunction with them.

Nutraceuticals are a popular therapy for MetS due to their low toxicity and minimal adverse effects. Using curcumin, resveratrol, quercetin, aged garlic extract, sulforaphane, and fruitflow may be a natural treatment for hypertension, platelet hyperactivity, and endothelial dysfunction,. More efforts must be undertaken in animal and clinical studies to evaluate the influence of these compounds on CVD risk factors. In addition, the prevention and treatment of underlying risk factors might lower CVD prevalence in the general population.

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Author contributions

AD conceptualized the review. DD, GJ, and NRS wrote the manuscript. SP, AB, GJ, and AD corrected the manuscript. All authors have 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

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Metabolic syndrome in relation to dietary acid load: a dose–response meta-analysis of observational studies

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Background and aim: Several studies have identified that dietary acid load (DAL) may be associated with the odds of metabolic syndrome (MetS); however, the evidence is inconclusive. This dose–response meta-analysis aimed to examine the relation of DAL to MetS.

Methods: A systematic literature search was carried out in PubMed and Scopus up to April 2023 for pertinent studies evaluating the relation of DAL scores, including potential renal acid load (PRAL) and net endogenous acid production (NEAP), to the odds of MetS. The odds ratios (OR) with 95% confidence intervals (CI) were pooled using a random-effects meta-analysis to test the association.

Results: Eight studies, with an overall sample size of 31,351 participants, were included in this meta-analysis. Higher DAL scores were significantly related to the elevated odds of MetS (NEAP: OR = 1.42, 95%CI = 1.12–1.79; PRAL: OR = 1.76, 95%CI = 1.11–2.78), with significant evidence of heterogeneity across studies. The linear dose–response analysis proposed that a 10 mEq/day elevation in NEAP and PRAL was linked to a 2% (OR = 1.02, 95%CI = 1.001–1.05) and 28% (OR = 1.28, 95%CI = 1.11–1.47) increased odds of MetS, respectively. No non-linear association was observed between MetS and NEAP (P-non-linearity = 0.75) and PRAL (P-non-linearity = 0.92).

Conclusion: This study revealed a significant direct relationship between DAL and MetS. Therefore, lower acidogenic diets are suggested for the prevention of MetS.

KEYWORDS

dietary acid load, NEAP, PRAL, metabolic syndrome, meta-analysis

1. Introduction

Metabolic syndrome (MetS), characterized by a cluster of metabolic abnormalities including insulin resistance, obesity, dyslipidemia, and hypertension, has become a prominent concern worldwide, owing to its prevalence and association with chronic diseases such as cardiovascular diseases, type 2 diabetes, and stroke (1–3). Genetics and environmental factors are both involved in the etiology of this disorder (4). Given its high prevalence and adverse health consequences, it is essential to identify preventive approaches against MetS.

Evidence has suggested the contribution of numerous dietary factors to the development of MetS; however, the focus should be on the dietary patterns rather than on single food items/ingredients as dietary patterns yield the whole effect of diet by considering the complex interactions between various food components (5–7). In recent years, the role of dietary acid load (DAL), as measured by the net endogenous acid production (NEAP) and potential renal acid load (PRAL), has been of particular interest. NEAP and PRAL reflect the dietary content of acid-or base-forming compounds that may affect the acid–base balance in the body (8). The PRAL is computed based on the dietary consumption of magnesium, potassium, phosphorous, calcium, and protein, while NEAP is computed using the intakes of potassium and total protein, which partly play a role in metabolic acidosis (9). A diet high in animal protein and grains and low in fruits and vegetables typically leads to a high acid load (8). Diet has been demonstrated to be a leading contributor to variations in endogenous acid production in different people (10).

Although associations between DAL and individual features of MetS have been reported, evidence regarding the link between DAL and the overall odds of MetS is scarce and highly controversial (11–13). The relation of DAL to MetS may differ by race, gender, geographic region, and other demographic features of various populations. Therefore, this meta-analysis aims to summarize the current evidence on the association between DAL, defined by NEAP and PRAL, and the odds of MetS.

2. Materials and methods

This meta-analysis was implemented by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) protocols (14).

2.1. Search strategy

We performed a systematic literature search with no language restriction through the Scopus and PubMed databases to find all pertinent studies published up to April 2023. The search strategy was as follows: ((((((((((("Dietary acid load"[Title/Abstract]) OR ("dietary acid–base load"[Title/Abstract])) OR ("dietary acidity"[Title/Abstract])) OR ("net acid load"[Title/Abstract])) OR ("acid excretion"[Title/Abstract])) OR ("potential renal acid load"[Title/Abstract])) OR (PRAL [Title/Abstract])) OR ("net endogenous acid production"[Title/Abstract])) OR (NEAP [Title/Abstract])) OR ("protein to potassium ratio"[Title/Abstract])) OR ("protein/potassium ratio"[Title/Abstract])) OR ("potential renal acid load"[Title/Abstract])) AND ((("Metabolic Syndrome"[Mesh]) OR (metabolic syndrome [Title/Abstract])) OR (insulin resistance syndrome [Title/Abstract])). The reference lists of the associated publications were also screened manually to evade missing any study.

2.2. Inclusion criteria

For the present meta-analysis, publications were expected to meet all the following criteria to be eligible for inclusion: (1) observational studies (prospective, case–control, or cross-sectional) investigating the relation of DAL, assessed by NEAP or PRAL (15, 16), to MetS; (2), studied reported odds ratios (OR), relative risk (RR), or hazard ratios (HR) and their 95% confidence intervals (CI) (or provided sufficient data to calculate them) for the relations of DAL indices, NEAP or PRAL, to the odds of MetS. We excluded reviews, letters, comments, conference papers, animal studies, and studies with irrelevant exposure/outcome during the screening of studies.

2.3. Data extraction and quality assessment

Data were extracted by two independent reviewers with the use of a standardized data extraction form, and inconsistencies were resolved by discussion among all authors. The following information was obtained from each publication: first author, publication year, type of exposure (NEAP or PRAL), mean or range of age, total sample size, number of MetS cases, gender, type of study, country, method of dietary assessment, the definition used for the diagnosis of MetS, confounder variables adjusted for in analyzes, and effect sizes (OR, RR, or HR with their 95%CI). If a publication applied both NEAP and PRAL for the evaluation of DAL, both effect sizes for NEAP and PRAL were extracted separately. When necessary, we contacted the corresponding authors to obtain publications. The quality of the studies was evaluated with the use of the Newcastle–Ottawa scale (NOS), in which scores of 0–3, 4–6, and 7–9 were considered as low, moderate, and high quality, respectively (17).

2.4. Statistical analysis

The included studies reported effect sizes for the associations in various models; for the present meta-analysis, we obtained the ORs and 95%CIs in the highest category of NEAP or PRAL scores, compared to the lowest category, in the most adjusted model. The ORs and 95%CIs in the highest vs. lowest N tiles of DAL were used as effect size in the meta-analysis to compute the pooled effect for the association. Heterogeneity across the studies was measured with the use of the Q-statistics and I^2 values, and $I^2 > 50\%$ or $p < 0.1$ were considered as statistically significant evidence of heterogeneity (18, 19). Because of the anticipated heterogeneity, data were pooled by the DerSimonian–Laird random-effects model (20). To instigate possible sources of heterogeneity, subgroup analysis by definition of MetS and the sex of participants was performed. Using the two-stage generalized least-squares trend estimation approach, a linear dose–response meta-analysis, as reported by Greenland and Longnecker (21), was carried out for the odds of MetS associated with each increment of 10 mEq/day in NEAP and PRAL. To obtain the overall average slope,

study-specific slope lines were first computed, and these lines were then blended using a random-effects model (22). To investigate non-linear associations, restricted cubic splines for each study with ≥3 categories of exposure were computed with the use of three fixed knots at 10, 50, and 90% through the total distribution of reported exposure, then combined with the use of multivariate meta-analysis (23–25). The distribution of cases and non-cases, the mean or median of the NEAP or PRAL scores, and the ORs with the 95%CIs for at least three exposure categories were needed for the dose–response analysis. Publication bias was also evaluated by funnel plots and Egger's test (26, 27). All statistical analyzes were performed with the Stata software (version 14). $p < 0.05$ was considered statistically significant for the relation of NEAP and PRAL to MetS.

3. Results

3.1. Characteristics of studies

The systematic literature search yielded a total of 210 publications. Of these, 47 studies were duplicates, and 139 studies were irrelevant based on the titles/abstracts, and these were thus excluded. The full texts of 24 potentially pertinent publications were reviewed, and finally, a total of eight studies (8, 9, 11–13, 28–30), with 31,351 participants, published from 2015 to 2022, were included in the meta-analysis according to the inclusion criteria. The study by Arisawa et al. reported the results for men and women separately; thus, two effect sizes were extracted from this study (8). The flow diagram of the study selection is reported in Figure 1. All studies were cross-sectional in design. Among the studies, five were performed in Iran (9, 12, 28–30), two were performed in Japan (8, 11), and one was from Italy (13). All analyzed studies reported effect sizes that were controlled for the potential covariate except for the study by Sanz et al. (13), which was based on a crude analysis without adjustment for confounders. The effect sizes for NEAP and PRAL were available in seven studies (with eight effect sizes) (8, 9, 11, 13, 28–30) and seven studies (9, 11–13, 28–30), respectively. Two publications reported effect sizes only for women (9, 12), one only for men (28), four for the combination of both genders (11, 13, 29, 30), and one for men and women separately (8). Dietary assessment was based on a food frequency questionnaire (FFQ) in seven studies (8, 9, 12, 13, 28–30) and a diet history questionnaire in one study (11). The definition of MetS was based on the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) in five studies (9, 12, 13, 28, 29), Joint Interim Statement Criteria (JIS) of 2009 in two studies (8, 11), and International Diabetes Federation (IDF) in one study (30). The quality of the included studies was moderate to high, with NOS scores ranging from 7 to 9 (Supplementary Table S1). The characteristics of the studies are presented in Table 1.

3.2. Findings from the meta-analysis

Among seven studies investigating the relation of NEAP to the odds of MetS, four studies (8, 11, 13, 28) identified a significant direct association between diets with high NEAP and odds of MetS. On the other hand, two studies (11, 13) out of seven studies on PRAL found a positive significant relationship between high PRAL and MetS. In the pooled analysis of available evidence by the random-effects model, a significant association between MetS and

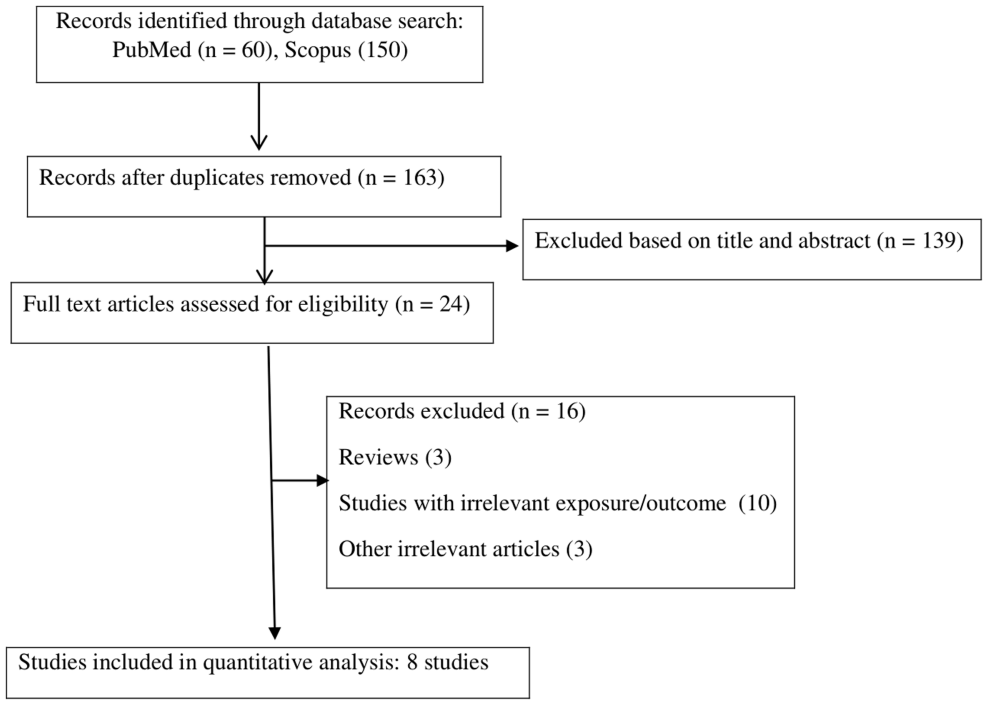

FIGURE 1
Flow chart for studies selection.

TABLE 1 Characteristics of included studies.

Author	Year	Study design	Location	Sex	No. of participants	Cases	Age (mean ± sd or range)	Exposure type	Exposure assessment	MetS diagnosis	Adjustment
Arisawa et al.	2020	Cross-sectional	Japan	Men	14,042	3,155	35–69	NEAP	*FFQ*	Joint Interim Statement Criteria of 2009	Adjusted for age, study site, smoking and drinking habits, physical activity level, total energy intake, and school career, plus nutrient pattern (fiber, iron, potassium, and vitamins pattern) scores
				Women	14,105	1,472					
Sanz et al.	2022	Cross-sectional	Italy	Both	448	74	55–80	NEAP PRAL	*FFQ*	NCEP ATP III	Crude
Iwase et al.	2015	Cross-sectional	Japan	Both	149	67	65.7 ± 9.3	NEAP PRAL	Diet history questionnaire	Joint Interim Statement Criteria of 2009	Adjusted for age, sex, serum uric acid and creatinine, total energy intake, carbohydrate intake, and sodium intake
Jafari et al.	2021	Cross-sectional	Iran	Men	357	NR	>60 years	NEAP PRAL	*FFQ*	NCEP ATP III	Adjusted for age, smoking, physical activity, socioeconomic status, marital status, energy, disease, anti-diabetic drugs, thyroid drugs, and heart disease drugs
Mozaffari et al.	2019	Cross-sectional	Iran	Women	371	NR	20–50	NEAP PRAL	*FFQ*	NCEP ATP III	Adjusted for energy intake, age, marital status, socioeconomic status, and BMI
Rezazadegan et al.	2022	Cross-sectional	Iran	Both	203	79	12 to 18 y	NEAP PRAL	*FFQ*	IDF	Adjusted for age, sex, energy intake, physical activity, socioeconomic status, and BMI
Tangestani et al.	2022	Cross-sectional	Iran	Women	246	79	36.49 ± 8.38	PRAL	*FFQ*	NCEP ATP III	Adjusted for age, physical activity, and socio-economic, marital, and education status
Mohammadifard et al.	2020	Cross-sectional	Iran	Both	1,430	205	38.70 ± 10.66	NEAP PRAL	*FFQ*	NCEP ATP III	Adjusted for age, physical activity, and BMI

BMI, body mass index; FFQ, food frequency questionnaire; NEAP, net endogenous acid production; PRAL, potential renal acid load; NCEPATP III, National Cholesterol Education Program Adult Treatment Panel III; IDF, International Diabetes Federation.

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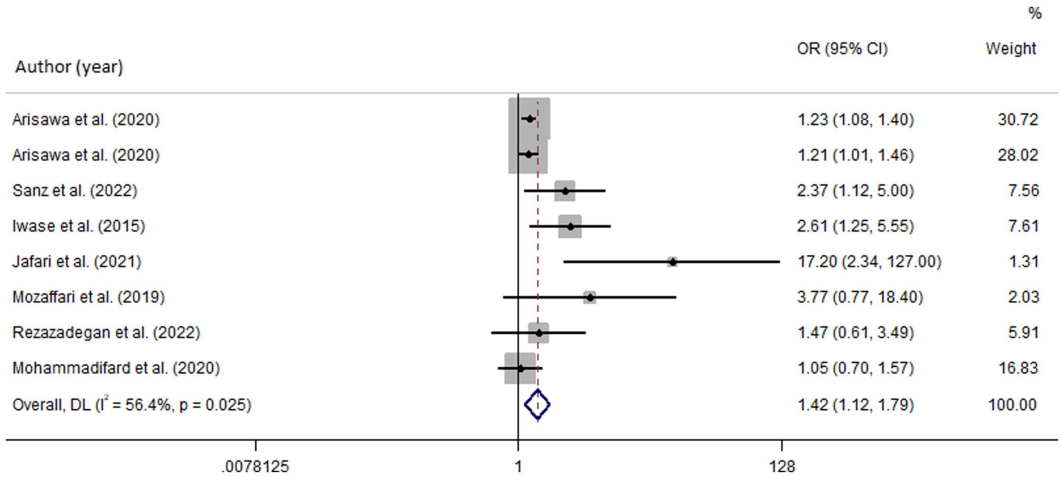

FIGURE 2
Forest plot of the pooled data for the association between high dietary acid load (based on NEAP) and odds of metabolic syndrome.

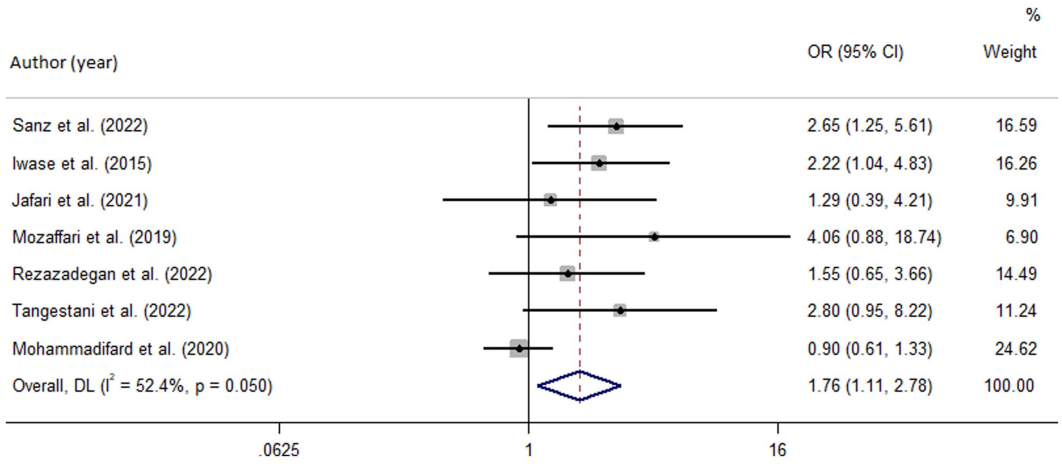

FIGURE 3
Forest plot of the pooled data for the association between high dietary acid load (based on PRAL) and odds of metabolic syndrome.

the indices of the dietary acid load was detected [NEAP: OR = 1.42, 95%CI = 1.12–1.79 (Figure 2); PRAL: OR = 1.76, 95%CI = 1.11–2.78 (Figure 3)]. There was significant evidence of heterogeneity across studies (NEAP: $I^2 = 56.4\%$, $p = 0.02$; PRAL: $I^2 = 52.4\%$, $p = 0.05$). In the linear dose–response meta-analysis, each 10 mEq/day increment in NEAP and PRAL was linked to a 2% (OR = 1.02, 95%CI = 1.001–1.05) and 28% (OR = 1.28, 95%CI = 1.11–1.47) increased odds of MetS, respectively (Figure 4). No evidence for a non-linear relationship was observed for NEAP (P-non-linearity = 0.75) and PRAL (P-non-linearity = 0.92) (Figure 5). The funnel plots revealed a remarkable asymmetry, with significant publication bias in studies on NEAP (Egger's test $p = 0.02$) and PRAL (Egger's test $p = 0.03$) (Figure 6).

3.2.1. Subgroup analysis

In the subgroup analysis by definition of MetS (Supplementary Figure S1) and the sex of participants (Supplementary Figure S2), the association of NEAP with MetS was supported by studies with NCEP ATPII definition for MetS (OR = 2.53, 95%CI = 1.003–6.43) and studies on both genders (OR = 1.65, 95%CI = 1.01–2.70), but not in men and women subgroups, as well as in studies with JIS and IDF criteria for MetS. Moreover, PRAL was significantly linked to the odds of MetS based on the JIS definition for MetS (OR = 2.22, 95%CI = 1.01–4.78) (Supplementary Figure S3) and in women (OR = 3.17, 95%CI = 1.31–7.65; Supplementary Figure S4).

3.2.2. Sensitivity analysis

In the sensitivity analysis, by removing single studies step by step from the main analysis, no individual study significantly affected the pooled effect sizes for the relation of NEAP and MetS (Supplementary Figure S5), showing the reliability of the findings; however, the sensitivity analysis remarkably affected the relation of PRAL to MetS (Supplementary Figure S6).

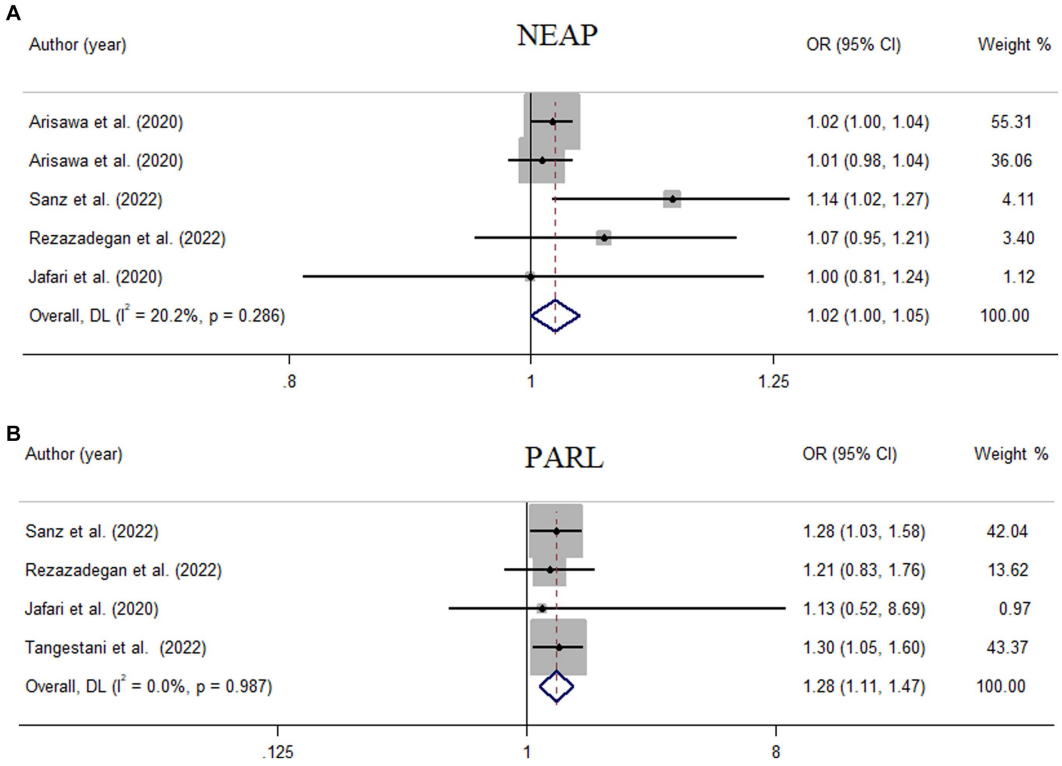

FIGURE 4
Meta-analysis of the linear association of dietary acid load for a 10-unit increment in NEAP **(A)** and PRAL **(B)** with odds of metabolic syndrome.

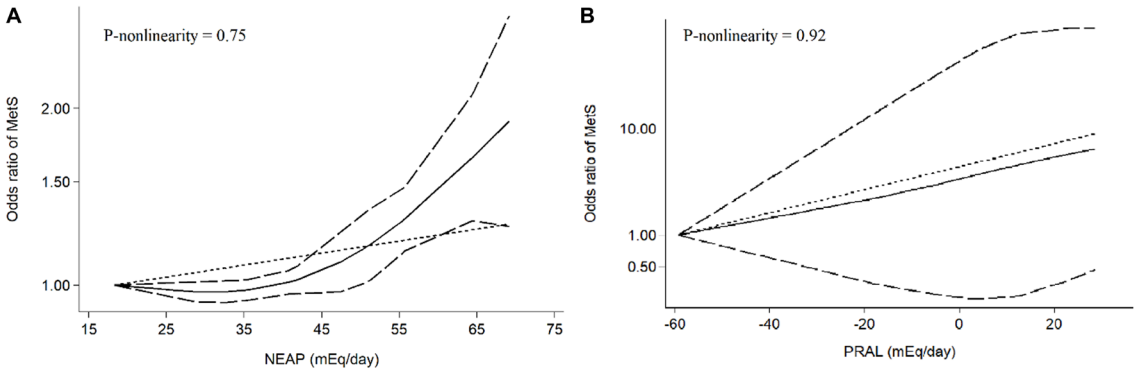

FIGURE 5
Meta-analysis of the non-linear association between dietary acid load [**(A)** NEAP, **(B)** PRAL] and odds of metabolic syndrome.

4. Discussion

The present meta-analysis aimed to investigate the relation of DAL to the odds of MetS. The results identified that higher DAL measured by PRAL and NEAP is significantly associated with elevated odds of MetS. There was a 28 and 2% elevated odds of MetS for each 10 mEq/day increment in PRAL and NEAP, respectively.

Diet is a main environmental factor influencing the odds of MetS (31). Recently, epidemiological evidence has suggested that DAL may play a role in MetS (28). Nevertheless, the available evidence in this regard is contradictory. Iwase et al. identified that, in Japanese patients with type 2 diabetes, higher scores for NEAP and PRAL were linked to the elevated prevalence of MetS (11). In contrast, four cross-sectional investigations on the Iranian population found no relationship between DAL and MetS (9, 12, 29, 30). In agreement with our findings, the study by Arisawa et al. on 14,042 men and 14,105 women revealed that metabolic acidosis, defined by high NEAP, is significantly related to increased odds of MetS (8). Recent meta-analyzes have also demonstrated a close direct relationship between DAL and single components of MetS, including increased obesity (32), impaired glucose metabolism (10), hypertension (33), and dyslipidemia (32), supporting our findings that a diet with a higher

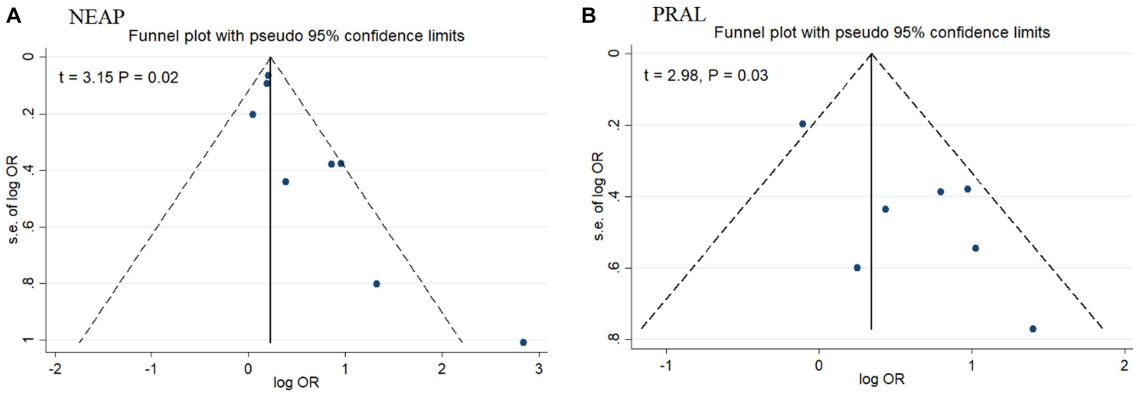

FIGURE 6
Funnel plot for publication bias in studies on NEAP **(A)** and PRAL **(B)**.

DAL may impair metabolic health. The diverging findings of previous studies might result from differences in gender, sociodemographic characteristics of the populations, and the sample size of the studies. Moreover, the varying results may be due to the high differences in the range of PRAL and NEAP across the available evidence. In the present meta-analysis, the strength of association between PRAL with MetS was stronger than NEAP. It has been recognized that PRAL is a more precise indicator of DAL since, in contrast to the NEAP score, which only takes dietary intake of protein and potassium into account, the PRAL score considers dietary intake of protein and several micronutrients, phosphorus, potassium, magnesium, and calcium, as well as the rate at which the nutrients are absorbed in the intestinal border (34). As a result, PRAL is a better predictor of the impacts of diet acidity on health outcomes (10, 35).

Regarding underlying biological mechanisms, high DAL values may be linked to the development of MetS through several interlinked mechanisms, including chronic low-grade inflammation (36), mineral imbalances (37), alterations of the gut microbiota (38), and insulin resistance (39). Chronic low-grade inflammation is a critical component of the pathogenesis of MetS (40). Acidic diets have been reported to induce inflammation by increasing the production of proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), activating the toll-like receptor (TLR) signaling pathway, and promoting the infiltration of immune cells into adipose tissue (28, 41).The inflammation may further aggravate MetS components such as insulin resistance, hypertension, and dyslipidemia (42). High DAL has been shown to induce insulin resistance, a central process contributing to MetS, by impairing insulin signaling pathways and promoting inflammation and oxidative stress (43). Furthermore, in response to acidic diets, the body releases cortisol (44), a stress hormone that inhibits insulin action, induces lipase activity, and promotes gluconeogenesis, leading to hyperglycemia, hyperinsulinemia, and hypertriglyceridemia (9, 45). The production of acids in the body increases hydrogen ion concentration, leading to a decrease in pH. To neutralize this acidity, the body utilizes alkaline reserves such as bicarbonate, calcium, and magnesium (29, 37). Acidic diets, which are high in animal protein and low in fruits and vegetables (46), tend to decrease the pH of the blood and urine, causing the depletion of alkaline reserves. Such mineral imbalances impair insulin action, disrupt lipid metabolism, and elevate blood pressure, all of which are components of MetS (8, 47). Lastly, a diet with high acidity may lead to dysbiosis of the gut microbiota (38), which has been linked to inflammation, insulin resistance, and dyslipidemia as components of MetS (38, 48). Another mechanism that leads to MetS in response to higher DAL is mediated by increasing adiposity (49), a fundamental component involved in the pathogenesis of all MetS components (9). Accordingly, reducing the intake of acid-forming dietary factors such as animal-based foods, which are high in Western diets, and increasing the consumption of alkaline-forming food items such as fruit, vegetable, potassium, calcium, and magnesium may help prevent or manage MetS (28, 50). Further research is needed to elucidate the mechanisms underlying the association between DAL and MetS and to develop appropriate interventions to mitigate the odds of this disease.

To the best of our knowledge, this was the first meta-analysis evaluating the relation of DAL to MetS. Both NEAP and PRAL scores, as indicators of DAL, were used to analyze the associations with the outcome. We also performed linear and non-linear dose–response analyzes to better understand the pattern of the relationship between DAL and MetS. Despite these strengths, some limitations of our study should be declared. First, all the included publications were cross-sectional in design, and causal inference could not be obtained from the results. While the analyzed data were obtained from large population-based publications with satisfactory quality, prospective and clinical trial studies are required to confirm our findings. Second, there was a remarkable heterogeneity across the studies. This heterogeneity may result from differences in the dietary assessment tools, criteria used to define MetS, genetic background, the level of adjustment for covariates, study population characteristics, and differences in FFQ items in the various populations. Third, significant evidence of publication bias was observed for studies on NEAP and PRAL; our search was limited to English-language publications, which may mean that some studies were ignored. Fourth, even though the majority of the studies controlled the results for potential confounders, residual and unknown confounding factors still might have influenced the findings. Fifth, the included studies were from limited geographic regions (Iran, Japan, and Italy); therefore, the pooled results might not be expandable to all populations. Sixth, calculations of DAL indices were based on self-reported retrospective questionnaires, which are at risk of recall bias. Moreover, the results of the subgroup analyzes should be interpreted with caution because of the small number of the included studies in each subgroup. Sensitivity analysis also revealed that the relation of PRAL to MetS was remarkably affected by single

studies, reducing the stability of the results. However, the results were stable for NEAP in the sensitivity analysis. Lastly, DAL is more reliable when diets supply the recommended dietary allowance of protein (0.8 g/kg body weight). In the case of extremely low intake of protein, PRAL would take negative scores; in such a condition, negative scores for PRAL are not representative of an alkaline situation but indicate an unhealthy condition. The included studies did not consider the sufficiency of dietary protein intake and thus may be at risk of an inaccurate estimation of diet-related acidosis.

5. Conclusion

The results of the present meta-analysis propose that high DAL is related to the increased prevalence of MetS. Additional studies, particularly prospective cohort and clinical trials, are needed to elucidate the association between DAL and MetS and to reveal the underlying mechanisms.

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

SA-H, FM, SSA, NG, and RA: conceptualization and software. ES, NG, SNA, KO, and RR-P: methodology. BH, NG, FM, SNA, and SSA: validation. NG, BH, FM, and RR-P: formal analysis. SA-H, RA, ES, and KO: investigation. SA-H, FM, SSA, RA, ES, and KO: data curation and writing–original draft preparation. SNA, NG, BH, and RR-P: writing–review and editing. SA-H: visualization and project administration. SA-H and NG: supervision and funding acquisition. 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.

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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.2023.1233746/full#supplementary-material

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Can flaxseed supplementation affect circulating adipokines in adults? An updated systematic review and meta-analysis of randomized controlled trials

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Introduction: The findings of randomized controlled trials (RCTs) regarding the effect of flaxseed on adipokine concentrations are conflicting. Therefore, the present meta-analysis was conducted to provide definite and conclusive results.

Methods: Systematically, Scopus, Embase, PubMed, Web of Science databases, and Google Scholar were searched for relevant literature published up to December 2022. Based on random-effect models, standard mean differences (SMDs) were calculated for net changes in adipokine concentrations.

Results: Overall, 13 RCTs (15 arms) were eligible to be included. The results indicated that leptin was significantly reduced after the intervention with flaxseed supplement (SMD = −0.69, 95% CI: −1.37, −0.01; $p = 0.048$; $I^2 = 92.0\%$, $p < 0.001$). In addition, flaxseed supplements had no considerable effect on plasma adiponectin (SMD = 0.52, 95% CI: −0.20, 1.25, $p = 0.159$; $I^2 = 92.0\%$, $p < 0.001$).

Discussion: Flaxseed significantly improves leptin but does not affect adiponectin concentrations. Additional future well-designed trials are required to further assess the potential benefits of flaxseed on adipokines in humans.

KEYWORDS

flaxseed, adiponectin, leptin, meta-analysis, systematic review

1. Introduction

The most common circulating hormone secreted by adipocytes is adiponectin. Adiponectin regulates many metabolic pathways, including fatty acid modulation and glucose oxidation (1). The high-molecular weight (HMW) of adiponectin is also considered a risk factor for metabolic syndrome (MetS) (2, 3). Previous studies have demonstrated that adiponectin levels are lowered in people with type 2 diabetes (T2DM), MetS, and cardiovascular disease (CVD) (4). High plasma levels of adiponectin have also been associated with a lower risk of myocardial infarction in men (5). Leptin is a hormone that regulates energy intake and consumption. It may also have an effect on the pathways that regulate glycolytic enzyme activity, glucose uptake, and the production of inflammatory cytokines (6–8).

Flaxseed (*Linum usitatissimum*), which is an oil seed or grain, has been suggested as a possible functional food since it contains bioactive components (9). Alpha-linolenic acid (ALA), which makes up ∼55% of the total fatty acid content, is present in high amounts. Lignans, a group of phytoestrogens, are also present. There is also dietary fiber that makes

up 28% of the weight, and up to one-third is soluble fiber (10). These characteristics suggest that flaxseed may have anti-inflammatory effects and clinical intervention trials have been conducted to ascertain whether flaxseed and flaxseed-derived products (flaxseed oil, whole flaxseed, or lignans) are effective in reducing a variety of cardiovascular risk factors, especially inflammatory indicators such as C-reactive protein (11–14).

In addition to the potential anti-inflammatory capabilities of flaxseed, adiponectin expression has been found to be induced by several flaxseed components in preclinical animal models (15). Additionally, flaxseed oil increased the expression of hepatic adiponectin receptors and circulating adiponectin (16). Other investigations have reached the conclusion that variations in leptin expression may contribute to the possible cardioprotective benefits of flaxseed supplementation (17). According to experimental research, ALA can bind to peroxisome proliferator-activated receptor gamma (PPARγ), which can enhance adiponectin expression and levels in the blood (18, 19). Other clinical trials have reported that ALA enhances adiponectin; in fact, ALA and adiponectin production was found to have a dose–response relationship (20). However, randomized controlled trials (RCTs) have shown conflicting outcomes. The effects of flaxseed on adiponectin and leptin levels were evaluated in a previous meta-analysis published in 2020 (21); however, several trials did not fully measure changes in their concentration. We conducted an additional study on the effects of flaxseed on leptin and adiponectin levels in adults as a result of the contradictory findings of the previous studies and the lack of a comprehensive meta-analysis. To determine the effect of flaxseed supplementation and flaxseed-derived products on adiponectin and leptin levels, the present study performed a comprehensive systematic review and meta-analysis of all relevant RCTs in adults.

2. Methods

This systematic review and meta-analysis was carried out and reported under the Preferred Reporting Items of Systematic Reviews and Meta-Analysis (PRISMA) statement guidelines (22).

2.1. Search strategy

We searched international databases, including Scopus, Embase, PubMed, Web of Science databases, and Google Scholar, from inception to December 2022, using the following keywords: (flax OR flaxseed OR linseed OR lignan OR whole flaxseed OR ground flaxseed OR flaxseed oil OR *L. usitatissimum*) **AND** (adiponectin OR adipocytokines OR leptin). The search strategy is presented in Supplementary Table S1. The search process was conducted by two researchers (VM and AHM). In addition, reference lists were searched from included studies.

2.2. Eligibility criteria

Retrieved studies were included in our meta-analysis if they met the following evidence-based PICOS criteria: (1) Patients: adult individuals >18 years old; (2) Intervention: flaxseed supplementation; (3) Control: placebo or control; (4) Outcomes: sufficient data for extraction regarding adiponectin and leptin levels; and (5) Study design: RCTs. *In vitro*, *in vivo*, and *ex vivo* studies, observational studies, quasi-experimental studies, and animal studies were excluded from this meta-analysis. Only articles in English were included in the study.

2.3. Data extraction

Two independent researchers (SA and VM) screened and extracted data from each qualified trial. First author's name, publication year, study location, study design, sample size in each group, dose and type of flaxseed, duration of intervention, average age, gender and baseline body mass index (BMI) of subjects, and mean and standard deviation (SD) of adipokines in both groups at baseline and at the end of the study and their changes from baseline were extracted from the selected RCTs. Any disagreement about the choice of studies was settled by consensus (AHF).

2.4. Quality assessment and assessment of the meta-evidence

The methodological quality assessments of each included study were performed independently by at least two researchers using the Cochrane Collaboration risk of bias tool, in which domains were judged as "low-risk, high-risk, or unclear" (23). The credibility of RCTs was evaluated using the Grading of Recommendations, Assessment, and Evaluation (GRADE) approach, which consisted of five factors as follows: risk of bias, consistency of results, directness, precision, and potential for publication bias. The evidence is categorized into four categories, namely high, moderate, low, or very low.

2.5. Statistical analysis

The STATA program (version 16) was used to conduct the statistical analysis (Stata Corp, College Station, TX). To assess the effect size for adipokines, SD and mean differences were determined for the two groups. Furthermore, a random-effects model was used to estimate standardized mean differences (SMDs) with 95% confidence intervals (CIs) (24). When standard error (SE) or confidence interval (CI) was reported, they were also transformed into SD. Heterogeneity between studies was assessed using I^2 and the p-value of Cochran's Q-test. We performed a subgroup analysis according to baseline BMI (<30, ≥30), study quality (high and low), intervention duration (<12 and ≥12 weeks), type of flaxseed (whole flaxseed and flaxseed oil), sample size (≤40 and >40), the health condition [T2DM, polycystic ovary syndrome (PCOS), obesity, and others], gender (men, women, and both), and average age (<50 and ≥50 years) to identify potential sources of heterogeneity. We also performed a sensitivity analysis to determine the effect of removing one particular study from

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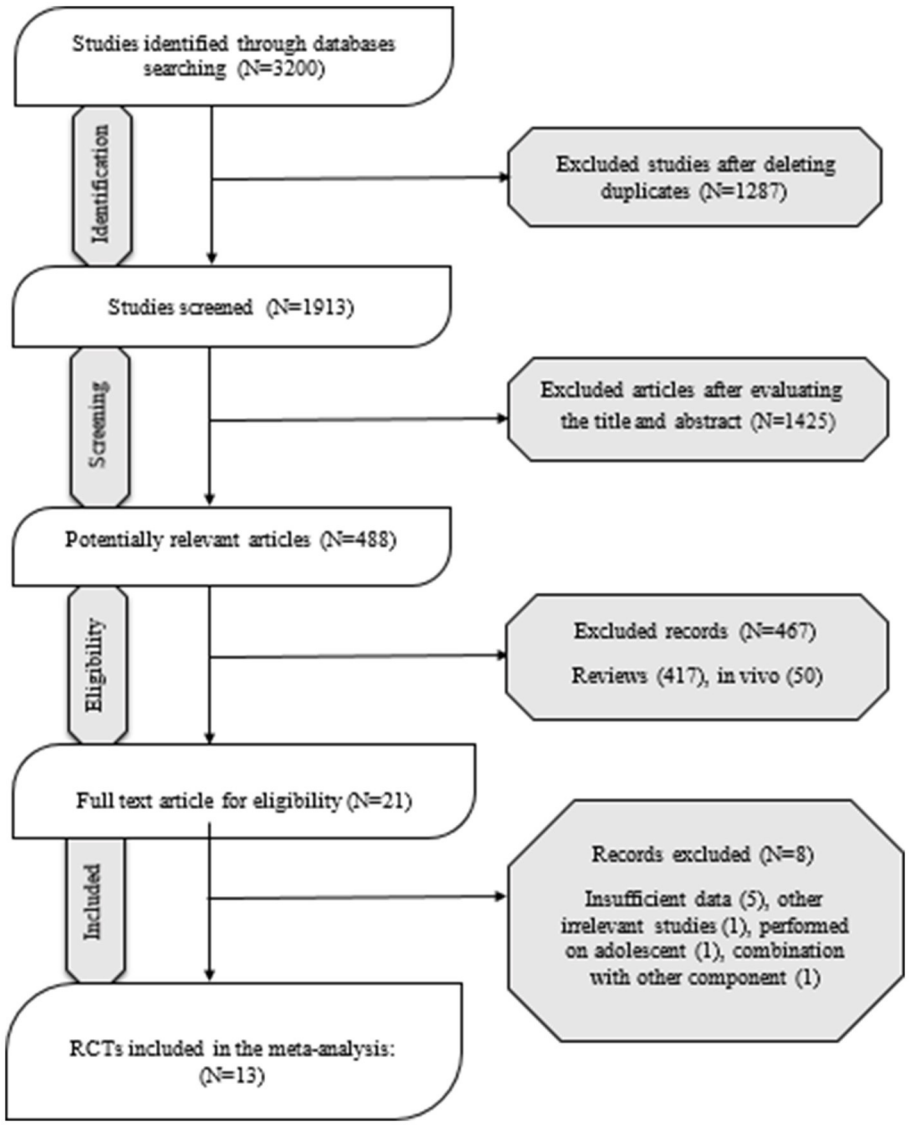

FIGURE 1
PRISMA flowchart diagram.

the overall SMDs. Begg's adjusted rank correlation and Egger's regression asymmetry test were applied to examine the results of the small study effect (25, 26). Publication bias was assessed by visual inspection of funnel plots. If there was evidence of publication bias, the "trim and fill" method was carried out. All statistical tests were two-sided, and a p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Flow and characteristics of included studies

A total of 3,200 studies were identified in the databases, and 1,287 duplicates were excluded. In total, 1,425 studies were evaluated based on the title and abstract, and 467 were deemed irrelevant. There were 21 studies that went through a full-text evaluation, and 8 were omitted. Finally, 13 studies were included in the analysis. Figure 1 shows the selection process of the study. Studies were conducted in Iran (27, 28), China (29), Canada (30), USA (31–34), Greece (35, 36), and Brazil (37–39). The range of intervention periods varied from 2 to 12 weeks. Whole flaxseed (27–30, 38, 39) and ground flaxseed (33) were used in four RCTs with doses from 13,000 to 60,000 mg/day. In the other studies, flaxseed oil (30–32, 34–37) was used, with doses of 3,500 to 14,200 mg/day. In this study, different patient populations were examined in eligible RCTs. Included subjects were patients with obesity (27, 29, 31, 38, 39), dyslipidemia (35), T2DM (30, 37), pre-diabetes (33), and PCOS (28, 32, 34), and healthy people (36). Detailed characteristics of the included studies are summarized in Table 1.

TABLE 1 Study characteristics of included studies.

Author, year	Design	Participants, *n*	Health condition	Age, year	Intervention		Baseline adiponectin	Baseline leptin	Duration (week)
					Treatment group	Control group			
Paschos et al. (35)	RA/SB/parallel	M: 35 Int: 18, Con: 17	Dyslipidemia	Int: 49, Con: 54	15 ml/day flaxseed oil	Safflower oil	Int: 5.97 μg/ml, Con: 5.98 μg/ml	–	12
Nelson et al. (31)	RA/parallel	M/F: 57 Int: 30, Con: 27	Overweight and obese	Int: 38.8, Con: 38.15	11,000 mg/day flaxseed oil (capsule)	Normal diet	Int: 10.12 μg/ml, Con: 7.93 μg/ml	–	8
Faintuch et al. (38)	RA/DB/crossover	M/F: 24 Int: 14, Con: 10	Obese	40.8	30,000 mg/day flaxseed flour	Manioc flour	–	Int: 27.3 ng/ml, Con: 27 ng/ml	2
Taylor et al. (30)	RA/parallel	M/F: 22 Int: 13, Con: 9 M/F: 21 Int: 12, Con: 9	T2DM	52.4	32,000 mg/day milled flaxseed 13,000 mg/day flaxseed oil	Placebo	Int: 10.5 μg/ml, Con: 9.8 μg/ml Int: 6.9 μg/ml, Con: 9.8 μg/ml	Int: 10 ng/ml, Con: 25 ng/ml Int: 10.3 ng/ml, Con: 25 ng/ml	12
Faintuch et al. (39)	RA/SB/parallel	M/F: 28 Int: 10, Con: 18	Obese	Int: 47.8, Con: 50.7	60,000 mg/day flaxseed powder	Cassava powder	–	Int: 44.4 ng/ml, Con: 27.6 ng/ml	12
Vargas et al. (32)	RA/DB/parallel	F: 34 Int: 17, Con: 17	PCOS	Int: 29.4, Con: 28.9	3,500 mg/day flaxseed oil (capsule)	Soybean oil	Int: 8 ng/ml, Con: 6.5 ng/ml	Int: 27.1 ng/ml, Con: 28.1 ng/ml	6
Kontogianni et al. (36)	RA/crossover	M/F: 37 Int: 19, Con: 18	Healthy	25.6	13,800 mg/day flaxseed oil	Olive oil	Int: 6.2 mg/L Con: 6.5 mg/L	–	6
Hutchins et al. (33)	RA/crossover	M/F: 25 Int: 13, Con: 12 • M/F: 25 Int: 13, Con: 12	Pre-diabetes	58.6	13,000 mg/day ground flaxseed 26,000 mg/day ground flaxseed	Placebo	Int: 8.4 μg/ml, Con: 9.4 μg/ml Int: 9.3 μg/ml, Con: 9.4 μg/ml	–	12
Gomes et al. (37)	RA/DB/parallel	M/F: 20 Int: 10, Con: 10	T2DM	Int: 47, Con: 50.1	6,000 mg/day linseed oil (capsule)	Placebo	Int: 10.61 μg/ml, Con: 12.04 μg/ml	–	8
Karakas et al. (34)	RA/DB/parallel	F: 34 Int: 17, Con: 17	PCOS	Int: 29.4, Con: 28.9	3,500 mg/day flaxseed oil (capsule)	soybean oil	Int: 8 ng/ml, Con: 6.5 ng/ml	Int: 27.1 ng/ml, Con: 28.1 ng/ml	6
Haidari et al. (28)	RA/parallel	F: 41 Int: 21, Con: 20	PCOS	Int: 27.21, Con: 26.13	30,000 mg/day brown milled flaxseed powder + lifestyle modification	Lifestyle modification	Int: 13.04 mg/ml, Con: 14.56 mg/ml	Int: 70.18 ng/ml, Con: 64.64 ng/ml	12
Kuang et al. (29)	RA/DB/parallel	M/F: 51 Int: 27, Con: 24	Overweight and obese	Int: 22.74, Con: 21.79	13,000 mg/day flaxseed meal (Biscuits)	Control	Int: 21.89 μg/ml, Con: 25.52 μg/ml	Int: 12.25 ng/ml, Con: 12.19 ng/ml	8
Ahmadniay motlagh et al. (27)	RA/DB/parallel	F: 52 Int: 29, Con: 23	Overweight and obese	Int: 38.28, Con: 41.74	30,000 mg/day brown milled flaxseed powder	Raw milled rice	Int: 12.11 ng/ml, Con: 12.48 ng/ml	Int: 53.76 ng/ml, Con: 51.48 ng/ml	12

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TABLE 2 Results of risk of bias assessment for randomized clinical trials included in the current meta-analysis on the effects of flaxseed supplementation on adipokines in adults.

Study	Random sequence generation	Allocation concealment	Reporting bias	Other sources of bias	Performance bias	Detection bias	Attrition bias
Paschos et al. (35)	L	L	L	H	L	H	L
Nelson et al. (31)	L	U	L	L	H	H	H
Faintuch et al. (38)	L	U	L	H	L	L	L
Taylor et al. (30)	L	U	L	H	U	U	H
Faintuch et al. (39)	L	U	L	H	L	H	L
Vargas et al. (32)	L	L	L	H	L	L	L
Kontogianni et al. (36)	L	L	L	H	U	U	L
Hutchins et al. (33)	L	L	L	H	U	U	L
Gomes et al. (37)	L	U	L	H	L	L	H
Karakas et al. (34)	L	L	L	L	L	L	L
Haidari et al. (28)	L	L	L	L	U	U	L
Kuang et al. (29)	L	L	L	H	L	L	L
Ahmadniay motlagh et al. (27)	L	L	L	L	L	L	L

Each study was assessed for risk of bias using the Cochrane Risk of Bias Assessment tool. Domains of assessment were included random sequence generation, allocation concealment, reporting bias, performance bias, detection bias, attrition bias, and other sources of bias. Each domain was scored as "high risk" if it contained methodological flaws that may have affected the results, "low risk" if the flaw was deemed inconsequential, and "unclear risk" if information was insufficient to determine. If a study got "low risk" for all domains, it is considered a high-quality study with low total risk of bias.

3.2. Risk of bias assessment and quality of evidence

Random allocation of participants was mentioned in all included trials. Most of the included studies had a low/unclear risk of allocation concealment and reporting bias. In addition, most studies showed a high risk of other sources of bias and detection bias. Out of the 13 RCTs in the current study, five were of high quality (27–29, 32, 34), six were of moderate quality (33, 35–39), and two were of low quality (30, 31). Detailed information regarding the quality of the included RCTs based on the Cochrane risk of bias assessment is shown in Table 2. GRADE quality of evidence was high for leptin and moderate for adiponectin (Table 3).

3.3. Flaxseed on adiponectin concentrations

Based on the result of 11 RCTs comprising 13 treatment arms, flaxseed could not significantly affect circulating adiponectin in adults (SMD = 0.52, 95% CI: −0.20, 1.25, $p = 0.159$; Figure 2). The results were heterogeneous ($I^2 = 92.0\%$, $p < 0.001$), and the sensitivity analysis results revealed no significant change following the removal of each study. Subgroup analysis indicated significant effects on adiponectin in RCTs administered with whole flaxseed (Table 4). Egger's and Begg's tests showed significant small-study effects ($p < 0.05$). The trim and fill method was performed (without imputed study) following the uneven distribution of the funnel plot (Figure 3).

3.4. Flaxseed on leptin concentrations

Eight RCTs with nine arms investigated the effect of flaxseed supplementation on leptin levels. The results indicated a significant reducing effect of flaxseed supplementation on leptin levels (SMD = −0.69, 95% CI: −1.37, −0.01; $p = 0.048$) with between-study heterogeneity ($I^2 = 86.4\%$, $p < 0.001$; Figure 4). Moreover, the overall effects of flaxseed on leptin were changed to not significantly impact by excluding studies using a one-study removal analysis (27–29, 32, 34). Whole flaxseed supplementation among RCTs with a sample size of >40 participants and age <50 years contributed to a robust reduction in leptin concentrations (Table 4). Begg's tests showed no significant publication bias ($p = 0.754$).

4. Discussion

The results of our pooled analysis showed that flaxseed supplementation, despite its non-significant effect on adiponectin, caused a significant decrease in circulating leptin. However, subgroup analysis showed that flaxseed had no significant effect on leptin levels in high-quality studies, and only low-quality studies showed ameliorating effects of flaxseed on leptin. Consequently, the interpretation of this result should be accompanied by caution, and studies with appropriate designs and a low risk of bias are needed to confirm our results on leptin. In addition, the

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TABLE 3 GRADE profile of flaxseed supplementation on plasma adipokines.

Adipokines	Summary of findings		Quality of evidence assessment (GRADE)					
	No. of patients (trials)	SMD* (95% CI)	Risk of bias[a]	Inconsistency[b]	Indirectness[c]	Imprecision	Publication bias[e]	Quality of evidence[f]
Adiponectin	420 (11)	0.52 (−0.20, 1.25)	Not serious	Not serious	Not serious	Serious[d]	Not serious	Moderate
Leptin	288 (8)	−0.69 (−1.37, −0.01)	Not serious	Not serious	Not serious	Not serious	Not serious	High

*Presented as standard mean difference (SMD) all outcomes.

[a]Risk of bias based on the Cochrane risk of bias tool. This tool assesses selection bias, performance bias, detection bias, attrition bias, and reporting bias. Five of the eight included studies had incomplete outcome data (attrition bias). Half of the included studies had performance bias.

[b]Downgraded if there was a substantial unexplained heterogeneity ($I^2 > 50\%$, $p < 0.10$) 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]There is no evidence of significant effects of flaxseed supplementation on adiponectin (95% CI including 0).

[e]Downgraded if there was evidence of publication bias using a funnel plot that affected overall results detected by trim and fill analysis.

[f]Since all included studies were randomized controlled 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, and very low.

examination of other subgroups showed that flaxseed significantly increases adiponectin in the form of whole flaxseed. Regarding leptin, the whole flaxseed caused a significant decrease in people of <50 years of age and in studies with a sample size of over 40. The results of the previous meta-analysis study in 2019 by Jalili et al. (21) showed that flaxseed supplementation has no significant effect on leptin and adiponectin levels and also on studied subgroups. However, the abovementioned study suggested that additional clinical trial studies should also be conducted for a definitive conclusion. Our study added four more clinical trials (27–29, 34) than Jalili et al.'s study of the pooled analysis which yielded different results in some aspects. In addition, the subgroup analysis of Jalili et al.'s study was only limited to the duration of the supplementation, the study population (healthy or unhealthy), and the type of intervention. However, our study added demographic variables (age and gender), sample size, body mass index, and study quality to subgroup analysis and examined the studied population more comprehensively than the aforementioned study in order to obtain generalizable results. In addition, unlike the abovementioned study, the quality of the obtained evidence in our investigation was checked with the GRADE tool.

In both studied biomarkers, whole flaxseed compared with flaxseed oil led to a significant improvement in leptin and adiponectin levels. Unlike flaxseed oil, which contains omega-3 fatty acids, especially polyunsaturated fatty acids (PUFAs), whole flaxseed contains PUFAs, soluble and insoluble fibers, proteins, various antioxidants, and phytoestrogenic lignans (40) that explain more improving effects on adipokines.

Studies have pointed out that the circulating levels of adiponectin and leptin are higher in women than in men (41, 42). Due to the existence of only one low-quality study (34) in the subgroup of men, the significant reduction of adiponectin in this subgroup cannot be a generalizable and valid result. No significant results have been reported for other gender subgroups either in leptin or adiponectin. However, it is suggested that future studies separate the effect of flaxseed supplements in men and women, in order to report a more accurate result. In terms of mean age, only one low-quality study (30) with two investigated arms included a <50 years of age subgroup in the leptin-pooled analysis. Therefore, a significant decrease in leptin in this subgroup is not highly worth noting. However, this finding can be a sign for future studies to clarify the effect of flaxseed on this age group.

The sample size is another important factor determining the true effect of flaxseed on leptin. Subgroup analysis showed that studies with a >40 sample size reported a significant decrease in leptin levels following flaxseed supplementation. As a general principle in epidemiological studies, large sample sizes lead to high power to show a true effect (43). However, a very high sample size can also lead to false conclusions (44).

The various compounds found in whole flaxseed lead to beneficial effects on circulating levels of leptin and adiponectin. Fatty acids through interaction with transcription factors such as PPARγ, CCAAT/enhancer-binding protein (C/EBP), and sterol regulatory element-binding transcription factor 1 (SREBPF1) can

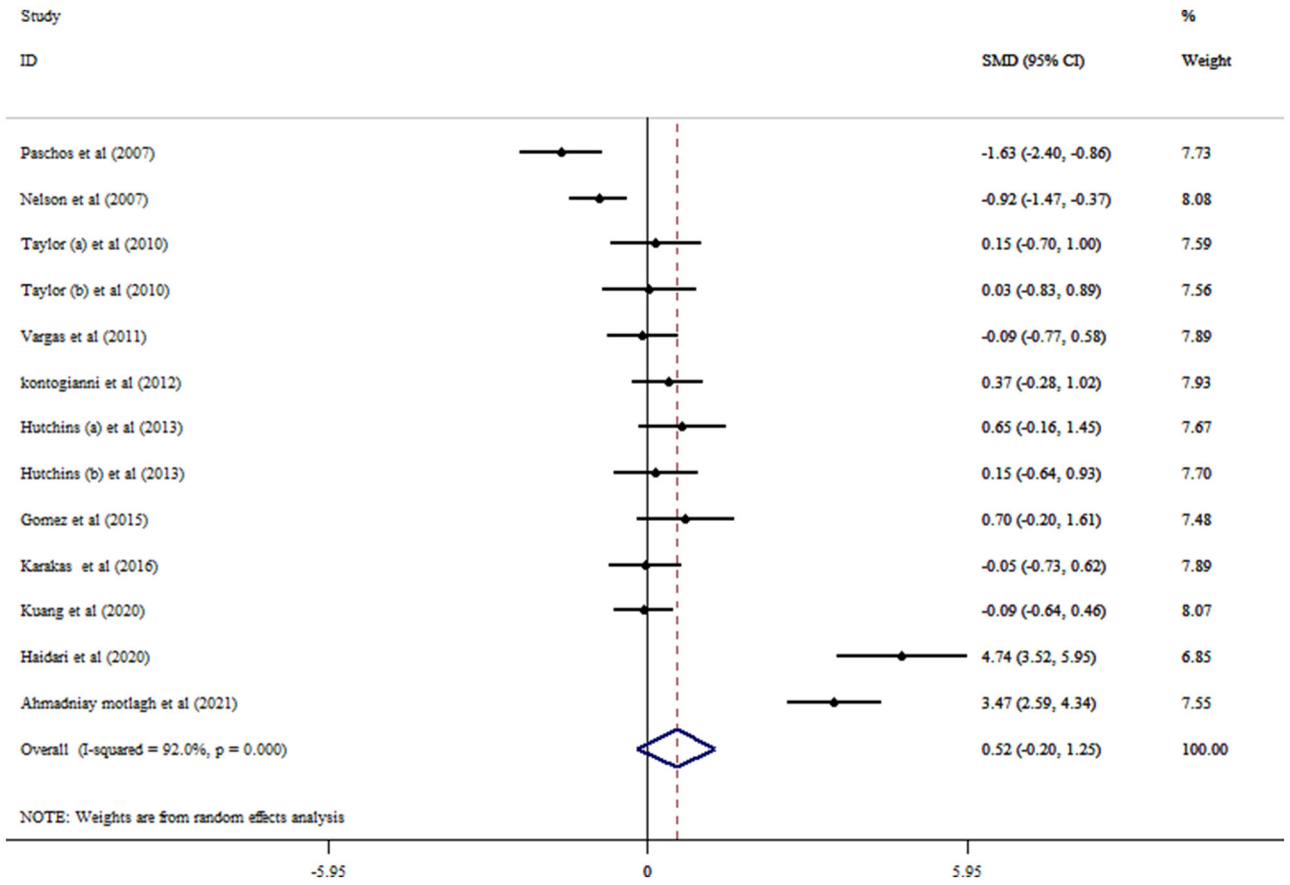

FIGURE 2
Forest plot detailing mean difference and 95% confidence intervals (CIs) and the effects of flaxseed supplementation on adiponectin levels.

alter the expression of leptin and adiponectin (45). Moreover, the anti-inflammatory properties of omega-3 fatty acids contribute to the regulation of adipokine production (46). Studies have reported that inflammatory conditions can lead to the inhibition of adiponectin production from adipocytes (47). In addition, pro-inflammatory cytokines have stimulating effects on leptin production (48). The main fatty acid of flaxseed oil is ALA, which is a poor activator of PPARγ compared with arachidonic acid as the main activator of PPARγ among fatty acids (49). This could explain the difference in the results observed between whole flaxseed and flaxseed oil. Dietary fibers can regulate the levels of adipokines in various ways, such as through changing body composition (50) and modifying gut microbiota (51). Due to the similar structure of phytoestrogens and estrogen, these compounds can bind to estrogen receptors (ERs) with a high affinity toward ERβ than ERα (52), leading to the inhibition of adipocyte differentiation and lipid accumulation in an *in vivo* model (53). However, phytoestrogens can directly bind to and activate PPARγ (52). This cross-talk between PPARγ and ERs focused on future studies to elucidate the precise effect of phytoestrogens on obesity-related pathways. The beneficial effects of plant polyphenols and antioxidants on the balance between different adipokines have been investigated in some studies (54, 55).

There were some limitations worth noting in our study that are suggested to cover in future studies. First, due to the lack of sufficient studies, an accurate comparison between men and women was not possible. As it is known, the expression of estrogen receptors between the two genders has a different pattern (56), and this can be effective in the effect of flaxseed on the circulating levels of adipokines. Second, it seems that the effect of flaxseed on other adipokines, such as visfatin and resistin, should also be taken into consideration in order to obtain a more comprehensive conclusion. Third, there were limited studied populations; therefore, additional studies on other diseases especially inflammatory conditions seem necessary.

Our study also has some worth noting strengths. First, the present study tried to cover all the limitations of the previous meta-analysis. Second, due to the low risk of bias in the included studies and the appropriate design of the current meta-analysis, the quality of our obtained results was moderate for adiponectin and high for leptin. Third, our study was registered in PROSPERO (code: CRD42023399735).

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TABLE 4 Subgroup analyses for the effects of flaxseed supplementation plasma adipokines.

	NO	SMD (95% CI)[a]	*p*-within[b]	I^2 (%)[c]	*p*-heterogeneity[d]
Flaxseed supplementation on adiponectin					
Overall	13	0.52 (−0.20, 1.25)	0.159	92.0	<0.001
Age (year)					
<50	9	0.67 (−0.36, 1.69)	0.203	94.6	<0.001
≥50	4	0.25 (−0.16, 0.66)	0.232	0.0	0.731
Gender					
Men	1	−1.63 (−2.40, −0.86)	<0.001	0.0	<0.001
Women	4	1.97 (−0.21, 4.15)	0.076	96.5	<0.001
Both	8	0.08 (−0.32, 0.48)	0.698	59.4	0.016
Intervention duration (week)					
<12	6	−0.06 (−0.50, 0.37)	0.774	63.3	0.018
≥12	7	1.05 (−0.42, 2.51)	0.161	95.1	<0.001
Intervention type					
Whole flaxseed	6	1.46 (0.06, 2.86)	0.040	94.5	<0.001
Flaxseed oil	7	−0.25 (−0.81, 0.32)	0.393	77.4	<0.001
Study population					
Overweight and obese	5	0.62 (−0.68, 1.93)	0.351	94.5	<0.001
PCOS	3	1.47 (−0.91, 3.85)	0.227	96.2	<0.001
T2DM	3	0.28 (−0.22, 0.78)	0.275	0.0	0.535
Other diseases	2	−0.62 (−2.57, 1.34)	0.537	93.4	<0.001
Sample size					
≤40	9	0.02 (−0.42, 0.46)	0.927	65.9	0.003
>40	4	1.75 (−0.54, 4.04)	0.135	97.5	<0.
BMI					
≤30	5	0.76 (−0.75, 2.26)	0.325	94.8	<0.001
>30	8	0.40 (−0.44, 1.24)	0.352	90.4	<0.001
Study quality					
Low	9	0.41 (−0.48, 1.30)	0.365	91.3	<0.001
High	4	0.78 (−0.66, 2.22)	0.289	94.5	<0.001
Flaxseed supplementation on leptin					
Overall	9	−0.69 (−1.37, −0.01)	0.048	86.4	<0.001
Age (year)					
<50	7	−0.84 (−1.68, −0.01)	0.047	89.0	<0.001
≥50	2	−0.16 (−0.93, 0.61)	0.686	36.4	0.210
Gender					
Women	4	−1.37 (−2.75, 0.01)	0.051	93.1	<0.001
Both	5	−0.21 (−0.76, 0.33)	0.442	56.9	0.055
Intervention duration (week)					
<12	4	−0.32 (−0.81, 0.17)	0.198	52.0	0.100
≥12	5	−1.06 (−2.37, 0.25)	0.112	91.6	<0.001

(Continued)

TABLE 4 (Continued)

	NO	SMD (95% CI)[a]	*p*-within[b]	I^2 (%)[c]	*p*-heterogeneity[d]
Intervention type					
Whole flaxseed	6	−1.03 (−2.02, −0.05)	0.040	89.6	<0.001
Flaxseed oil	3	−0.09 (−0.50, 0.33)	0.686	0.0	0.690
Study population					
Overweight and obese	4	−0.48 (−1.20, 0.23)	0.181	74.9	0.007
PCOS	3	−1.47 (−3.57, 0.63)	0.170	95.3	<0.001
T2DM	2	−0.16 (−0.93, 0.61)	0.686	36.4	0.210
Sample size					
≤40	6	−0.06 (−0.39, 0.26)	0.693	0.0	0.727
>40	3	−2.02 (−3.55, −0.50)	0.009	92.6	<0.001
BMI					
≤30	2	−2.53 (−5.73, 0.66)	0.120	96.1	<0.001
>30	7	−0.25 (−0.68, 0.18)	0.257	55.5	0.036
Study quality					
Low	4	−0.65 (−1.16, −0.14)	0.013	61.8	0.049
High	5	−0.78 (−2.23, 0.66)	0.289	92.1	<0.001

[a]Obtained from the Random-effects model.
[b]Refers to the mean (95% CI).
[c]Inconsistency, percentage of variation across studies due to heterogeneity.
[d]Obtained from the Q-test.
SMD, standard mean differences; CI, confidence interval; NR, not reported; NAFLD, non-alcoholic fatty liver disease.

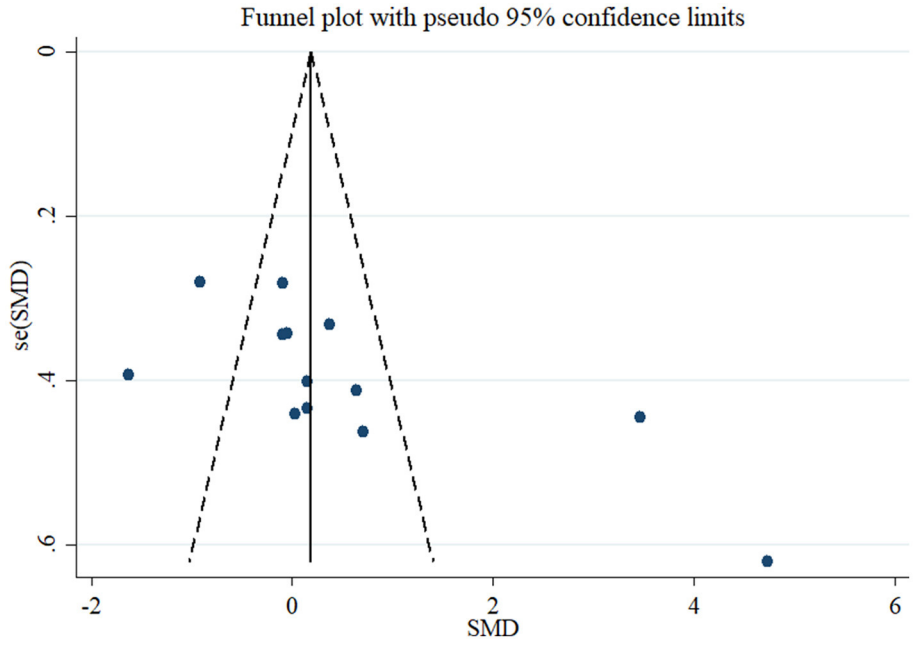

FIGURE 3
Funnel plot displaying publication bias in the studies reporting the effects of flaxseed supplementation on adiponectin levels.

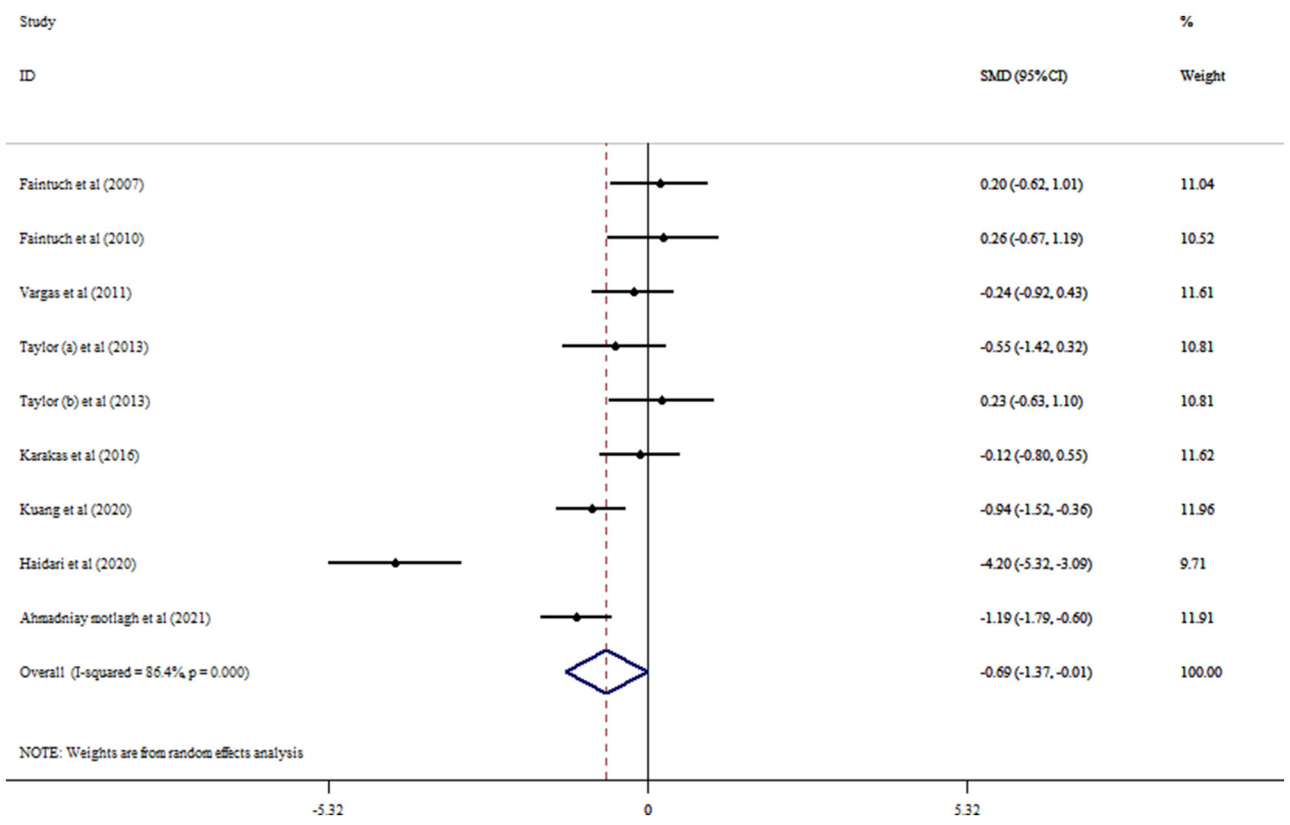

FIGURE 4
Forest plot detailing mean difference and 95% confidence intervals (CIs) and the effects of flaxseed supplementation on leptin levels.

5. Conclusion

Whole flaxseed is effective in improving the levels of adiponectin and leptin. Flaxseed oil cannot change circulating levels of adipokines. The quality of our obtained results is moderate for adiponectin and high for leptin.

Data availability statement

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

Author contributions

VM was responsible for designing and coordinating the study. VM, KK, SA, HJ, and AH were responsible for the statistical study and writing of the manuscript. AF was responsible for reviewing the manuscript. KK was responsible for the statistical work and for writing the manuscript. All authors were responsible for data collection, data analysis, and data interpretation of the manuscript, 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.

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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.2023.1179089/full#supplementary-material

SUPPLEMENTARY TABLE S1
The search strategy.

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The effects of NAD+ precursor (nicotinic acid and nicotinamide) supplementation on weight loss and related hormones: a systematic review and meta-regression analysis of randomized controlled trials

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Background: Despite the fact that obesity and overweight are serious major health problems worldwide, fighting against them is also considered a challenging issue. Several interventional studies have evaluated the potential weight-reduction effect of nicotinamide adenine dinucleotide (NAD+) precursor. In order to obtain a better viewpoint from them, this study aimed to comprehensively investigate the effects of NAD+ precursor supplementation on weight loss, adiponectin, and leptin.

Methods: Scopus, PubMed/Medline, Web of Science, Cochrane, and Embase databases were searched using standard keywords to identify all controlled trials investigating the weight loss and related hormones effects of NAD+ precursor. Pooled weighted mean difference and 95% confidence intervals were achieved by random-effects model analysis for the best estimation of outcomes.

Results: Twenty two treatment arms with 5,144 participants' were included in this systematic review and meta-regression analysis. The pooled findings showed that NAD+ precursor supplementation has an effect on lowering BMI (weighted mean difference (WMD): −0.19 kg/m2, 95% confidence interval (CI): −0.29 to −0.09, $p < 0.001$) and increasing adiponectin (WMD: 1.59 μg/mL, 95% CI: 0.49 to 2.68, $p = 0.004$) in humans compared with control groups. However, no significant effect was observed on body weight and leptin. There was a significant relationship between doses of intervention with changes in BMI. In addition, subgroup analysis showed that BMI reduction was greater when receiving nicotinic acid (NA) supplementation than nicotinamide (NE) supplementation.

Conclusion: NAD+ precursor had significant effects on weight management with the reduction of BMI and increasing adiponectin.

KEYWORDS

NAD+ precursor, niacin, obesity, obesity hormone, weight loss

Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. A body mass index (BMI) over 25 is considered overweight, and over 30 is obese. The issue has grown to epidemic proportions, with over 4 million people dying each year as a result of being overweight or obese in 2017 according to the global burden of disease (1). Adiposity, particularly abdominal adiposity, is associated with an increased risk of most chronic diseases such as type 2 diabetes (T2DM), cancer, cardiovascular disease (CVD), atherosclerosis, and metabolic syndrome (2). Moreover, low-grade chronic inflammation linked to adiposity, along with adipocyte development and a change in the pattern of activity of adipokines especially, leptin and adiponectin, leads to adipose tissue (AT) dysfunction (3). Following these changes, serious disorders are generally observed, such as a decrease in mitochondrial biogenesis and an increase in oxidative stress and inflammation (4–6). Therefore, one might hypothesise that elements such as nicotinamide adenine dinucleotide (NAD)+and their related components, which regulate mitochondrial function, metabolism, cellular stress response, carbohydrates and fatty acids synthesis, ATP generation, and ketogenesis, have been significantly involved in the adjustment of metabolic complications associated with obesity (7–9). NAD+ is synthesised in two major pathways in the human body: *de novo* synthesis and salvage, the latter of which has a more effective role in maintaining levels of NAD+ in the body. The compounds that are created from the salvage pathway are called NAD+ precursors (10). Furthermore, the presence of Sirtuins, NAD+-dependent protein deacetylases (SIRT1–7), is crucial for NAD+'s biological function in humans (11). Studies have shown that NAD+ precursors and Sirtuins gene expression were significantly down-regulated in obese subjects (12–14), whereas their expression increased progressively in subjects with a weight loss programme (15). However, the effects of NAD+ precursor supplementation on weight and other related factors, as well as adipokines, are still not completely clear. Therefore, the objective of this systematic review and meta-analysis of randomized controlled trials is to analyze and evaluate the effects of NAD+ precursor supplementation on weight loss and obesity hormone.

Methods

Search strategy

The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) criteria were followed for conducting this study (16). Without regard to language or time restrictions, a thorough search was carried out in the PubMed/MEDLINE, Web of Science, SCOPUS, and Embase databases from the beginning to February 2023. Additionally, similar papers and gray literature were considered in the search. Medical subject headings (MeSH) and Emtree (Embase subject headings) were selected to search the online databases, as follow: ("NAD" OR "NAD precursor" OR "Nicotinic Acids" OR "Niacin" OR "Niacinamide" OR "Nicotinamide Mononucleotide") AND ("weight" OR "Waist Circumference" OR "Body Mass Index" OR "Adiponectin" OR "Leptin") AND ("Clinical Trials as Topic" OR "Cross-Over Studies" OR "Double-Blind Method" OR "Single-Blind Method" OR "Random Allocation" OR "Clinical Trial") (The specific search strategy is described in the Supplementary Appendix S1). The reference lists of the publications retrieved and linked review studies were manually searched to identify potentially overlooked qualifying trials.

Eligibility criteria

Using titles, abstracts, or the complete texts of the research, two writers separately removed duplicate articles before finding and reviewing relevant publications. In the end, the papers were separated based on the following standards: (1) Randomized clinical trials studies; (2) NAD+ precursor supplementation (nicotinic acid (NA) or nicotinamide (NE) supplementation) was given as an intervention in individual's aged 18 and over; and 3) baseline and post measurements in both group (intervention and control) weight, BMI, adiponectin, and leptin were recorded. The most recent or longest follow-up period was used when a research revealed results at more than one follow-up time. Studies with duplicated data, studies with ambiguous information, studies in which NAD+ precursor was used as an intervention alongside other commonly prescribed medications, non-randomized trial designs, animal studies, studies without a control group, reviews, and meta-analysis studies were also excluded. The PICOS criteria for inclusion and exclusion of studies were as follows. Population: individual's aged 18 and over; Intervention: NAD+ precursor supplementation (nicotinic acid (NA) or nicotinamide (NE) supplementation); Comparator: other intervention or placebo; Outcomes: weight, BMI, adiponectin, and leptin; Study design: randomized clinical trials studies.

Data extraction

The qualifying studies were examined by two authors independently. The first author's name, the study's location, the year it was published, the sample size (for the intervention and control groups), the participant characteristics (such as the percentage of men, the participant's BMI, age, and health status), the type of outcomes, duration of the intervention, the dosage and type of the intervention, and the means and standard deviations (S.D.s) of the intended outcomes at baseline, post-intervention, and/or changes between baseline and post-intervention, were all extracted.

Quality assessment

The details of the evaluation of the study's quality are presented in Table 1. Using the Cochrane risk-of-bias test for randomized trials (RoB 2), version 2, the quality of the included RCTs was methodologically evaluated (17). Based on the following potential sources of bias: blinding of outcome assessment, allocation concealment, participant and staff blinding, random sequence generation, incomplete outcome data, selective reporting, and other bias, two authors independently rated each study as having a low, high, or unclear risk of bias. A "High risk" rating indicates significant bias that may invalidate the results. These studies have serious errors in design, analysis, or reporting; have large amounts of missing information; or have discrepancies in reporting. Studies are assessed as at unclear risk of bias when too few details are available to make a judgement of 'high' or 'low' risk; when the risk of bias is genuinely unknown despite sufficient information about the conduct; or when an

TABLE 1 Risk of bias assessment according to the Cochrane collaboration's risk of bias assessment tool.

Study, Year (reference)	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Overall assessment of risk of bias
El-Kady et al. (2022)	Low	Low	Low	Low	Unclear	Low	Low
Liao et al. (2021)	Low	Unclear	Low	Low	Unclear	Low	Unclear
Canner et al. (2003)	Low	Low	Low	High	Unclear	Low	Unclear
Linke et al. (2008)	Low	Low	Low	Low	Unclear	Low	Low
Fabbrini et al. (2010)	Low	Unclear	Low	Low	Unclear	Low	Unclear
Aye et al. (2014)	Low	Low	Low	Low	Unclear	Low	Low
Vittone et al. (2007)	Low	High	Low	Low	Unclear	Low	Low
Vaccari et al. (2007)	Low	Low	High	Low	Unclear	Low	Unclear
Superko et al. (2004)	Low	Low	Unclear	Low	Unclear	Low	Low
Savinova et al. (2015)	Low	Unclear	Low	Low	Unclear	Low	Unclear
Bays et al. (2010)	Low	Low	Low	Low	Unclear	Low	Low
Okabe et al. (2022)	Low	Unclear	Unclear	Low	Unclear	Low	Low
Ko et al. (1998)	Low	High	High	Low	High	Low	High
Kei et al. (2011)	Low	Low	High	Unclear	Unclear	Low	Unclear
Chauhan et al. (2011)	Low	Low	Unclear	Unclear	Unclear	Low	Unclear
Osar et al. (2004)	Low	Low	High	Low	Unclear	Low	Unclear
Westphal et al. (2007)	Low	Low	Unclear	Low	Unclear	Low	Low
Lee et al. (2009)	Low	Low	Low	Low	Unclear	Low	Low

entry is not relevant to a study (for example because the study did not address any of the outcomes in the group of outcomes to which the entry applies). A "Low risk" study has the least bias, and results are considered valid. Any discrepancies were discussed with a third author in order to come to a consensus. The GRADE (Grading of Recommendations Assessment, Development, and Evaluation) grading method was also used to evaluate the quality of the current analytic research (18). A reliable 10-point assessment system that assesses elements affecting study quality is the GRADE checklist. This scale has seven components: (1) risk of bias, (2) precision, (3) heterogeneity, (4) directness, (5) publishing bias, (6) funding bias, and (7) study design.

Data synthesis and statistical analysis

The data were examined using STATA version 12.0 software. Different data types were converted using a predetermined procedure to the mean and standard deviations (S.D.s) (19, 20). For instance, in the absence of standard deviations, we calculated the change using the method below: The definition of standard deviation changes is square root [(S.D. baseline ^{2} + SD final ^{2}) - (2R S.D. baseline 2 S.D. final)]. The following formula is used to convert the standard error of the mean (SEM) to standard deviation: S.D. is equal to SEM $\times \sqrt{n}$, where n is the total number of participants in each group. The random-effects model was employed in the meta-analysis of research results. The weighting of the research followed the typical inverse variance technique. The data from the longest time point were used for the analysis, which allowed for the handling of many assessments within a single study group. Using Q Statistics and I-squared (I^2), the degree of study heterogeneity was evaluated. Insignificant, low, moderate, and high heterogeneity were found with I^2 values ranging from 0% to 25, 26 to 50%, 5 to 75%, and 76 to 100%, respectively (21). To identify possible causes of heterogeneity, a pre-defined subgroup analysis based on the dosage, duration, and type of the intervention was conducted. A sensitivity analysis was done to determine the contribution of each research to the overall mean difference. In order to establish if there was publication bias, we utilized the official Egger's test (22).

Results

Figure 1 depicts a flowchart of the research selection process with exclusion criteria. This value indicates that the aforementioned electronic databases generated 1781 articles. After removing publications with duplicate research, there were 1,259 total. Following an assessment of the research's titles and abstracts, 1,220 papers were dropped since they did not meet the inclusion requirements. 39 articles were found utilizing the full-text search during the secondary screening. For the reasons listed above, 21 of the investigations were dropped. Finally, 18 papers with 22 treatments arm were included in the quantitative meta-analysis since they matched the qualifying requirements.

Study characteristics

The features of the pooled articles are shown in Table 2. Our surveys reveal that seven studies have been carried out in USA, 2

articles in the Germany and UK, respectively, and other studies were conducted in Japan, China, India, Egypt, Turkey, Hong Kong, and Greece. All articles were published between 1998 and 2022 and follow up intervention ranged from 4 to 144 weeks. The mean age and percentage of male participants ranged from 31 to 65 years and 0–100%, respectively, at the baseline. The doses prescribed in the studies were between 250 and 3,000 mg per day, and in 4 studies the supplement type was in the form of NE and the rest were in the form of NA. Five studies conducted on patients with metabolic syndrome, five articles on people with a history of cardiovascular disease or dyslipidemia, two studies on each of type 2 diabetes (T2DM) and non-alcoholic fatty liver (NAFLD) as well as healthy samples, and two studies on patients with impaired glucose tolerance and polycystic ovary syndrome.

The findings of the evaluation of the eligible studies' quality are shown in Table 1. Additionally, a score of 8.8 (very good quality) was determined after the GRADE score system was used to assess the quality of the current meta-analysis.

Meta-analysis results

Pooled findings from the random-effects model indicated that body mass index (BMI) (weighted mean difference (WMD): $-0.19\,kg/m^2$, 95% confidence interval (CI): −0.29 to −0.09, $p < 0.001$) were significantly reduced after NAD+ precursor supplementation compared to control group. However, no significant effect was observed on body weight (WMD: 0.07 kg, 95% CI: −0.40 to 0.55, $p = 0.766$), leptin (WMD: 1.74 ng/mL, 95% CI: −3.45 to 6.92, $p = 0.512$) compared to the control group. Also, Pooled findings indicated that compared to the control group, adiponectin (WMD: 1.59 μg/mL, 95% CI: 0.49 to 2.68, $p = 0.004$) was significantly increased after with NAD+ precursor supplementation. Furthermore, significant heterogeneity was found among the studies for adiponectin (Cochran Q test, $p < 0.001$, $I^2 = 88.3\%$). However, low heterogeneity was reported for weight (Cochran Q test, $p = 0.984$, $I^2 = 0.0\%$), BMI (Cochran Q test, $p = 0.395$, $I^2 = 5.1\%$), and leptin (Cochran Q test, $p = 0.579$, $I^2 = 0.0\%$) (Figures 2–4).

Subgroups analysis

The findings of the subgroup also show the greater effect of NAD+ precursor supplementation on BMI decrease in a dose ≥2 g and a duration of intervention >12 weeks. In addition, subgroup analysis showed that BMI reduction was greater when receiving nicotinic acid (NA) supplementation than nicotinamide (NE) supplementation (Supplementary Table).

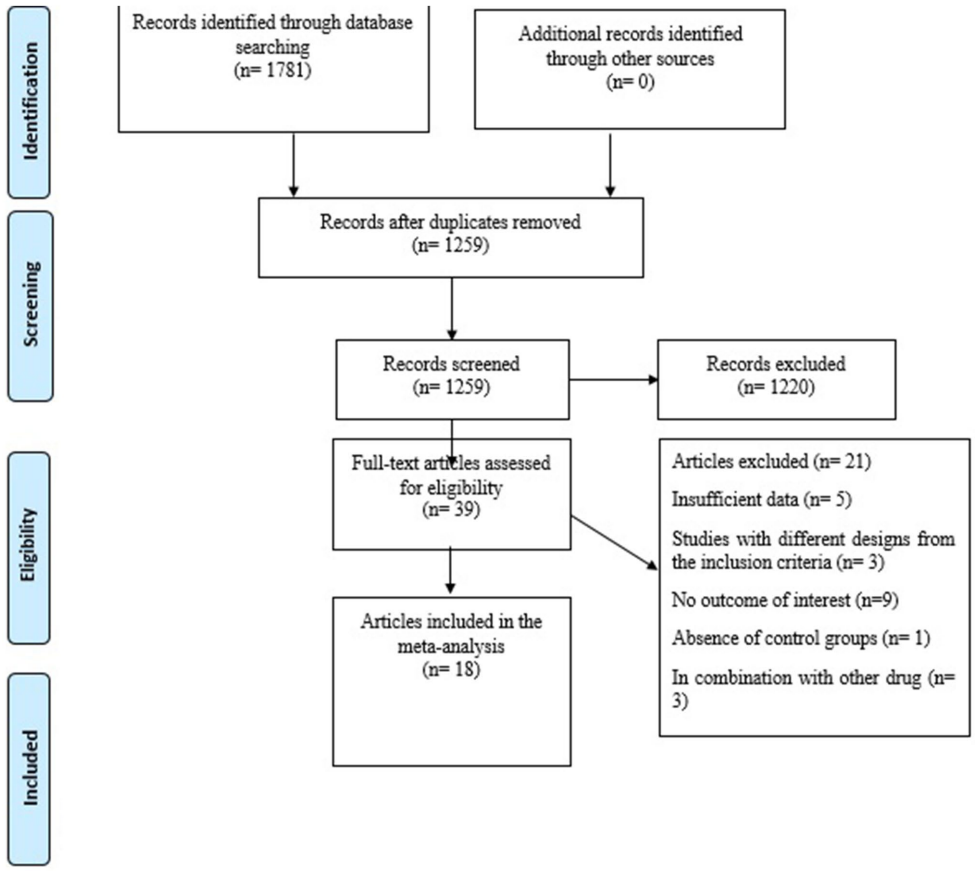

FIGURE 1
Flow chart of the study, including identification, screening, eligibility, and the final sample included.

TABLE 2 Characteristics of eligible studies.

Author (year)	Country	Population	Mean age year	Sex (Male %)	Sample size study (intervention/ control)	Follow up of intervention (Weeks)	Type and dose (mg/day) of intervention	Baseline of BMI (kg/m^2)	Outcomes
El-Kady et al. (2022)	Egypt	NAFLD	45.6	41.9	31/30	12	1,000 mg NE	32.69	BMI, weight, Adiponectin
Liao et al. (2021)	China	Healthy Subjects	37	83.3	12/12	6	300, 600, 1,200 mg NE	22	BMI, weight
Canner et al. (2003)	USA	Metabolic Syndrome and Healed Myocardial Infarction	NR	NR	964/2468	48	2000 mg NA	NR	BMI
Linke et al. (2008)	Germany	Patients with impaired glucose tolerance	45.5	70	30/30	24	1,000 mg NA	37.9	BMI, weight, Adiponectin, Leptin
Fabbrini et al. (2010)	USA	NAFLD	43	30	9/9	16	2000 mg NA	35.8	BMI, weight
Aye et al. (2014)	UK	Polycystic ovary syndrome	31	0	13/12	12	1,000 mg NA	35.8	BMI
Vittone et al. (2007)	USA	Patients with metabolic syndrome	54	86.2	80/80	144	2000 mg NA	29.7	BMI
Vaccari et al. (2007)	USA	Patients with metabolic syndrome	32	56	30/15	52	1,000 mg NA	29.7	BMI, Adiponectin
Superko et al. (2004)	USA	Hypercholesterolemic subjects	53	71.6	60, 59/61	14	1,500, 3,000 mg NA	28	BMI
Savinova et al. (2015)	USA	Patients with metabolic syndrome	47	57	14/14	16	2000 mg NA	32.7	BMI
Bays et al. (2010)	USA	Dyslipidemic patients without metabolic syndrome	57.7	62.4	221, 320/110, 160	24	2000 mg NA	31.5	BMI
Okabe et al. (2022)	Japan	Healthy Subjects	42.9	26.6	15/15	16	250 mg NE	21.3	BMI, weight
Ko et al. (1998)	Hong Kong	T2DM	59.2	36.3	32/30	12	750 mg NA	25.7	BMI
Kei et al. (2011)	Greece	Dyslipidemic patients	58	46.6	30/30	12	2000 mg NA	29.1	weight
Chauhan et al. (2011)	India	Hyperlipidemic patients	NR	NR	17/20	12	2,250 mg NA	NR	weight
Osar et al. (2004)	Turkey	Patients with Poorly Controlled Type 2 Diabetes Mellitus	58	46.6	15/15	4	3,000 mg NE	30	weight
Westphal et al. (2007)	Germany	Patients with metabolic syndrome	55	100	20/10	6	1,500 mg NA	32.4	Adiponectin, Leptin
Lee et al. (2009)	UK	Patients with coronary artery disease	65	94	22/29	48	2000 mg NA	31	Adiponectin

BMI, body mass index; NAFLD, Nonalcoholic fatty liver disease; TD2M, Type 2 diabetes; NR, not report; NA, Nicotinic acid; NE, Nicotinamide.

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Meta-regression

Meta-regression between NAD+ precursor and absolute mean differences in body weight and BMI based on dosage and duration of intervention was performed. Only, there was a significant relationship between dose of intervention with changes in BMI (coefficient (Coef) = −0. 0001847, $p = 0.033$). However, meta-regression analysis did not show a significant linear relationship between dose and duration of intervention with weight changes (Supplementary Figures S1, S2).

Sensitivity analysis

To discover the effect of each article on the pooled effect size for the levels of weight, BMI, adiponectin, and leptin, we step-by-step discarded each trial from the analysis. The leave-one-out sensitivity analysis indicated the robustness of the results (Supplementary Figure S3).

Publication bias

Evaluating the publication bias by visual inspection of the funnel, no evidence for publication bias based on the Egger's tests was detected for weight ($p = 0.531$), BMI ($p = 0.621$), adiponectin ($p = 1.00$), and leptin ($p = 0.602$; Supplementary Figure S4).

Discussion

The results of our systematic review and meta-analysis showed that nicotinamide adenine dinucleotide (NAD+) precursor supplementation has an effect on lowering BMI and increasing adiponectin in humans compared with control groups. Furthermore, there was a significant relationship between doses of intervention with changes in BMI. The findings of the subgroup also show the greater effect of NAD+ precursor supplementation on BMI decrease in a dose ≥2 g and a duration of intervention >12 weeks.

Currently, there is no meta-analysis on the effect of NAD+ precursors on obesity in the human body. Earlier meta-analysis investigated and described the effect of NAD+ precursor supplementation on improving TG, TC, LDL, and HDL levels in humans, but resulted in hyperglycemia, compared with placebo or no treatment (23, 24). Animal studies evaluating obese mice have shown an association between NAD+ supplementation and improved indices of obesity as well as molecular regulation of adipocytes (25, 26).

Nicotinamide adenine dinucleotide (NAD+) is an important molecule in energy and signal transduction, besides acting as substrate for enzymes such as sirtuins, poly-ADP ribose polymerases (PARPs), and cyclic ADP ribose synthetases that regulate key cellular processes of energy metabolism, DNA damage repair, and calcium signaling (27). Improving NAD+ availability *via* NAD+ precursor supplementation has emerged as a potential strategy to augment tissue-specific NAD+ homeostasis and improve physiological function (28). Thus, NAD+ supplementation therapy is a new treatment for obesity in recent years because acts *via* activation of the NAD + -dependent sirtuin enzyme family, thereby regulating oxidative metabolism (29, 30).

NAD+ precursors such as nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) is a recently discovered vitamin B3, are available as over-the-counter dietary supplements, and oral supplementation with these precursors reduction in fat mass and an increase in lean mass; improved glucose tolerance and alleviated

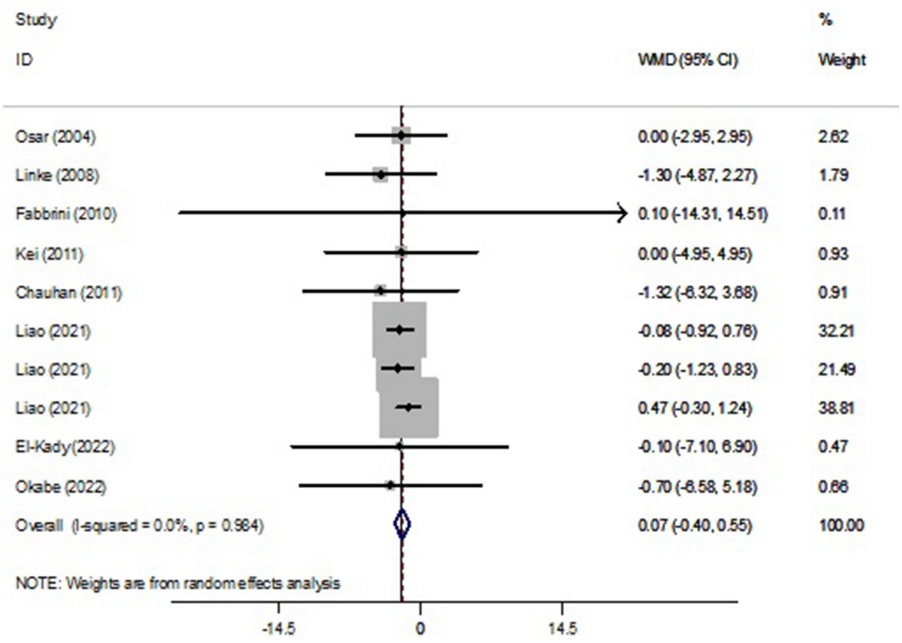

FIGURE 2
Forest plot of randomized controlled trails investigating the effects of NAD+ precursor supplementation on weight (kg).

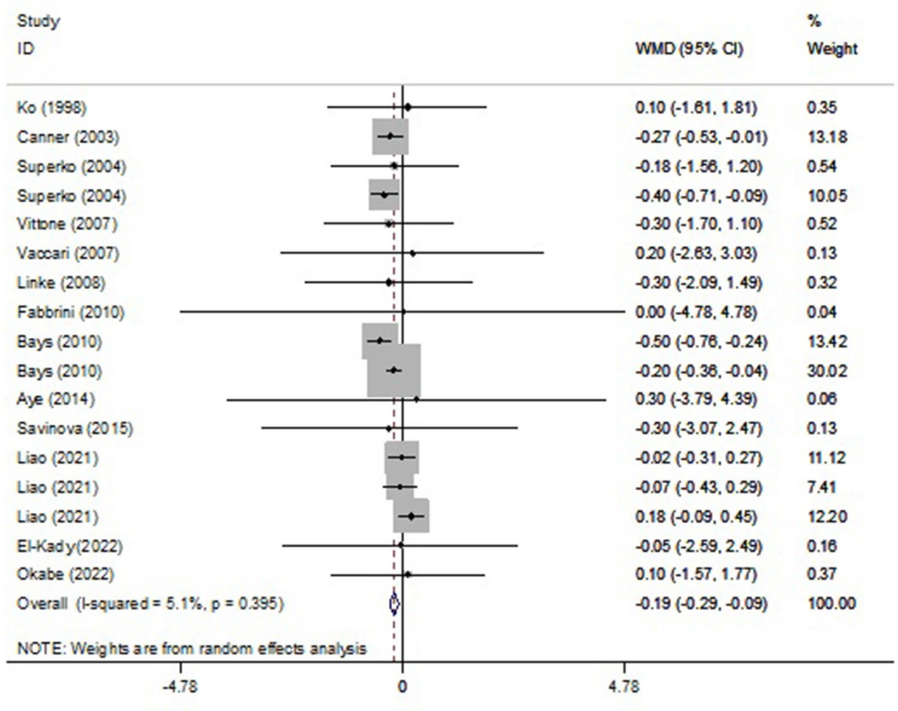

FIGURE 3
Forest plot of randomized controlled trails investigating the effects of NAD+ precursor supplementation on body mass index (BMI) (kg/m^2).

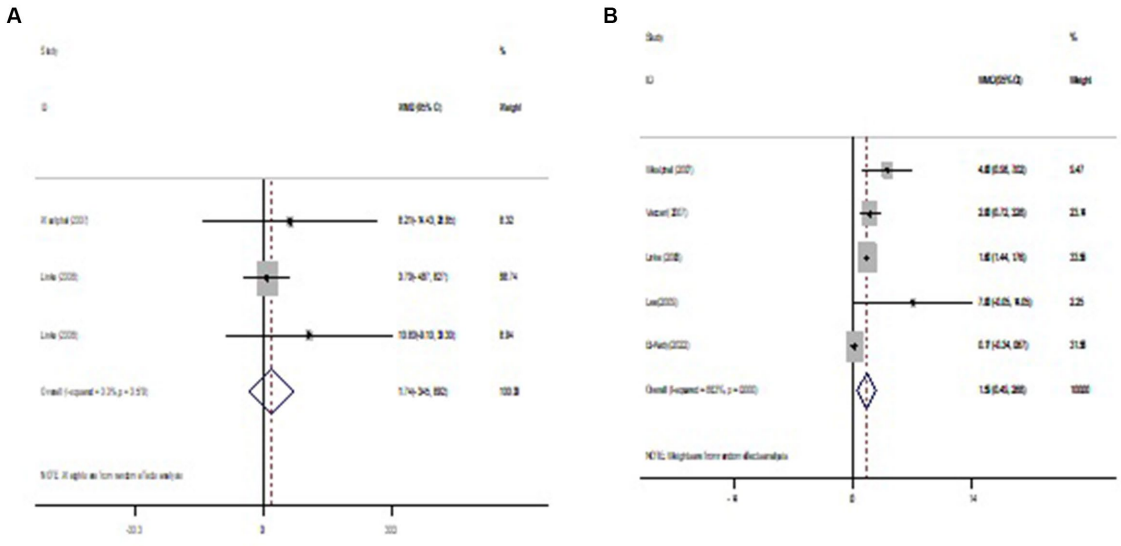

FIGURE 4
Forest plot of randomized controlled trails investigating the effects of NAD+ precursor supplementation on **(A)** leptin (ng/ml) and **(B)** adiponectin (μg/mL).

adipose tissue inflammation in pre-clinical models with obesity (26). Remie et al. (31) has demonstrated that NAD+ supplementation therapy with 1,000 mg/d for 6 weeks in healthy overweight or obese men and women increased skeletal muscle NAD+ metabolites, affected skeletal muscle acetylcarnitine metabolism, and induced minor changes in body composition.

Consistent with the effect of NAD+ precursor supplementation on body composition observed by Remie et al. (31), we have shown lowering BMI at 0.19 kg/m^2 and BMI decrease was higher in a dose ≥2 g and a duration of intervention >12 weeks. In another study in 2018, which was conducted to investigate dietary nicotinamide riboside (NR) supplementation in a 12-week period on the

improvement of insulin sensitivity and other metabolic parameters in obese and insulin-resistant men, the results showed that NR in doses of 2 g/day had no effect on resting energy expenditure, lipolysis, lipid oxidation, or body composition (32). In a study, with the aim of determining the effect of long-term NR supplementation on increasing mitochondrial biogenesis and metabolic health on twenty monozygotic twins discordant with BMI with an increasing dose of NR (250 to 1,000 mg per day) for 5 months, NR did not improve obesity or metabolic health (33). Udin et al. (34) evaluated the effects of NAD+ supplementation in rats fed a high-fat diet. The results showed that NAD+ supplementation reduced the BMI of the rats, suggesting that NAD+ supplementation may be an effective strategy for weight loss and improving metabolic health. In another animal study on diet-induced obese rats, NA decreased visceral adipose tissue and improved adiponectin levels and lipid profile (35). However, a study on developing rats did not show any useful results regarding the effect of NE on factors related to obesity (36).

However, despite the promising result for BMI lowering in the face of supplementation, no significant effect was observed on body weight. Despite previous evidence from preclinical studies on promoting resistance to weight gain no significant effect on body weight in human studies was observed in our meta-analysis. We believe that the discrepancy between the results observed for the BMI and body weight outcomes is related to the smaller number and consequent smaller effect size of studies selected for the body weight outcome in relation to the BMI outcome (37). Moreover, we observed that for the outcome of body weight, the majority of weight of the studies obtained from the effect size was concentrated in the evidence demonstrated by Liao et al. (38), which was responsible for 92.5% of the overall effect (% weight), this can overestimate the total effect.

To investigate the role of the effect of NAD+ precursor supplementation on obesity, we evaluated leptin and adiponectin. Adipose tissue is a metabolic and endocrine organ that secretes a number of adipokines that contribute to the etiology of obesity-related metabolic complications (39, 40). Leptin is a hormone produced by adipose tissue that plays an important role in regulating appetite and energy metabolism. Elevated leptin levels are associated with suppressed appetite and increased energy expenditure, while reduced leptin levels are associated with increased appetite and decreased energy expenditure (41). Adiponectin, on the other hand, is a hormone secreted by fat cells that has been linked to the regulation of metabolism and insulin sensitivity (42). Dysfunction of leptin signaling and reduced adiponectin levels may contribute to the development of obesity. In our review we observed no effect on leptin levels. In contrast, increased levels were observed in patients who used supplementation. Elevated levels of adiponectins in humans may correlate with inflammation or the body's reaction to exacerbation on contact with new substances, in the case of NAD+ supplementation, so that in the supplemented group there was an increase in its levels, as an attempt to combat the effects of pro-inflammatory cytokines, there is an increase in adiponectins (43). In addition, the fact that a low BMI is associated with an increase in adiponectin can be explained, identifying that the secretion of this adipokine is related to the quality, not the quantity, of adipose tissue, and in addition to BMI, in situations of greater age this also tends to occur (42).

Another interesting finding regarding the effects of NAD+ precursors on BMI was their greater magnitude with the use of NA when compared to NE. However, it is important to highlight that only five RCTs evaluating the effect of NE supplementation on BMI were included in this meta-analysis. In fact, when compared to NA, NE has been less studied regarding its effect on BMI. Thus, more studies evaluating the effect of NE supplementation would be important to better investigate its therapeutic potential in obesity outcomes, allowing a better comparison in relation to other NAD+ precursors. In addition, unlike NE, NA reduces the levels of lipid profiles, including cholesterol and triglycerides, which can explain its greater effect on BMI and be effective on factors related to obesity.

Our study had some limitations that jeopardized the extraction of robust conclusions. Clinically and statistically significant heterogeneities was found for adiponectin. These may be explained by the differences in the intervention-specific factors (e.g., type, dose, administration route, and duration of drugs) and weight-specific factors (e.g., age, sex, physiology, genetics, familial history, race/ethnicity, physical activity, socioeconomic status, dietary intakes, and drug, tobacco, or alcohol consumption) (44). Nonetheless, we attempted to identify some possible sources of heterogeneity in data by performing a subgroup analysis. In addition, lack of registration of the current study in PROSPERO due to time limit was another limitation of this study.

Despite its limitations, the current study has several positive features: a rigorous methodology was used based on the PRISMA guidelines; a comprehensive literature search included different independent databases; search, selection, and data extraction applied to the selected studies were performed separately, and in duplicate, by two researchers; a third party was accessed to solve disagreements. Furthermore, the present study likely included the largest effect size for each outcome assessed at obesity.

Conclusion

The finding of this paper showed that NAD+ precursor supplementation has an effect on lowering BMI and increasing adiponectin in humans compared with control groups. However, no significant effect was observed on body weight and leptin. Given the evidence of decreased BMI in humans and increased adiponectin, it is important to emphasize that NAD+ supplementation should not be seen as a one-time solution for weight loss and should be combined with healthy eating habits and exercise under professional guidance from a dietitian.

Data availability statement

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

Ethics statement

This study was approved by the research council and ethics committee Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Author contributions

MS and YB contributed in conception, design, and statistical analysis. MS, YB, ST, MG, and NS contributed in data collection and manuscript drafting. MS supervised the study. 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.2023.1208734/full#supplementary-material

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Boosting plant food polyphenol concentration by saline eustress as supplement strategies for the prevention of metabolic syndrome: an example of randomized interventional trial in the adult population

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Introduction: Phenolic compounds in lettuce can increase by the application of positive stress (eustress) such as moderate saline stress. Phenolic compounds possess antioxidant capacity that is a key factor in the detoxification of excess reactive oxygen species. A double-blinded randomized interventional and placebo- controlled study design was carried out to compare the effect of daily dietary eustress lettuce ingestion in hepatic, lipid, bone, glucose, and iron metabolism.

Methods: Forty-two healthy volunteers, 19 female and 23 male participants, were divided into two groups. Participants were randomized into a polyphenol-enriched treatment (PET) arm or control arm. Each arm consumed 100 g/day of control or eustress (polyphenols enriched treatment = PET) lettuce for 12 days. Primary study outcomes were serological analysis for assessing hepatic, lipid, bone, iron, and glucose markers at baseline and after 12 days. Secondary outcomes assessed body composition.

Results: Salinity stress reduced plant yield but increased caffeic acid (+467%), chlorogenic acid (+320%), quercetin (+538%), and rutin (+1,095%) concentrations. The intake of PET lettuce reduced PTH, low-density lipoprotein (LDL), cholesterol, alanine transaminase (ALT), and aspartate transaminase (AST) enzyme levels and increased vitamin D and phosphate levels, while iron and glucose metabolism were unaffected.

Discussion: Supplementation with eustress lettuce by increasing polyphenols concentration ameliorates hepatic, lipid, and bone homeostasis. Body composition was not affected.

Clinical trial registration: https://classic.clinicaltrials.gov/ct2/show/NCT06002672, identifier: NCT06002672.

KEYWORDS

MetS, diet, body homeostasis, functional food, lettuce, phytochemicals, nutritional intervention

1 Introduction

In recent years, the demand for fresh vegetables has been increasing due to consumers' self-awareness that fibers, mineral salts, and vitamins have a beneficial effect on health by reducing, for example, the risk of age-related diseases (1). Due to their bioactive properties, horticultural crops are in the interest of the major lines of international food manufacturing. Therefore, farmers together with scientists are working to characterize new market products with enhanced nutraceutical activity (2) in order to maintain sustainability for the preservation of natural resources.

In this view, it is necessary to point out the gap between research and market offering (3). At present, horticultural crops are well characterized for the quality component, while the information about the functional properties comes from *in vitro* (cells) or *in vivo* animal studies (rodents) and, therefore, translated to human metabolism. Thus, the effects of crops enriched with nutraceutical compounds on human body homeostasis and on disease prevention need to be addressed.

Lettuce (*Lactuca sativa* L.) is a green leafy vegetable belonging to the *Asteraceae* family, which is consumed worldwide by most of the population in all seasons. The high fiber content, which promotes satiety, and the presence of vitamin C, flavonoids, and phenolic acids place lettuce at the top of healthy food items (4, 5).

Groundwater salinization caused by salts such as NaCl and $CaCl^2$ is a growing concern in several regions of the ecosphere, comprising those of the Mediterranean basin (6). These salts can be derived from different fonts such as seawater infiltration, dissolution of minerals in rocks, and anthropogenic actions (7). The high electrical conductivity of irrigation water and agricultural soils is mainly due to the excessive use of fertilizers and determines salt stress in plants, affecting their growth and yield. However, the salinity can also be used to increase the content of bioactive compounds (8). The application of positive stress (eustress) can modulate the biosynthesis and accumulation of secondary metabolites *via* the activation of plant defense mechanisms (9). Therefore, the nutritional management of horticultural crops and the application of eustress, such as salinity, provide valuable and cost-effective tools to manipulate plant phytochemical content and product quality, which will contribute to meeting the growing market trends toward high value-added products (8). In fact, it was shown that moderate salinity resulted in an increase of ascorbic acid, α-tocopherol content, and antioxidant activity of *Cichorium spinosum* leaves (10). Furthermore, an increase in anthocyanin and ascorbic acid contents in green and red lettuce leaves (11, 12) was documented.

Several studies strongly suggest that a diet rich in polyphenols may have beneficial effects in the prevention of metabolic syndrome and related diseases (13), including cancer. Data on the effects of the consumption of polyphenols-enriched lettuce on human health are missing. Therefore, the specific objective of this study was to test whether polyphenols-enriched treatment (PET) lettuce supplementation influences human health. Two outcomes were measured: Primary outcomes investigating whether the consumption of PET lettuce could influence key regulators of body homeostasis, in particular, liver, lipid, bone, glucose, and iron metabolism in the adult population by assessing serological levels of hepatic, lipid, bone, iron, and glucose markers at baseline and after 12 days. Secondary outcomes evaluated the body composition.

2 Materials and methods

2.1 Cultivation practice, experimental site, and design and yield of lettuce

The lettuce plants were grown on a farm belonging to the Department of Agricultural, Food, and Forestry Sciences of the University of Palermo (SAAF), located near Palermo. The growing cycle was carried out in a tunnel covered with a transparent polyethylene film. Before transplanting, aged manure was added as a soil amendment at a rate of 10 t ha^{-1}. On March 15, 2021, 300 plug plants of "Canasta" lettuce (*Lactuca sativa* L.) (Syngenta Seed, Basel, Switzerland), at the stage of four to five true leaves, were transplanted at a density of 20 plants m^{-2}. Considering the short duration of the lettuce growing cycle and the nitrogen available in the soil (2‰), no fertilizers were added. During the growing cycle, the irrigation management was conducted in accordance with standard lettuce cultivation practices. After 7 days of transplanting, half of the plants were irrigated with tap water (0 mM NaCl; EC 0.68 dS m^{-1}), while the remaining plants were exposed to salinity stress (25 mM NaCl; EC 3.15 dS m^{-1}). All plants were harvested on May 31, 2021 and immediately weighed (10 plants per replication) and transferred to the Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies, University of Palermo to start the clinical trial. The agronomic trial was arranged in a randomized complete block design, and each block was replicated three times, containing 50 plants each.

2.2 Design of the study and participants

This is a randomized and placebo-controlled study design. The protocol was approved with the number of approbation protocol 02/2020 by the Ethics Committee of the University of Palermo Hospital P. Giaccone and was conducted in accordance with the Declaration of Helsinki. Participants provided, prior to study inclusion, written informed consent. The participants were recruited in March 2021. The study is registered at clinicaltrial.gov NCT06002672.

Based on previous studies (14–16), for the estimation of fasting insulin levels, *a priori* power calculation utilizing a level of statistical significance α of 5% and a probability β of 20% was performed, which estimated a sample size of eight people. In order to reduce type two error risks and to improve the evaluation power for the secondary outcomes, we decided to include not <12 persons in each group of the study. Forty-two healthy volunteers, 19 female and 23 male participants, joined the study. A team of physicians and nutritionists individually instructed the volunteers for the compilation of a food and lifestyle diary and for not changing lifestyle habits throughout the study period. This is because having a trained interviewer review increases the quality of the report (17). The information about food diaries was collected 8 days

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TABLE 1 Criteria of eligibility for participation in the recruitment process.

Criteria of selection for participation in the study	Inclusion criteria	Exclusion criteria
Italian ethnicity	Absence of gastrointestinal, cardiac and blood dysfunction, absence of metabolic disorders, and viral infection	Presence of metabolic or chronic disease
Not taking medications and supplements	Age range: 18–65 years	Use of vitamins and minerals supplements and use of medications
Clinically healthy	Body mass index: 18.5–28 kg/m^2	Pregnancy and breastfeeding condition

before the beginning of the study as previously reported (15) and until the end of the study in order to monitor compliance and adherence, showing eventual food habits and lifestyle modification. More specifically, participants were asked to record in detail the foods and beverages they consumed during the day at designated times and to report physical activity type and duration. The team of nutritionists conducted meal plan analysis of food diaries for each participant to monitor energy, macronutrients, micronutrients, and water and fiber consumption by using the WinFood software from Medimatica s.r.l Colonnella (TE) (Supplementary Table 1). This was done in order to check that subjects maintained their usual diet and lifestyle during the supplementation period. The flowchart of the recruitment and assignment of applicants to study groups is described in Figure 1. The criteria of eligibility for participation in the study were the absence of gastrointestinal, cardiac and blood dysfunction, absence of metabolic disorders, viral infection, use of medication including minerals and vitamins supplements and exogenous hormones, and the absence of pregnancy and breastfeeding condition. The criteria are summarized in Table 1.

2.3 Experimental research design procedures

Participants attended the ambulatory Nutrition Age and Bone (NABbio) of the University of Palermo, STEBICEF department in the morning between 7:00 and 8:00 a.m. (18, 19) under controlled conditions. They were in fasting condition from the dinner of the day before (12-h fasting) as previously reported (15). A sample of venous whole blood was collected in the appropriate tubes for serum and plasma, and the anthropometric measurements including body lean and fat mass, weight, and height (20) were recorded for each participant. To randomize the participants, a random number generator computer program (excel) was used to generate random numbers from the list for each group. Then, the participants were randomly assigned to the control or experimental group and given random numbers by a third party, who encoded the lettuces with matching random numbers. The medical staff, the investigator, and the participants in the study were blinded to the allocation during the whole data collection period. The investigators were also blinded during the data analysis and the sample assessment. Thus, participants in a double-blinded manner were allocated to one group, were provided with the crops of lettuce (∼2 kg), were instructed to store them (21), and to eat 100 g each day for 12 days. In the control group, 12 volunteers (8 female and 12 male participants) who received canasta lettuce were allocated. In the PET lettuce group, 22 people (11 female and 11 male participants) were allocated, but 2 subjects dropped out due to personal problems, thus leaving a total of 20 volunteers (9 female and 11 male participants). The study outcome is represented by a sample of blood collected at time zero and after 12 days of nutritional intervention (15), as shown in Figure 2. Specifically, the primary outcome assessment involved measuring the serological levels of hepatic, lipid, bone, iron, and glucose markers at baseline and after 12 days. secondary outcome measured body mass.

Authors had no access to information that could identify individual participants during or after data collection. To not be individuated and be anonymous, the samples were identified by code numbers.

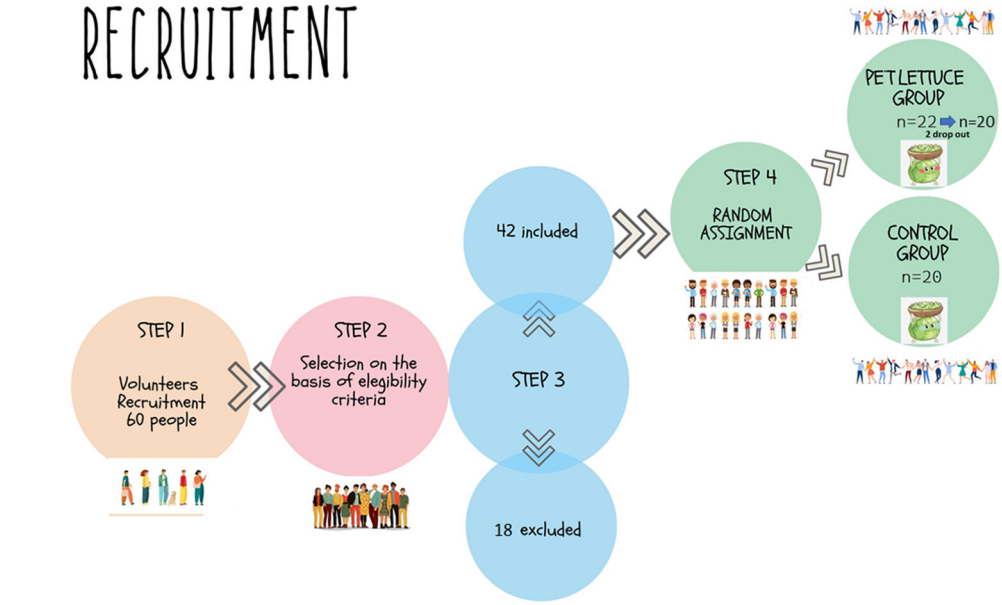
2.4 Hematological and biochemical analysis of the samples

For the analysis of the samples, to obtain serum, blood was collected in specific tubes without anti-coagulant and centrifugated at room temperature, 1.300 g for 15 min. An automated procedure on the Roche COBAS c503, according to standard commercially available assays supplied by Roche Diagnostics, was performed to measure glucose and insulin, markers of hepatic, lipid, and iron metabolism, and markers of bone metabolism (14–16, 20, 22, 23).

2.5 Analysis of polyphenols in lettuces

Lettuce samples were stored immediately on reception at −80°C in a properly numbered plastic jar until analysis. Samples were properly homogenized before analysis using a laboratory blender. The blank was analyzed to confirm the absence of polyphenols.

In the sample extraction procedure, 0.1 g of lettuce grounded sample was used. Then, 10 mL of acetone/water/HCl (70:29.9:0.1 v/v/v) solution was added, and this mixture was shaken in a vortex, sonicated for 30 min, and centrifuged at 3,500 rpm for 15 min at +5°C. The supernatant was incubated overnight at 4°C. After incubation, samples were filtered using a 0.45 μ filter, and finally, 10 μL of the extract was injected into the UHPLC/HRMS system. For the analysis of polyphenols in lettuces, an accela pump HPLC binary pump, with an XBridge BEH column (Thermo) C18 2.5 μm (2.1 × 50 mm) was used. The oven temperature was +40°C and the flow rate was 300 μL min^{-1}. The mobile phases were aqueous 0.1% formic acid (A) and ACN hypergrade (B), and the gradient program was as follows (time min, % B): (0, 5); (0.33, 30); (1.63, 100); (9.63, 100); (11.63, 5). The total run time was 13.63 min. The chromatographic system was coupled to a hybrid quadrupole-Orbitrap HRMS instrument (Q-Exactive, Thermo Scientific, Bremen, Germany), with heated electrospray



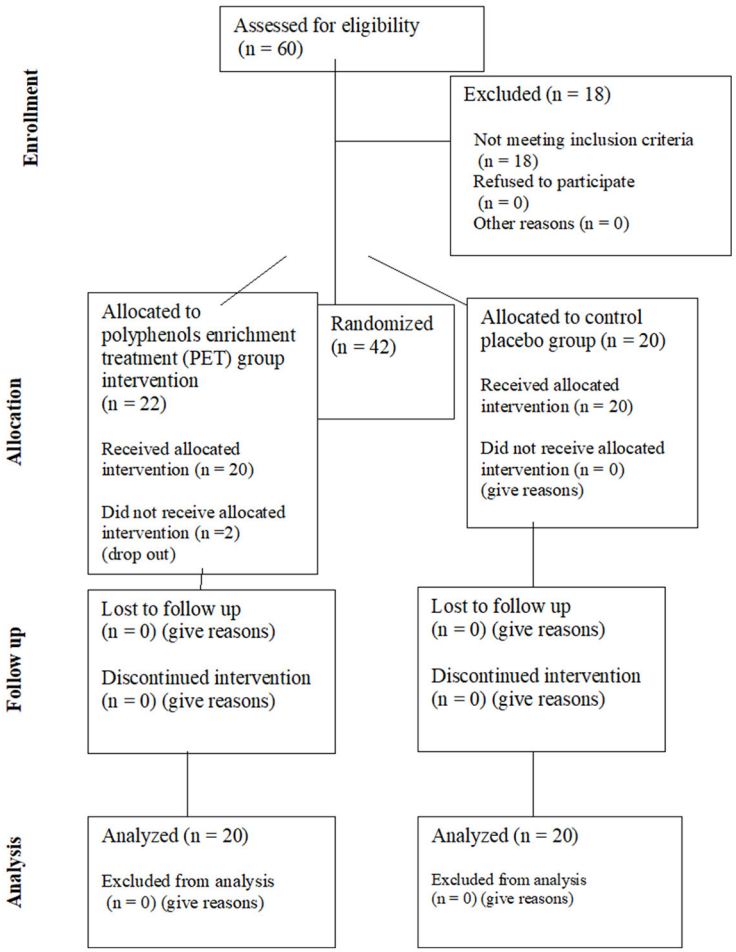


FIGURE 1
Flow chart recruitment and random assignment of participants to the study.

ionization (HESI) in the positive and negative ionization mode. The full MS-ddMS2 acquisition mode was applied with a resolution of 70,000 (m/z 200, FWHM), automatic gain control (AGC) target 3e6, maximum injection time (IT) 200 ms, and a scan range of 100–1,000 m/z. Nitrogen was obtained from a Zephyr generator (Thermo Fisher Scientific, San Jose, CA, USA). Compounds were

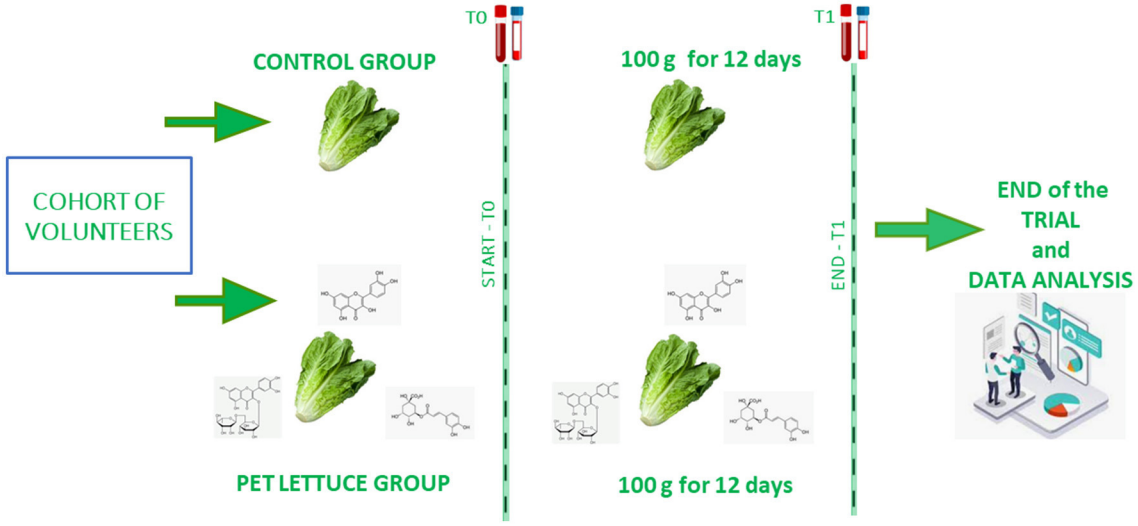


FIGURE 2
Schematic overview of the nutritional interventional in the control and eustress polyphenols-enriched treatment (PET) lettuce groups of study. Canasta lettuce (above) or PET lettuce (below) was administered to the volunteers every day, 100 g a day, for a total of 12 days. The samples of blood were collected at time zero (start or baseline) and at time one (the end of the intervention).

identified by retention time (RT) and accurate mass. In the experiment, a mass accuracy of 5 ppm was used. The analysis was carried out on the basis of calibration curves (https://doi.org/10.6084/m9.figshare.24219346).

2.6 Statistical analyses

Student *t*-tests were used for the comparison of polyphenols in control and eustress lettuce. For the human study, the groups of study were first compared at baseline by using Student *t*-tests, while the differences between T0 and 1 were compared by using one-way ANOVA followed by Tukey's posttest by using GraphPad Prism software. A *p*-value for a dataset of ≤ 0.05 indicates that the results were statistically significant. Data are expressed as mean ± standard deviation (S.D).

3 Results

3.1 Phenolic quantification analysis in lettuces

The quantification of the phenolic compounds was performed in control and eustress (polyphenols enriched treatment) lettuces in order to observe differences in the concentration of polyphenols. Eustress (polyphenols enriched treatment) lettuce showed significantly increased concentrations of caffeic acid, chlorogenic acid, quercetin, and rutin compared to the control lettuce. No difference was observed in apigenin and catechin concentrations between eustress lettuce and control (Table 2).

TABLE 2 Polyphenols quantification in control and eustress (polyphenols enriched treatment PET) lettuce.

Polyphenols content (μg/100 g of fresh weight)	Control lettuce mean ± SEM	PET lettuce mean ± SEM	*p*-value
Apigenin	114.4 ± 4.0	107.4 ± 1.2	n.s.
Caffeic acid	27.4 ± 1.1	128 ± 0.8	<0.000001
Catechin	18 ± 4.2	25.1 ± 2.0	n.s.
Chlorogenic acid	260 ± 4.1	834.1 ± 96.6	0.000144
Quercetin	28.2 ± 3.3	151.8 ± 7.8	<0.000001
Rutin	14.6 ± 1.6	160 ± 5.9	<0.000001
Total polyphenols	462.4 ± 7.0	1406.5 ± 106.9	0.000005

Data are means ± SEM. Student t-tests were used to compare the two groups of study. A p-value higher than 0.05 denotes that the change is not statistically significant (n.s.) and indicates strong evidence for the null hypothesis.

3.2 Anthropometric characteristics of the study population

As shown in Table 3, the two groups of the study were homogeneous in nature. The study started in March 2021 and ended in June 2021. No difference was observed in the percent of body fat and lean mass, in body weight among the two groups of subjects enrolled in the study at baseline and at the end of the intervention (Table 3).

TABLE 3 Characteristics of subjects in the two groups (control and polyphenols-enriched treatment, PET lettuce groups) at baseline (T0) and following 12 days of lettuce administration (100 g/day) (T1).

Study group information	Control group T0 ($n = 20$; 8 females; 12 males) Mean ± S.D.	PET lettuce group T0 ($n = 20$; 9 females; 11 males) Mean ± S.D.	Control group T1 ($n = 20$; 8 females; 12 males) Mean ± S.D.	PET lettuce group T1 ($n = 20$; 9 females; 11 males) Mean ± S.D.	*p*-value
Age (years)	45 ± 12	37 ± 11	-	-	n.s.
Body weight (kilograms)	75 ± 13	72 ± 15	75 ± 12	72 ± 15	n.s.
Body mass index	28.2 ± 3.8	26.0 ± 5.1	-	-	n.s.
Height (centimeters)	164 ± 8	166 ± 8	-	-	n.s.
Body fat mass (percent)	24.2 ± 4.7	23.4 ± 4.2	24.3 ± 4.8	23.6 ± 4.3	n.s.
Body lean mass (percent)	75.7 ± 4.7	76.5 ± 4.2	75.7 ± 4.8	76.4 ± 4.3	n.s.

All the values are indicated as means ± standard deviations (SD). Student t-tests were used to compare control and PET lettuce groups at baseline. When appropriate differences between and within the groups (T0 and T1) were compared by using one-way ANOVA followed by Tukey's posttest. A p-value higher than 0.05 means that the change is not statistically significant and reported as not statistically significant (n.s.). A p-value for a dataset of ≤0.05 indicates that the results were statistically significant.

3.3 PET lettuce consumption in adults and its impact on hepatic and lipid homeostasis

PET-enriched lettuce consumption, 100 g/day, for 12 days improved liver function. Specifically, a reduction of ∼30% in AST and ∼34% in ALT enzymatic levels following 12 days of PET lettuce consumption from baseline (Table 4) was observed. Ingestion for 12 days of control lettuce was without effects on hepatic function. No difference was observed in ALP, GGT, TP, and albumin within or among the two groups (Table 4). The nutritional intervention with PET lettuce ameliorates lipid metabolism. In fact, it reduced total cholesterol and LDL levels and did not affect triglycerides and HDL concentration (Table 5).

3.4 PET lettuce intake in the adult population and its impact on bone homeostasis

Daily intake of PET lettuces reduced serum PTH and increased vitamin D and phosphate levels compared to baseline and control lettuce (both baseline and after 12 days) (Table 6). However, there was no modification in the markers of bone remodeling. There was no change in serum levels of bone formation (osteocalcin) and resorption (CTX), calcium, potassium, and calcitonin compared to the baseline or control group.

3.5 PET lettuce intake in the adult population and impact on glucose and iron homeostasis

PET lettuce intake did not affect blood glucose concentration. In fact, no differences were observed in fasting glucose and insulin levels among the two groups of study that consumed control or PET lettuce (Table 7). Moreover, the nutritional intervention with PET lettuce did not modify markers of iron metabolism such as iron, ferritin, and transferrin serum level concentration as well as transferrin saturation if compared to the control arm (Table 7).

4 Discussion

We are dealing with global warming and new pathology, and the COVID-19 pandemic is the latest example. These are signals that there is a need to increase sustainability. Therefore, new approaches to vegetable growth and novelty in their consumption are necessary in order to find new treatments for the prevention of metabolic syndrome. In the present study, we aimed to understand the effect of natural eustress, such as a mild increase in salinity, in one of the most popular consumed vegetables such as lettuce. In particular, the novelty of the study was to verify the ability of eustress to increase functional content, in particular, polyphenols, and to analyze the impact on human physiological homeostasis in an attempt to find new nutritional strategies for the prevention of metabolic syndrome.

Data showed that salinity-stressed plants had a lower yield than control plants. These findings are coherent with a previous study (24) that reported a yield reduction in iceberg lettuce plants stressed with NaCl. This effect could be attributed to the osmotic, ionic, and oxidative stress caused by salinity (25). Moreover, eustress significantly increased the concentration of caffeic acid, chlorogenic acid, quercetin, and rutin with respect to control lettuce. Therefore, as previously shown by Santander et al. (26), we confirm that mild eustress improves the functional quality of lettuce and specifically it acts by increasing polyphenol concentration. This effect is linked to the modification of plant physiological mechanisms and metabolism, permitting plants to grow better under sub-optimal environmental conditions through the activation of antioxidant systems (8).

To investigate the potential effect of PET lettuce supplementation in the prevention of metabolic syndrome, the cohort of adults consumed two different types of lettuce every day in a quantity that we know is well accepted by the participants (23), 100 g a day for 12 days. One group ate the control lettuce, which was grown without salinity eustress, while the other

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TABLE 4 Markers of hepatic function were measured in the study population baseline (T0) and following 12 days of control and polyphenols-enriched treatment (PET) lettuce administration (100 g/day) (T1).

Markers of hepatic function	Control group T0 ($n = 20$) Mean ± SD	Control group T1 ($n = 20$) Mean ± SD	*p*-value	PET lettuce group T0 ($n = 20$) Mean ± SD	PET lettuce group T1 ($n = 20$) Mean ± SD	*p*-value
Aspartate aminotransferase (AST) (U/l)	24 ± 7	25.6 ± 6	n.s.	23 ± 7	16.4 ± 5	**0.0108**
Alanine aminotransferase (ALT) (U/l)	25 ± 7.0	23.6 ± 8.0	n.s.	24 ± 8.4	15.8 ± 11	**0.0309**
Alkaline phosphatase (ALP) (U/l)	66 ± 9	61 ± 11	n.s.	66 ± 11	64 ± 13	n.s.
Gamma-glutamyl transferase (GGT) (U/L)	17 ± 6	17.5 ± 6	n.s.	17 ± 10	15 ± 7	n.s.
Total protein (TP) (g/L)	72 ± 5	71 ± 4	n.s.	72 ± 3	70 ± 3	n.s.
Albumin (g/L)	46 ± 3	44 ± 3	n.s.	45 ± 2.4	44 ± 2.5	n.s.

All the values are indicated as means ± standard deviations (SD). Differences between and within the groups (T0 and T1) were compared by using one-way ANOVA followed by Tukey's posttest. A p-value higher than 0.05 means that the change is not statistically significant and reported as not statistically significant (n.s.). A p-value for dataset ≤ 0.05 indicates that the results were statistically significant.

TABLE 5 Markers of lipid metabolism were measured in the study population baseline (T0) and following 12 days of control and polyphenols-enriched treatment (PET) lettuce administration (100 g/day) (T1).

Markers of lipid metabolism	Control group T0 ($n = 20$) Mean ± SD	Control group T1 ($n = 20$) Mean ± SD	*p*-value	PET lettuce group T0 ($n = 20$) Mean ± SD	PET lettuce group T1 ($n = 20$) Mean ± SD	*p*-value
Triglycerides (mg/dL)	104 ± 49	97 ± 20	n.s.	91 ± 56	95 ± 29	n.s.
Total cholesterol (mg/dL)	194 ± 27	189 ± 14	n.s.	198 ± 32	167 ± 33	0.0035
Low-density lipoproteins (LDL) cholesterol (mg/dL)	116 ± 28	113 ± 24	n.s.	124 ± 32	90 ± 27	0.0010
High-density lipoproteins (HDL) cholesterol (mg/dL)	65 ± 13	61 ± 13	n.s.	63 ± 11	60 ± 14	n.s.

All the values are indicated as means ± standard deviations (SD). Differences between and within the groups (T0 and T1) were compared by using one-way ANOVA followed by Tukey's posttest. A p-value higher than 0.05 means that the change is not statistically significant and reported as not statistically significant (n.s.). A p-value for dataset ≤ 0.05 indicates that the results were statistically significant.

group consumed—for the same period—the PET lettuce. The two groups of study have a similar age range and anthropometric characteristics. The participants did not report differences in the taste or appearance of lettuces. Moreover, they did not report differences in diet and/or lifestyle during the supplementation periods as reported in the food diary. Both the groups, control and PET lettuce, maintained their usual lifestyle. From the physiopathology point of view, the group that consumed the PET lettuce showed improved body homeostasis. In fact, PET lettuce consumption ameliorated liver function by reducing the AST and ALT levels. It also acted in lipid metabolism by reducing total cholesterol and LDL compared with control lettuce. Our results are in accordance with several reports (27), which showed that polyphenols, due to their beneficial properties when regularly consumed in humans, reduced the risk of several metabolic disorders associated with non-alcoholic fatty liver disease. The added value of this study is that polyphenols are naturally increased by eustress, and lettuce can be easily consumed during meals with a normal diet. Thus, regular PET lettuce consumption could be an optimal solution for the prevention of NAFLD and metabolic syndrome.

Despite the health-promoting qualities of polyphenols, it was postulated that the consumption of polyphenols may be associated with decreasing the absorption and bioavailability of iron (28, 29) and affects iron metabolism. Therefore, the markers of iron metabolism were measured in order to verify if consumption of eustress lettuce may be associated with negative health consequences. We did not find differences in iron, transferrin, ferritin, and ceruloplasmin levels between the groups of study ruling out that PET lettuce consumption could interfere with iron status. We also did not find modification in glucose and insulin circulating levels between the control and PET lettuce group. The effects of polyphenols-enriched food in glycemic control during clinical trials are still unclear (30).

In consideration of the tight link between lipid and bone metabolism (31–33) and the lack of clinical trials to define a clear link between these phytonutrients and bone health (34), we analyzed if consumption of PET lettuce impacts markers of bone remodeling and metabolism. We measured serum PTH. This hormone is secreted from the parathyroid glands and is a key regulator of bone metabolism and calcium–phosphorus homeostasis (35). It upregulates bone turnover (36). In particular, PTH has a stimulatory effect on bone resorption. PET lettuce consumption significantly reduced the concentration of PTH. Therefore, its reduction, within the physiological range, suggests that PET lettuce intake exerts a positive effect on the maintenance

TABLE 6 Markers of bone remodeling and metabolism were measured in the study population baseline (T0) and following 12 days of control and polyphenols-enriched treatment (PET) lettuce administration (100 g/day) (T1).

Markers of bone metabolism	Control group T0 ($n = 20$) Mean ± SD	Control group T1 ($n = 20$) Mean ± SD	*p*-value	PET lettuce group T0 ($n = 20$) Mean ± SD	PET lettuce group T1 ($n = 20$) Mean ± SD	*p*-value
Carboxy-terminal collagen crosslinks (CTX) (μg/L)	0.45 ± 0.1	0.43 ± 0.1	n.s.	0.5 ± 0.2	0.46 ± 0.2	n.s.
Osteocalcin (μg/L)	22 ± 6.5	20.5 ± 6.2	n.s.	24 ± 6.6	23.3 ± 8	n.s.
PTH (ng/ml)	43.2 ± 9.3	40 ± 9.5	n.s.	40.3 ± 12	29.8 ± 11	0.0177
Vitamin D (μg/L)	32.2 ± 4.4	31.6 ± 5.5	ns	32.5 ± 3.3	41.3 ± 8.9	<0.0001
Phosphate (mg/dL)	3.2 ± 0.7	3.1 ± 0.8	n.s.	3.2 ± 0.6	3.8 ± 0.5	0.0079
Aa calcium (mg/dL)	8.7 ± 1.3	8.4 ± 1.1	n.s.	9.1 ± 0.2	9.2 ± 0.2	n.s.
Potassium (mmol/L)	4.0 ± 0.3	3.9 ± 0.4	n.s.	3.7 ± 1.1	4.2 ± 0.3	n.s.
Calcitonin (ng/L)	2.3 ± 2.4	1.6 ± 1.5	n.s.	2.3 ± 2.3	1.6 ± 1.4	n.s.

All the values are indicated as means ± standard deviations (SD). Differences between and within the groups (T0 and T1) were compared by using one-way ANOVA followed by Tukey's posttest. A p-value higher than 0.05 means that the change is not statistically significant and reported as not statistically significant (n.s.). A p-value for dataset ≤ 0.05 indicates that the results were statistically significant.

TABLE 7 Markers of glucose and iron metabolism were measured in the study population baseline (T0) and following 12 days of control and polyphenols-enriched treatment (PET) lettuce administration (100 g/day) (T1).

Markers of glucose and iron metabolism	Control group T0 ($n = 20$) Mean ± SD	Control group T1 ($n = 20$) Mean ± SD	*p*-value	PET lettuce group T0 ($n = 20$) Mean ± SD	PET lettuce group T1 ($n = 20$) Mean ± SD	*p*-value
Glucose (mg/dL)	85.7 ± 8.4	86 ± 9.7	n.s.	87.7 ± 9.0	88.6 ± 9.7	n.s.
Insulin (mUI/L)	9.2 ± 3.5	9.4 ± 3	n.s.	8.7 ± 4	8.5 ± 5.7	n.s.
Iron (μg/dL)	80 ± 21	75 ± 17	n.s.	88 ± 43	83 ± 36	n.s.
Ferritin (ng/dL)	87 ± 29	84 ± 19	n.s.	81 ± 38	83 ± 17	n.s.
Transferrin (mg/dL)	250 ± 42	242 ± 37	n.s.	267 ± 39	254 ± 41	n.s.
Transferrin saturation (%)	22 ± 7	19 ± 3.5	n.s.	24 ± 9.5	23 ± 10	n.s.

All the values are indicated as means ± standard deviations (SD). Differences between and within the groups (T0 and T1) were compared by using one-way ANOVA followed by Tukey's posttest. A p-value higher than 0.05 means that the change is not statistically significant and reported as not statistically significant (n.s.). A p-value for dataset ≤ 0.05 indicates that the results were statistically significant.

of skeletal homeostasis (37). In fact, we did not observe a difference in CTX (the marker of bone resorption) and osteocalcin (the marker of bone formation) after 12 days of intervention.

In order to investigate the potential mechanism of action, we measured vitamin D levels. In fact, PTH synthesis and secretion are regulated by vitamin D. Specifically, vitamin D acts at the parathyroid gland by suppressing the synthesis of PTH by repressing its gene (38). In our study, the consumption of eustress lettuce increased vitamin D levels. Therefore, it is possible to suppose that the consumption of PET lettuce by increasing vitamin D levels suppresses the synthesis of PTH (38), and reduces PTH secretion.

Maintenance of skeletal homeostasis through bone remodeling is a tight coupling process that requires subtle coordination between osteoblasts and osteoclasts (39). Following PET lettuce treatment, an increase in the level of phosphate, within the physiological range, in the adult population was observed, while it was not observed for calcium. This is probably because vitamin D is able to enhance the efficiency of intestinal absorption of calcium up to 30–40% and of phosphate to nearly 80% (40). Therefore, we registered significant changes in the level of phosphate but not calcium concentration. Our results are consistent with previous *in vitro* and *in vivo* animal studies, which showed that bioactive phenolics have beneficial effects on bone health (41).

About the mechanism of action by which the supplementation with PET lettuce exerts its effects, we found that it may act at different levels to modulate homeostasis because multiple targets have been reported for polyphenols. They could affect lipid metabolism by modulating oxidative stress (42) by reducing ROS. In fact, polyphenols are powerful regulators of LDL oxidation (43). This could ameliorate lipid metabolism. Moreover, they could impact liver homeostasis by decreasing liver pro-inflammatory cytokines (27) and this in turn could reduce liver enzymes. Elevated liver enzymes often indicate inflammation or damage to liver cells. Polyphenols can protect bone health through modulation of osteoimmunological action, osteoblastogenesis, and osteoclastogenesis. Thus, polyphenols by inducing a reduction of oxidative stress could modulate bone metabolism due to their

antioxidant activity and probably also by reduction of inflammation by modulating pro-inflammatory signaling in the bone (41) as they do in liver and adipose tissue. It is interesting to underline that the second cause of osteoporosis is liver disease. In fact, ∼30% of patients affected by chronic liver disease suffer from osteoporosis (44). Thus, the use of natural polyphenols, supplied by the vegetable matrix, could be useful in the prevention of liver disease and its associated osteoporosis.

In the PET lettuce, we found an increase in caffeic acid, chlorogenic acid, quercetin, and rutin concentration. Thus, about the mechanism of action, we found that each of them could take part in the observed effects. In fact, caffeic acid is a potent antioxidant and anti-inflammatory molecule. In obese animal models improves lipid profile, and liver biomarker enzymes by decreasing lipoperoxyl radicals (45). In nutritional intervention studies caffeic acid acts by inhibiting low-density lipoprotein (LDL) oxidation and impacts bone metabolism by reducing oxidative stress on bone cells (46). In particular, caffeic acid in *in vitro* and animal models reduces osteoclastogenesis and bone resorption, as well as osteoblast apoptosis (47).

Chlorogenic acid is able to improve lipid metabolism. In diabetic mice, it acts by enhancing fatty acid oxidation and triglycerides lipolysis. Moreover, it reduces liver triglyceride synthesis and fatty acid transportation, alleviating hepatic inflammatory response and oxidative stress (48). Similar to our results, in healthy male subjects (aged 20–31 years), chlorogenic acid, supplied in coffee, decreased LDL-cholesterol (49). In obese rats, it reduced cholesterol and triacylglycerol concentrations in the liver and plasma (50).

About the effects of quercetin, it was found, in accordance with our results, that supplementation with quercetin, in patients with MetS and related disorders, significantly reduced total cholesterol and LDL cholesterol, but did not affect triglycerides and HDL cholesterol (51). In animal models, quercetin exerts antioxidative properties favoring an increase in osteogenic activities and a decrease in osteoclastogenic activities (52).

Rutin enhances proliferation and ossification markers in bone cells. In fact, *in vitro* studies showed that rutin increases osteocyte and osteoblast-related gene expression and, similar to our study, increases vitamin D levels (53). In animal models, rutin reduces reactive oxygen species by inhibiting inflammatory cytokines (54). In rats, rutin reduces plasma total cholesterol and LDL and exerts hepatoprotective effects that seem to be related to antioxidant activity (55).

There are limitations to the clinical trial. The intervention was short-term. It was performed for 12 days. Although it provides information in acute about the effect of PET consumption, longer trials are necessary. Moreover, it could be interesting to analyze the effects of the PET in a cohort of seniors.

To the best of our knowledge, this is the first interventional study that has investigated the benefits of the consumption of dietary polyphenols, carried by a vegetable matrix, in human bone metabolism. The effects that were observed in the liver, and lipid and bone metabolism could pave the way for future application of this natural resource for prevention of osteoporosis and NAFLD.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://doi.org/10.6084/m9.figshare.24219346.v1.

Ethics statement

The studies involving humans were approved by Ethics Committee of the University of Palermo Hospital P. Giaccone. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Palermo University Hospital (No. 2/2020).

Author contributions

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Conflict of interest

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Supplementary material

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Dietary intervention improves metabolic levels in patients with type 2 diabetes through the gut microbiota: a systematic review and meta-analysis

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Background: Poor dietary structure plays a pivotal role in the development and progression of type 2 diabetes and is closely associated with dysbiosis of the gut microbiota. Thus, the objective of this systematic review was to assess the impact of dietary interventions on improving gut microbiota and metabolic levels in patients with type 2 diabetes.

Methods: We conducted a systematic review and meta-analysis following the PRISMA 2020 guidelines.

Results: Twelve studies met the inclusion criteria. In comparison to baseline measurements, the high-fiber diet produced substantial reductions in FBG (mean difference −1.15 mmol/L; 95% CI, −2.24 to −0.05; $I^2 = 94\%$; $P = 0.04$), HbA1c (mean difference −0.99%; 95% CI, −1.93 to −0.03; $I^2 = 89\%$; $P = 0.04$), and total cholesterol (mean difference −0.95 mmol/L; 95% CI, −1.57 to −0.33; $I^2 = 77\%$; $P = 0.003$); the high–fat and low–carbohydrate diet led to a significant reduction in HbA1c (mean difference −0.98; 95% CI, −1.50 to −0.46; $I^2 = 0\%$; $P = 0.0002$). Within the experimental group (intervention diets), total cholesterol (mean difference −0.69 mmol/L; 95% CI, −1.27 to −0.10; $I^2 = 52\%$; $P = 0.02$) and LDL-C (mean difference −0.45 mmol/L; 95% CI, −0.68 to −0.22; $I^2 = 0\%$; $P < 0.0001$) experienced significant reductions in comparison to the control group (recommended diets for type 2 diabetes). However, no statistically significant differences emerged in the case of FBG, HbA1c, HOMA-IR, and HDL-C between the experimental and control groups. The high dietary fiber diet triggered an augmented presence of short-chain fatty acid-producing bacteria in the intestines of individuals with T2DM. In addition, the high-fat and low-carbohydrate diet resulted in a notable decrease in Bacteroides abundance while simultaneously increasing the relative abundance of Eubacterium. Compared to a specific dietary pattern, personalized diets appear to result in the production of a greater variety of beneficial bacteria in the gut, leading to more effective blood glucose control in T2D patients.

Conclusion: Dietary interventions hold promise for enhancing metabolic profiles in individuals with T2D through modulation of the gut microbiota. Tailored dietary regimens appear to be more effective than standard diets in improving glucose metabolism. However, given the limited and highly heterogeneous nature of the current sample size, further well-designed and controlled intervention studies are warranted in the future.

KEYWORDS

type 2 diabetes, dietary intervention, gut microbiota, short-chain fatty acids, systematic review

1 Introduction

Type 2 diabetes mellitus (T2DM) is a non-infectious metabolic disease characterized by elevated blood glucose levels caused by impaired insulin secretion, insulin resistance, or both (1). The etiology of T2DM is multifaceted, and recent studies have identified the gut microbiota as a potential contributor to its pathophysiology (2). In addition to chronic inflammation and metabolic disturbances, individuals with T2DM experience dysregulation of the gut microbiota and compromised intestinal barrier function, leading to increased intestinal permeability (3). Specifically, gut microbiota imbalances manifest as reduced microbial abundance and diversity, particularly the depletion of butyric acid-producing bacteria (4). On one hand, patients exhibit diminished production of short-chain fatty acids (SCFAs) by beneficial gut bacteria, resulting in decreased levels of glucagon-like peptide-1 (GLP-1). On the other hand, the proliferation of harmful bacteria in the gut leads to increased intestinal permeability and the release of endotoxins (LPS), causing chronic inflammation in pancreatic islets, ultimately contributing to pancreatic β-cell damage and reduced insulin sensitivity (5, 6). Additionally, the gut microbiota is now recognized as a novel endocrine organ that influences human health by secreting metabolites such as SCFAs, bile acids, indoleacetic acid, branched-chain amino acids, and trimethylamine-N-oxide (7).

Furthermore, poor dietary structure and eating habits play significant roles in the onset and progression of T2DM, and an unbalanced diet is closely associated with gut microbiota dysbiosis. This dietary structure is characterized by chronic excessive consumption of carbohydrates and fats (particularly saturated fats) and inadequate intake of dietary fiber (8). Consequently, dietary interventions for patients with T2DM should focus on the overall dietary pattern rather than isolated nutrients or specific foods (9). This is because different dietary combinations and the nutrients contained within them interact synergistically to influence the health of individuals with T2DM (10).

While numerous meta-analyses and systematic reviews have examined the effects of dietary patterns or individual nutrients on glycemia in T2DM patients, few have explored the collective impact of daily dietary patterns on both gut microbiota and metabolic levels in this population. Hence, the objective of this systematic review is to investigate the efficacy of dietary patterns or combinations in improving gut microbiota and metabolic levels in patients with type 2 diabetes, while also exploring the potential mediating role of gut microbiota and its metabolites in the diet-metabolism relationship. Specifically, we will analyze changes in gut microbiota diversity, composition, and function. Probiotic interventions directly affecting the gut microbiota have been excluded from the inclusion criteria.

2 Materials and methods

This Meta-analysis and systematic review adhered to the latest PRISMA 2020 guidelines for reporting (11).

2.1 Eligibility criteria

The following criteria were used for inclusion and exclusion:

Experiment type: Population intervention trials such as randomized controlled trials. Experiments conducted on animals and *in vitro* were excluded.

Population: Inclusion encompassed patients diagnosed with type 2 diabetes or pre-diabetes, while exclusions were applied to individuals with type 1 diabetes, gestational diabetes, and other diabetes types. It was imperative for subjects with type 2 diabetes to possess a clinical diagnosis managed through dietary control, oral medications, and/or insulin therapy.

Intervention: Eligible interventions included specific dietary patterns or combinations of diets. Supplementation with nutrients, nutritional supplements, or probiotics in isolation was not considered for inclusion.

Outcomes: The primary outcomes were changes in the gut microbiota (including changes in the diversity and abundance of the gut microbiota, gut microbiota composition and gut microbiota function) and metabolic levels. Among the metabolic levels were blood glucose (glycated hemoglobin, fasting glucose, and insulin resistance homeostasis model assessment) and lipid profile (total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol). Secondary outcomes were changes in anthropometric indicators and inflammatory markers (endotoxin levels, tumor necrosis factor alpha, interleukin 6, and C-reactive protein).

2.2 Information sources and search strategy

Two authors independently searched electronic databases including PubMed, EMBASE, Web of Science, and ScienceDirect. The search was limited to articles published in English, with the completion date set as October 10, 2023. Medical subject headings (MeSH) and their synonyms were used as search terms, combined using Boolean operators (OR/AND) based on the study requirements. The search strategy is: "type 2 diabetes" combined with Boolean operator OR to similar terms ("type 2 diabetes mellitus"; "T2D"; "T2DM"; "pre-diabetes"; "prediabetes"; "prediabetic state"); AND "dietary pattern" combined with Boolean operator OR to similar terms ("feeding pattern"; "eating behavior"; "diet"; "dietary habit"; "food selection"); AND "gut microbiota" combined with Boolean operator OR to similar terms ("gut microbiome"; "gut microbiota"; "Intestinal flora"; "Gut microbiota"; AND "randomized controlled trial" combined with Boolean operator OR to similar terms ("RCT"; "trial"; "intervention").

2.3 Selection process and data extraction

All titles and abstracts obtained from the literature search were initially screened manually, following the inclusion and exclusion criteria. Subsequently, the literature selected from the initial screening underwent a second screening through full-text reading. Finally, the studies identified in the secondary screening were organized using Microsoft Excel software. We extracted information from the included studies, including authors, country,

year, diet and subgroup, study type, subject characteristics, and study outcomes (Table 1). The data extracted for analysis included relevant indicators such as baseline and post-dietary intervention measurements of gut microbiota, glucose, lipids, and inflammation.

2.4 Data analysis

Meta-analysis was conducted using Review Manager 5.4 software. All included studies were randomized controlled trials, except for the study by Ismael S et al., which was a single-arm trial with only one experimental group in its study subgroup. In our meta-analysis, two distinct approaches were employed. Firstly, we conducted a comparative assessment between the changes observed in the experimental group pre- and post-intervention and those in the control group (following a recommended diet for patients with type 2 diabetes mellitus) before and after their respective interventions. Secondly, we compared changes before and after the intervention of two nutritionally characterized diets (high-fiber diets, high-fat and low-carbohydrate diets) within groups. The specific indicators scrutinized encompassed alterations in fasting blood glucose, glycated hemoglobin, the homeostasis model insulin resistance index, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and body mass index. The mean and standard deviation were calculated using the conversion formula in an Excel sheet (24, 25), and the standard error was converted to standard deviation using a specific formula. Fasting glucose units were standardized to mmol/L.

2.5 Risk of bias and quality assessment

Review Manager 5.4 software was used as an assessment tool for evaluating the quality of clinical trial studies. Two authors independently assessed the risk of bias and quality of each included article. Areas assessed included selection bias (random sequence generation and allocation concealment), implementation bias (blinding of participants and personnel), measurement bias (blinding of outcome assessment), missing visit bias (incomplete outcome data), reporting bias (selective reporting), and other biases. Each assessment was categorized as "low risk," "high risk," or "uncertain risk."

3 Description and classification of dietary interventions

In light of the intricate and multifarious nature of various dietary patterns, this study categorizes them into two distinct classes: specific dietary patterns and individualized dietary patterns, contingent upon whether the groups underwent uniform dietary interventions. Furthermore, within the category of specific dietary patterns, a finer classification was employed to delineate high-fiber diets, high-fat and low-carbohydrate diets, and low-fat low-carbohydrate diets, guided by the nutritional characteristics of the dietary interventions encompassed. Table 2 describes the characteristics of the main nutrients in dietary interventions.

4 Results

4.1 Study selection

Using the literature search strategy, we initially identified 785 relevant citations from four databases: Pubmed, EMBASE, Web of Science, and Science Direct. After removing duplicate citations, 532citations underwent title and abstract screening based on the inclusion and exclusion criteria, resulting in the exclusion of 502 citations. Finally, 12clinical studies were included in the meta-analysis after a thorough evaluation of the full text to ensure they met the specified study types, interventions, and study outcomes. Figure 1 presents the PRISMA flow chart illustrating the selection process of the included studies.

4.2 Study characteristics

4.2.1 Participant characteristics

The study involved a total of 676 individuals with either type 2 diabetes mellitus (T2DM) or prediabetes, ranging in age from 25 to 80 years. One study reported a BMI of 18.5–24.9 kg/m^2 (15), while 6 studies provided a BMI range of 25–45 kg/m^2, and the remaining five did not report BMI values. These findings indicate that at least half of the participants were overweight or obese.

4.2.2 Intervention characteristics

The duration of the interventions ranged from 18 days to 3 years. The interventions consisted of various dietary patterns or combinations, including 3 Mediterranean diets (Two of them were compared with ketogenic diet and personalized diet, respectively), 1 low-energy diets, 1 low-carbohydrate diet based on almonds, 1 low-fat diet, 1 Ma-Pi 2 diet, 2 high dietary fiber diets, 1 diet incorporating sardines, 1 diet with reduced branched-chain amino acids, and 2 individualized diet. Supplementary Table S1 provides details of the nutritional intake for each experimental group after the dietary intervention, as anticipated in the experiment.

4.3 Risk of bias in studies

We assessed the quality of the 11 included studies using the Cochrane Collaboration Risk of Bias tool (Supplementary Figure S1) (26). All studies were determined to have low risk of bias in terms of missing visit bias (incomplete outcome data) and reporting bias (selective reporting). Only three studies (13, 19, 20) explicitly mentioned using random sequence generation for allocation, and two of these studies (13, 19) also reported concealing the allocation scheme, indicating low risk of selection bias. Implementation bias (blinding of participants and personnel) and measurement bias (blinding of outcome assessment) were rated as lower in four studies, while two studies exhibited a higher risk of bias associated with blinding of participants and personnel (14, 18).

TABLE 1 Description and characteristics of the included studies.

References/ Country	Dietary patterns and groupings	Nutritional characteristics of the intervention group	Study Design	Subject characteristics	Main microbiota results	Main clinical outcomes
Candela et al. (12) Italy	①Experimental group: fiber-rich longevity Ma-Pi 2 diet ($n = 28$) ②Control group: Italian Professional Association for T2D Therapy recommended diet CTR ($n = 28$) ③Healthy group: normal diet ($n = 13$)	High dietary fiber	Open randomized controlled trial, 21 days	①T2DM, $n = 56$, BMI of 27–45 kg/m^2, 55–70 years old; ②Healthy group: normal weight, 21-40 years old.	Both dietary interventions demonstrated effectiveness in alleviating gut microbiota dysbiosis and promoting the restoration of bacteria that produce short-chain fatty acids (SCFAs) in individuals with T2DM.	The reduction in HOMA-IR, total cholesterol and LDL/HDL ratio was significantly higher in the Ma-Pi 2 diet group than in the CTR group. the Ma-Pi 2 diet significantly reduced TNF-α, plasma CRP and IL-6 levels, while only TNF-α was significantly reduced in the CTR group.
Zhao et al. (13) China	①Experimental group: high dietary fiber diet ($n = 27$) ②Control group: 2013 version of the Chinese Diabetes Association dietary guidelines for patients to manage their diet ($n = 16$)	High dietary fiber	Randomized controlled trial, 84 days	T2DM, $n = 43$, acarbose as a treatment drug	The high dietary fiber intervention increased 15 strains of acetate and butyric acid-producing bacteria, inhibited perindole- and hydrogen sulfide-producing bacteria, and promoted GLP-1 and PYY secretion to improve blood glucose, the abundance and diversity of which correlated significantly with clinical outcomes.	Indicators such as HbA1c improved faster and better in the experimental group than in the control group, and this clinical effect could be reproduced in mice by colony transplantation.
Chen et al. (14) China	①Experimental group: high dietary fiber diet ($n = 9$) ②Control group: 2013 version of the Chinese Diabetes Association dietary guidelines for patients to manage their diet ($n = 8$)	High dietary fiber	Randomized controlled trial, 4 weeks	T2DM, $n = 17$, acarbose as a therapeutic agent	The ratio of *Firmicutes* to *Bacteroidota* was significantly lower in the treatment group, and the number of *Proteus* was reduced; the proportion of beneficial microorganisms of several genera increased, and the relative abundance of all other opportunistic pathogens decreased.	Glucose homeostasis, glucose homeostasis and systemic inflammation levels were significantly improved in the treatment group compared to the control group.
Medina-Vera et al. (15) Mexico	①Experimental group: Functional food diet ($n = 81$) (T2DM) ②Control group: normal diet (healthy population)	High dietary fiber, low carb, high unsaturated fat	Randomized controlled trial, 12 weeks	①T2DM, $n = 81$, 30-60 years old, and BMI of 18.5-24.9 Kg/m^2 ②Healthy control group, 20-40 years old	Compared to the control group, the experimental group showed a significant increase in the α-diversity of the gut microbiota and significant changes in the abundance of specific flora, which were not associated with antidiabetic drugs. Among them, P Copri decreases, while *Faecalibacterium praussnitzii* and *Akkermansia* with anti-inflammatory effects increase.	The intervention group also had significantly lower blood glucose, total and LDL cholesterol, FFA, HbA1c, triglycerides and area under the CRP curve, and increased antioxidant activity compared to the control group.

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TABLE 1 (Continued)

References/ Country	Dietary patterns and groupings	Nutritional characteristics of the intervention group	Study Design	Subject characteristics	Main microbiota results	Main clinical outcomes
Jian et al. (16) Finland and 8 other countries	Low energy diet for the first 8 weeks and weight maintenance for the last 148 weeks ($n = 211$)	Low-carbohydrate, low-fat	Multicenter randomized controlled trial, 3 years	Prediabetes overweight adult patients, $n = 211$, 25–70 years old, BMI $\geq$ 25 kg/m^2	There was a significant increase in the relative abundance of several genera linked to enhanced metabolism. Changes in microbiota composition and predicted function were strongly correlated with weight loss. The initial characteristics of the gut microbiota accounted for approximately 25% of the variability in overall changes in adiposity prior to low-energy diet treatment.	Subjects lost an average of 11.5% of body weight and 22% of total body fat during the intervention, with significant improvements in all metabolic parameters. 76 subjects returned to normal blood glucose levels. Substantial interindividual variability was observed in the changes induced by the low-energy diet in variables related to glucose metabolism and total body fat.
Ismael et al. (17) Portugal	Mediterranean diet ($n = 9$)	High fiber and unsaturated fat	Single-arm trial, 12 weeks	T2DM, $n = 9$ (6 males, 3 females), 40–80 years old (mean 66±9 years), except for 1 subject, all received oral hypoglycemic drugs	After 4 weeks, there was an increase in the abundance of intestinal bacteria, and the ratio of *Prevotella*/*Bacteroides* also increased. Bacterial diversity showed a negative correlation with HbA1c, while bacterial abundance exhibited negative correlations with FBS and HOMA-IR. Changes in gut microbiota seemed to precede alterations in a conventional biomarker for type 2 diabetes, namely HbA1c.	HbA1c and HOMA-IR were significantly reduced after 12 weeks. Blood lipid profiles showed no concomitant changes. Alkaline phosphatase activity (a marker of intestinal inflammation and permeability) in fecal samples was negatively correlated with HbA1c and positively correlated with bacterial diversity.
Deledda et al. (18) Italy	①Experimental group: ketogenic diet ($n = 6$) ②Control group: Mediterranean diet ($n = 5$)	Very low-carb, high-fat	Randomized controlled trial, 12 weeks	T2DM newly diagnosed and without complications, $n = 11$ (6 males, 5 females), 45-65 years, BMI $\geq$ 28 Kg/m^2	In the ketogenic diet group, there was a significant increase in beneficial microbiota groups, along with a decrease in microbiota groups associated with obesity (*Firmicutes* and Actinobacteriota) or other diseases. The Mediterranean diet group exhibited a significant increase in *Actinobacteria* and *Firmicutes*.	The beneficial effects of the ketogenic diet on anthropometric parameters were more significant than those of the Mediterranean diet, but there were no statistically significant differences in biochemical improvements. Macrogenomic alterations associated with certain metabolic pathways were found only in the ketogenic diet group.
Ren M, et al. (19) China,	①Experimental group: almond-based low-carbohydrate diet a-LCD ($n = 22$) ②Control group: low-fat diet LFD ($n = 23$)	Low-carb, high-fat	Randomized controlled trial, 12 weeks	T2DM, $n = 45$, $\geq$18 years old	The consumption of a low-calorie diet (a-LCD) notably augmented the presence of short-chain fatty acid-producing bacteria, including *Roseburia*, *Ruminococcus*, and *Eubacterium*.	HbA1c levels were significantly lower during the study period in both groups compared to baseline. At Month 3, the a-LCD group had higher GLP-1 concentrations than the LFD group, had a greater decrease in HbA1c levels than the LFD, and significantly improved depressive symptoms.

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TABLE 1 (Continued)

References/ Country	Dietary patterns and groupings	Nutritional characteristics of the intervention group	Study Design	Subject characteristics	Main microbiota results	Main clinical outcomes
Balfegó et al. (20) Spain	①Experimental group: sardine diet ($n = 19$) ②Control group: general diet recommended for diabetes without sardines ($n = 16$)	High unsaturated fat	Randomized controlled trial, 6 months	T2DM, $n = 35$ (16 males, 19 females), BMI of 26–35 kg/m^2, 40–70 years old, not receiving insulin and oral hypoglycemic drugs.	Both dietary interventions effectively lowered the concentrations of phylum *Firmicutes* and E. coli compared to their respective baselines. Moreover, the intervention group displayed a reduced *Firmicutes*/*Bacteroidetes* ratio and an increased abundance of *Bacteroides-Prevotella*.	There was no significant difference in glycemic control between the groups. Plasma insulin and HOMA-IR were reduced in both groups at 6 months after baseline. Plasma lipocalin increased only in the intervention group (+40.7%) compared to baseline levels. Omega-3 index increased by 2.6% in the experimental group and by 0.6% in the control group.
Karusheva et al. (21) Germany	①Experimental group: reduced branched-chain amino acid diet (BCAA-) ②Control group: complete amino acid diet (BCAA+)	Reduction in branched-chain amino acids	Crossover test, 4 weeks	T2DM, $n = 12$, 40–60 years old, BMI of 28–35 kg/m^2, disease duration<5 years.	In comparison to the BCAA+ diet, the BCAA- diet intervention demonstrated an 11% decrease in the abundance of *Firmicutes* and a remarkable 40% increase in the abundance of *Bacteroidetes*.	After the BCAA-diet, insulin secretion was reduced, postprandial insulin sensitivity was increased, and mitochondrial efficiency in adipose tissue was stimulated.
Meleshko et al. (22) Ukraine	①Experimental group: personalized diet ($n = 35$) ②Control group ($n = 21$)	NA	Randomized controlled trial, 18 days	T2DM, $n = 56$, 39–68 years old, all female.	Enterococcus faecalis, Escherichia coli, lac+, and Candida spp. significantly decreased, while *Lactobacillus* spp. significantly increased.	Significant improvements in blood glucose, lipid profile (cholesterol, LDL, HDL, VLDL, triglycerides) and inflammatory markers (IL-1 β, IL-10, IgA, TNF-α).
Shoer et al. (23) Israel	①Experimental group: personalized diet ($n = 100$) ② Control group: Mediterranean diet ($n = 100$)	NA	Randomized controlled trial, 6 months	Prediabetes, $n = 200$, adults	The personalized diet had a greater effect on the gut microbiota than the Mediterranean diet. The personalized diet resulted in a significant increase in the relative abundance and alpha diversity of 19 gut microbiota species. flavonifractor plautii, Roseburia hominis, Ruthenibacterium lactatiformans and Faecalibacterium prausnitzii increased significantly in abundance. The Mediterranean diet resulted in a significant increase in the relative abundance of four gut microbiota species.	Compared to the Mediterranean diet, the personalized diet had a greater effect on glycemic control (HbA1c).

FBS, fasting blood sugar; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; FFA, free fatty acids; plasma CRP, C-reactive protein; IL-6, interleukin; Functional food diet: Rich in soluble fiber, prebiotics, plant protein, and n-3 unsaturated fatty acids; Personalized diet: Using developed algorithms, personalized diets are selected based on the patient's gut microbiota, immune, and biochemical parameters.

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TABLE 2 The characteristics of the main nutrients in dietary intervention.

Categorization of dietary patterns	Dietary intervention measures	Specific dietary characteristics	Main nutrient characteristics			
			Dietary fiber	Carbohydrate	Lipid	Protein
Specific dietary patterns	Fiber-rich longevity Ma-Pi 2 diet (12)	High dietary fiber; Rich in vegetables, fruits, grains, and white meat; No added sugar.	High-fiber	NA	Low saturated fat	NA
	High dietary fiber diet (13)	High dietary fiber; Rich in whole grains and prebiotics.	High-fiber	NA	NA	NA
	High dietary fiber diet (14)	A high fiber diet composed of whole grains and prebiotics.	High-fiber	NA	NA	NA
	Functional food diet (15)	Rich in soluble fiber, prebiotics, plant protein, and n-3 unsaturated fatty acids.	High-fiber	Low carbohydrate	High unsaturated fat	NA
	Mediterranean diet (17)	Rich in fiber, unsaturated fatty acids, and phytochemicals; Very low red meat and processed foods.	High-fiber	NA	High unsaturated fat	NA
	ketogenic diet (18)	Extremely low in carbohydrates and high in fat.	NA	Low carbohydrate	High fat	NA
	Mediterranean diet (18)	Rich in fiber, unsaturated fatty acids, and phytochemicals; Very low red meat and processed foods.	High-fiber	NA	High unsaturated fat	NA
	Low energy diet (16)	8 weeks of full meal replacement diet, followed by a low calorie diet for the next 148 weeks.	NA	Low carbohydrate	Low fat	NA
	Almond-based low-carbohydrate diet (19)	A large amount of nuts, low-carbon water, and high fat.	NA	Low carbohydrate	High fat	NA
	Low-fat diet (19)	Low fat.	NA	NA	Low fat	NA
	Sardine diet (20)	Take 100 g of sardine 5 days a week.	NA	NA	High unsaturated fat	NA
	BCAA- diet (21)	Reduce branched chain amino acids in dietary protein.	NA	NA	NA	Reduce branched chain amino acids
Personalized dietary patterns	Personalized diet (22)	Using developed algorithms, select personalized diets based on the patient's gut microbiota, immune, and biochemical parameters.	NA	NA	NA	NA
	Personalized diet (23)	Using developed algorithms, select personalized diets based on the patient's gut microbiota, immune, and biochemical parameters.	NA	NA	NA	NA

NA, Not Applicable.

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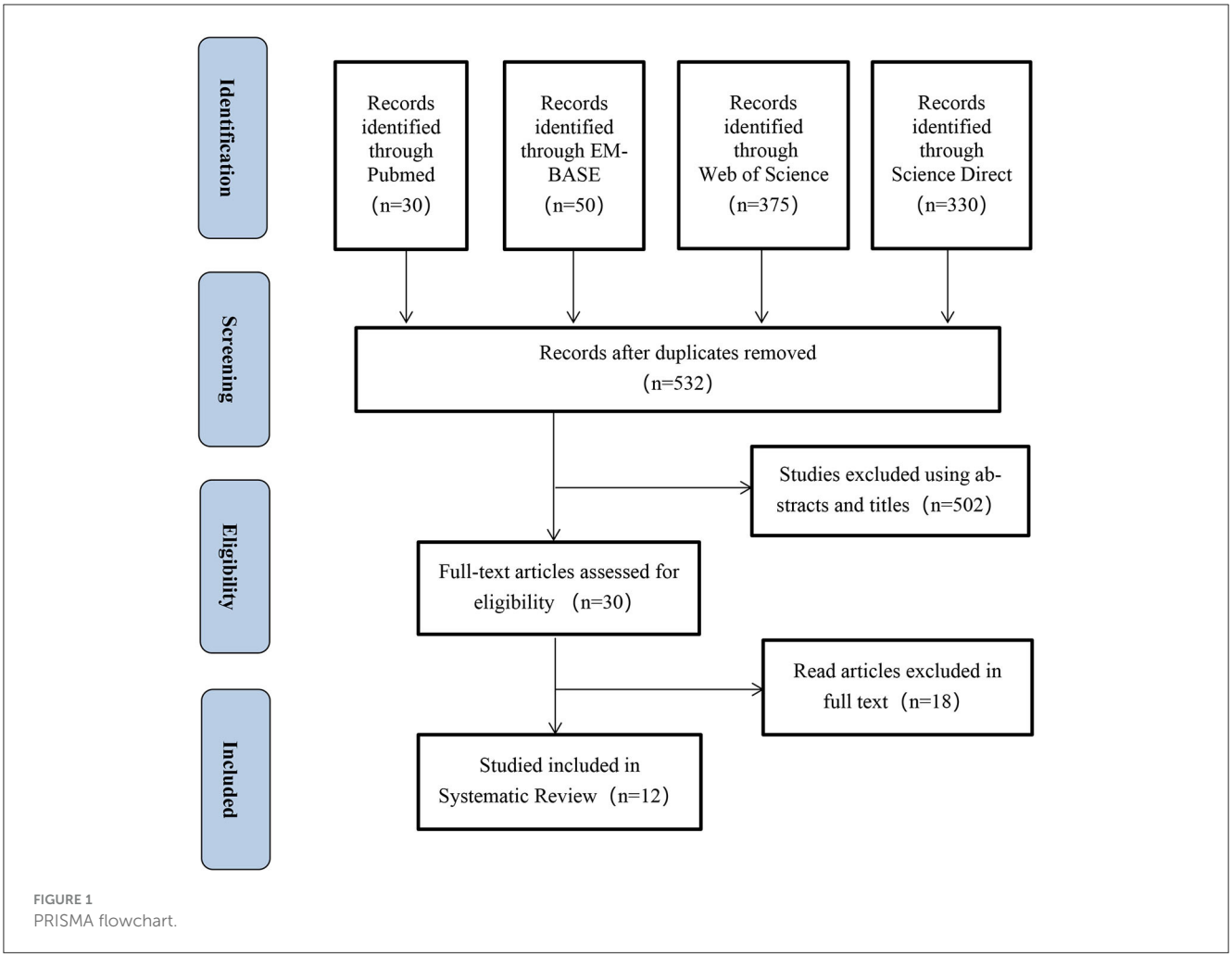

FIGURE 1
PRISMA flowchart.

4.4 Effect of dietary intervention on gut microbiota

4.4.1 Changes in gut microbiota diversity and richness

Five studies investigated gut microbiota diversity, while two studies examined gut bacterial richness. In a multicenter randomized clinical trial (16) involving 211 pre-diabetic overweight adult patients, a low-energy diet intervention resulted in significant increases in alpha diversity (microbiota composition diversity within individuals), beta diversity (differences in microbiota structure between individuals), and gut microbiota richness (all $P < 0.001$). Ren et al. (19) observed increased alpha diversity of the gut microbiota in patients with T2DM following both an almond-based low-carbohydrate diet and a low-fat diet, although there was no significant difference in gut microbiota structure (beta diversity) between the two diet groups. Candela M et al. (12) compared the fiber-rich long-life Ma-Pi 2 diet with the Italian T2D Therapy Professional Association recommended diet and found a tendency for both dietary patterns to increase gut microbiota alpha diversity in T2DM patients, but without significant differences between time points.

Ismael et al. (17) conducted a single-arm trial of a Mediterranean diet intervention, where no difference in gut microbiota diversity was observed at the end of the intervention, while bacterial richness tended to increase from baseline to 12 weeks. However, Deledda et al. (18) also implemented a Mediterranean diet intervention, comparing it with a ketogenic diet. The results indicated no significant difference in the alpha diversity of the gut microbiota over time in either group, but a significant difference in beta diversity of the flora between the two intervention groups ($p = 0.013$).

4.4.2 Changes in the composition of the gut microbiota

Table 3 displays the effects of different dietary interventions on the gut microbiota at the phylum, genus, and species levels. Candela et al. (12) reported that a fiber-rich Ma-Pi 2 diet effectively countered the reduction of *Faecalibacterium*, *Bacteroides*, and *Dorea* in T2DM patients and promoted the presence of SCFA-producing bacteria, such as *Faecalibacterium prausnitzii* and *Lachnospiraceae bacterium*. Zhao et al. (13) demonstrated that a high dietary fiber intake stimulated the production of 15 strains of acetate and butyrate-producing bacteria, while inhibiting the production of indole and hydrogen sulfide-producing bacteria. Chen et al. (14) found that a high dietary fiber intake increased the proportion of several beneficial bacteria in the intestines of T2DM

TABLE 3 Effect of different diets on part of the gut microbiota in T2DM patients at Phylum, Genus and Species levels.

Phylum	Genus	Ma-Pi 2 diet (12)	High fiber diet (13)	High fiber diet (14)	Functional food diet (15)	Low energy18mm diet (16)	Mediterranean diet (17)	Ketogenic diet (18)	Mediterranean diet (18)	Low Carbohydrate diet (19)	Low-fat diet (19)	Sardine diet (20)	BCAA+ and BCAA- (21)	Personalized diet (22)	Personalized diet (23)
Bacteroidetes		NR	NR	NR	NR	↑***	NR	↓	↓*	↓*	↓	↓	BCAA- has a 40% higher abundance of *Bacteroidetes* than BCAA+.	NR	NR
	Bacteroides	↑	NR	↑	*Bacteroides* fragilis↑	↑***	↑	↓*	↓*	↓*	↓	NR	NR	NR	NR
	Prevotella	NR	NR	↓	*Prevotella copri* reduced by 13%↓	NR	NR	NR	NR	NR	NR	↑	NR	NR	NR
Firmicutes		NR	NR	NR	NR	↓***	↓	↓*	↑*	NR	NR	↓**	The abundance of *Firmicutes* in BCAA - is 11% lower than that in BCAA+.	NR	NR
	Ruminococcus	↓	NR	↑	NR	↑***	↑	↓*	NR	↑	↓*	NR	NR	NR	*Ruthenibacterium lactatiformans*↑*
	*Faecalibacterium*①	↑	NR	NR	NR	↓***	↓	NR	NR	NR	NR	NR	NR	NR	NR
	*Roseburia*①	↑	NR	NR	NR	NR	↑	NR	NR	↑*	↓**	NR	NR	NR	Roseburia hominis↑*
	*Clostridium leptum*①	NR	*Faecalibacterium prausnitzii*↑	NR	*Faecalibacterium prausnitzii*↑	NR	NR	NR	NR	NR	NR	*Faecalibacterium prausnitzii*↑	NR	NR	*Faecalibacterium prausnitzii*↑*
	*Eubacteriu*①	NR	↑	NR	NR	NR	NR	↑*	NR	↑**	↑	NR	NR	NR	NR
	*Lachnospir*①	↑	*Lachnospiraceae* bacterium↑	NR	NR	↓***	NR	NR	NR	NR	NR	NR	NR	NR	NR
	*Pseudobutyrivibrio*①	NR	NR	NR	NR	↓***	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Lactobacillus	NR	NR	NR	NR	NR	NR	NR	NR	NR	↓	NR	NR	↑*	NR
Verrucomicrobia	*Akkermansia*	↑	NR	NR	*A.Muciniphila* increased by 125% ↑	↑***	↑	↑*	NR	NR	NR	NR	NR	NR	NR
Actinobacteria		NR	NR	NR	NR	↓***	↑	↓*	↑*	NR	NR	NR	NR	NR	NR
	Bifidobacterium	NR	↑	↑	*Bifidobacterium* longum↑	↓***	NR	NR	NR	NR	NR	NR	NR	NR	NR

All studies are within-group comparisons except for BCAA+ vs. BCAA- for between-group comparisons: ↑indicates increased bacterial abundance compared to baseline, ↓indicates decreased bacterial abundance compared to baseline. In studies where p-value sizes are provided, *indicates $p \leq 0.05$ compared to baseline, **indicates $p \leq 0.01$ compared to baseline, and ***indicates $p \leq 0.001$ compared to baseline. ①indicates butyric acid-producing bacteria. NR, Not reported.

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patients, while decreasing the proportion of certain opportunistic pathogenic bacteria.

Medina-Vera et al. (15) observed that a Functional food diet increased the levels of *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* (associated with anti-inflammatory effects) by 125%, while decreasing the levels of *Prevotella copri* by 13%. Additionally, the intake of *Bifidobacterium* longum (linked to improved insulin signaling) and *Bacteroides* fragilis (with a robust capacity for multiple dietary polysaccharides) also increased. Jian et al. (16) demonstrated that after 8 weeks of a low-energy diet intervention, there was a significant increase in the abundance of *Verrucomicrobia* and *Bacteroidetes* ($P < 0.001$) at the phylum level, while *Actinobacteria* and *Firmicutes* significantly decreased in abundance ($P < 0.001$). At the genus level, *Akkermansia, Ruminococcus, Bacteroides*, and *Christensenellaceae R-7* showed significant increases, whereas *Faecalibacterium*, *Bifidobacterium*, and butyrate-producing bacteria (*Lachnospira, Pseudobutyrivibrio*, and *Blautia*) were significantly reduced.

Ismael et al. (17) compared changes in *Bacteroides* flora at 4 and 12 weeks of Mediterranean diet intervention with baseline. The ratio of *Prevotella* to *Bacteroides* significantly increased after 4 weeks, and the increase in the ratio of *Firmicutes* to *Bacteroidetes* after 12 weeks was clinically significant. The relative abundance of *Bacteroides*, *Ruminococcus*, *Roseburia*, *Akkermansia*, and *Actinobacteria* showed an increasing trend after 12 weeks of Mediterranean diet intervention, while *Faecalibacterium* and *Firmicutes* exhibited a decreasing trend. Deledda et al. (18) divided the ketogenic and Mediterranean diets into two intervention groups, and both groups showed consistent reductions in *Bacteroidetes* and *Bacteroides*. The ketogenic diet group exhibited a significant increase in beneficial microbiota such as *Akkermansia* and *Eubacterium*, as well as a decrease in microbial taxa associated with obesity (*Firmicutes* and *Actinobacteria*). The Mediterranean diet group showed significant increases in *Firmicutes* and *Actinobacteria*. Ren et al. (19) compared an almond-based low-carbohydrate diet with a low-fat diet. After 3 months of intervention, the low-carbohydrate diet group exhibited significantly higher relative abundances of *Ruminococcus* and *Roseburia* ($P < 0.01$) compared to the low-fat diet group. Several short-chain fatty acid-producing bacteria, including *Eubacterium*, were significantly increased in the low-carbohydrate diet group compared to baseline.

Karusheva et al. (21) found an 11% decrease in the abundance of *Firmicutes* and a 40% increase in the abundance of *Bacteroidetes* after BCAA- intervention compared to BCAA+. Meleshko et al. (22) reported that the use of a personalized diet resulted in a significant increase in the abundance of *Lactobacillus* spp., Enterococcus faecalis, Escherichia coli, lac+, and Candida spp., while there was a significant decrease in abundance. In a study by Shoer et al. (23), it was observed that personalized dietary regimens elicited a more pronounced influence on gut microbiota in comparison to the Mediterranean diet. The personalized diet instigated a noteworthy rise in the relative abundance of 19 gut microbiota species, notably including Flavonifractor plautii, Roseburia hominis, Ruthenibacterium lactatiformans, and Faecalibacterium prausnitzii. In contrast, the Mediterranean diet led to a notable increase in the relative abundance of only four gut microbiota species.

Upon comprehensive analysis of alterations in gut microbiota composition across the included studies, it became evident that high dietary fiber-based diets (such as the Ma-Pi 2 diet, high dietary fiber diet, functional food diet, and Mediterranean diet) conferred an augmented presence of intestinal Bacteroides, Faecalibacterium prausnitzii, Akkermansia, and Bifidobacterium in patients with T2DM. In contrast, high-fat low-carbohydrate diets, encompassing ketogenic diets and almond-based low-carbohydrate diets, markedly diminished the relative abundance of Bacteroides and substantially augmented that of Eubacterium.

4.4.3 Changes in gut microbiota function

Three studies conducted comparative predictive analyses of the functional macrogenome of the gut microbiota following dietary interventions, and some of the metabolic pathways that were significantly altered are collated in Supplementary Table S2 in the Appendix. Deledda et al. (18) reported a significant increase in 22 metabolic pathways commonly associated with the ketogenic diet group, while 17 pathways showed a significant decrease at months 2 and 3 compared to baseline. Notably, pathways involved in the degradation of limonene and ethylbenzene, biosynthesis of cephalosporin and penicillin, and carbohydrate digestion and absorption exhibited strong negative correlations, but displayed strong positive correlations with steroid and carotenoid biosynthesis as well as non-homologous end-joining pathways. No significant correlations with metabolic pathways were observed in the Mediterranean diet group.

Jian et al. (16) discovered that low-energy dietary intake significantly increased the abundance of *Akkermansia* (which promotes the glycosaminoglycan degradation pathway) and decreased the abundance of *Pseudobutyrivibrio* (which promotes flagellar assembly). Furthermore, the body mass index (BMI) and body weight of the subjects exhibited a negative correlation with the glycosaminoglycan degradation pathway and a positive correlation with flagellar assembly, indicating a connection between changes in the human gut microbiota and body weight.

Candela et al. (12) demonstrated that the Ma-Pi 2 diet reduced the abundance of gut microbiota marker bacteria associated with type 2 diabetes mellitus (involved in polyketide biosynthesis, sphingolipid biosynthesis, arachidonic acid metabolism, and alanine metabolism), while increasing the abundance of bacteria that improved metabolism (involved in taurine, cysteine, methionine, valine, leucine, isoleucine metabolism, and unsaturated fatty acid biosynthesis). Consequently, the Ma-Pi 2 diet provided the body with additional essential amino acids and vital nutrients.

4.5 Effect of dietary intervention on glycemic control

Candela et al. (12) compared the fiber-rich Ma-Pi 2 diet with the diet recommended by the Italian Professional Association for the Treatment of T2DM. Both diets led to a significant reduction in fasting blood glucose (FBG) levels in patients. The reduction in FBG ($P = 0.007$) and homeostatic model assessment of insulin resistance (HOMA-IR) ($P = 0.0004$) was more pronounced in the

Ma-Pi 2 diet group compared to the control group. Zhao et al. (13) observed a significant decrease in both glycated hemoglobin (HbA1c) and FBG levels in the high dietary fiber group ($P < 0.001$) and the control group (following the 2013 edition of the Dietary Guidelines for Patients with Chinese Diabetes Society) ($P < 0.001$). However, the high dietary fiber group exhibited a greater reduction in HbA1c levels starting from day 28 of the intervention (−1.91±0.24). Chen et al. (14) found that high dietary fiber significantly lowered HbA1c and FBG levels in patients.

Medina-Vera et al. (15) reported significant reductions of −15.6% in free fatty acids (FFA) and −7.2% in HbA1c levels among patients following a high fiber low-energy diet intervention compared to baseline. Jian C et al. (16) observed significant reductions in HbA1c, FBG, and HOMA-IR in patients with T2DM after a low-energy diet ($P<0.001$). Ren M et al. (19) compared low-carbohydrate and low-fat diets and found that the low-carbohydrate group exhibited a greater decrease in HbA1c levels compared to the low-fat diet group after 3 months of intervention ($P < 0.01$). Both groups demonstrated significantly lower HbA1c levels during the intervention ($P < 0.01$ and $P < 0.05$, respectively).

Ismael et al. (17) implemented a 12-week Mediterranean diet intervention in T2DM patients, resulting in a significant decrease in HOMA-IR (mean change −1.03±2.64, $P<0.05$, Cohen's d = −0.41) and HbA1c compared to baseline levels (mean change −0.67±0.98, $p < 0.05$, Cohen's d = −0.70), although the decrease in FBG was not significant. Deledda et al. (18) demonstrated a 1.1% decrease in HbA1c in the ketogenic diet group after dietary intervention (from 6.6 ± 0.9 to 5.5 ± 0.5, $p = 0.012$), while the change in HbA1c in the Mediterranean diet group was not significant. No significant changes in FBG were observed in either group.

Balfegó et al. (20) found no significant difference in glycemic control between the sardine diet and the control diet. Both groups exhibited significantly lower homeostatic model assessment of insulin resistance (HOMA-IR) and fasting insulin levels compared to baseline, but the reduction was greater in the sardine diet group (mean change in fasting insulin −6.1 ± 1.8 mU/L, P=0.01; mean change in HOMA-IR −2.3 ± 0.7, $P = 0.007$). Karusheva et al. (21) demonstrated that a reduced branched-chain amino acid diet (BCAA-) led to reduced insulin secretion and increased postprandial insulin sensitivity when compared to a full amino acid diet (BCAA+). Meleshko et al. reported a significant reduction in blood glucose levels in patients with T2DM after personalized dietary intervention (mean change −2.36 ± 2.13 mmol/L, $P < 0.05$). Shoer et al. (23) reported that a personalized diet was more effective in managing glycemic control (HbA1c) compared to the Mediterranean diet.

Regarding the meta-analysis of glucose metabolism, no significant differences were observed in FBG, HbA1c, and HOMA-IR between the experimental group (intervention diet) and the control group (recommended diet for type 2 diabetic patients), as depicted in Supplementary Figure S2. Following the high-fiber dietary intervention, FBG (mean difference −1.15 mmol/L; 95% CI, −2.24 to −0.05; $I^2 = 94\%$; $P = 0.04$) and HbA1c (mean difference −0.99%; 95% CI, −1.93 to −0.03; $I^2 = 89\%$; P=0.04) exhibited significant reductions compared to baseline levels (Figure 2). Moreover, the high–fat low–carbohydrate HbA1c (mean difference −0.98; 95% CI, −1.50 to −0.46; $I^2 = 0\%$; $P = 0.0002$) was notably lower after the dietary intervention (Figure 2).

4.6 Effect of dietary intervention on lipids

Medina-Vera et al. (15) observed a decrease in total cholesterol (−7.8%), triglycerides (−23%), and LDL-C (−9.9%) compared to baseline values in 81 subjects who received a high fiber, low-energy diet. Additionally, Candela et al. (12) reported a significantly greater reduction in total cholesterol, HDL-C, and LDL-C in the Ma-Pi 2 diet group compared to the control group ($p < 0.05$).

In the context of the meta-analysis on lipid metabolism, the experimental group (intervention diet) exhibited a noteworthy reduction in total cholesterol (mean difference −0.69 mmol/L; 95% CI, −1.27 to −0.10; I^2 =52%; P=0.02) and LDL–C (mean difference −0.45 mmol/L; 95% CI, – 0.68 to −0.22; I^2 =0%; $P<0.0001$) compared to the control group (recommended diet for type 2 diabetic patients), as illustrated in Figure 3. After the high–fiber dietary intervention, total cholesterol (mean difference −0.95 mmol/L; 95% CI, −1.57 to −0.33; I^2 =77%; $P = 0.003$) demonstrated a significant decrease relative to baseline levels. Nonetheless, no significant differences were observed in HDL-C between the experimental and control groups, nor in the changes before and after the high-fiber diet intervention, as depicted in Supplementary Figure S2.

4.7 Effect of dietary intervention on inflammatory indicators

According to Candela et al. (12), the consumption of a high-fiber Ma-Pi 2 diet significantly reduced the levels of TNF-α (-18.63 ± 27.59 pg/mL), CRP (−4.43±7.81 mg/L), and IL-6 (−0.286±3.86 pg/mL) ($p < 0.01$). Medina-Vera et al. (15) demonstrated that a low-energy diet high in fiber, polyphenols, and plant proteins effectively reduced inflammation levels in T2DM patients. The intervention group showed a 65% reduction in endotoxin (LPS) levels compared to baseline. Similarly, Chen et al. (14) found that a high dietary fiber diet significantly decreased IL-6, IL-1, and TNF-α, resulting in reduced systemic inflammation. Meleshko et al. (22) designed a personalized diet that significantly reduced TNF-α levels (−6.9 ± 0.91 pg/mL, $P < 0.05$), along with IL-6 and IL-1, indicating the efficacy of dietary interventions rich in dietary fiber and phytochemicals in improving inflammation levels in T2DM.

4.8 Effect of dietary intervention on anthropometrics

Deledda et al. investigated the impact of the Mediterranean diet intervention, which resulted in significant reductions in weight (−3.1 ± 17.6 kg, $P = 0.02$), body mass index (−2.1 ± 4.53 kg/m², $P = 0.02$), and waist circumference (−4.7 ± 9.08 cm, $P = 0.004$) compared to baseline. However, the ketogenic diet intervention

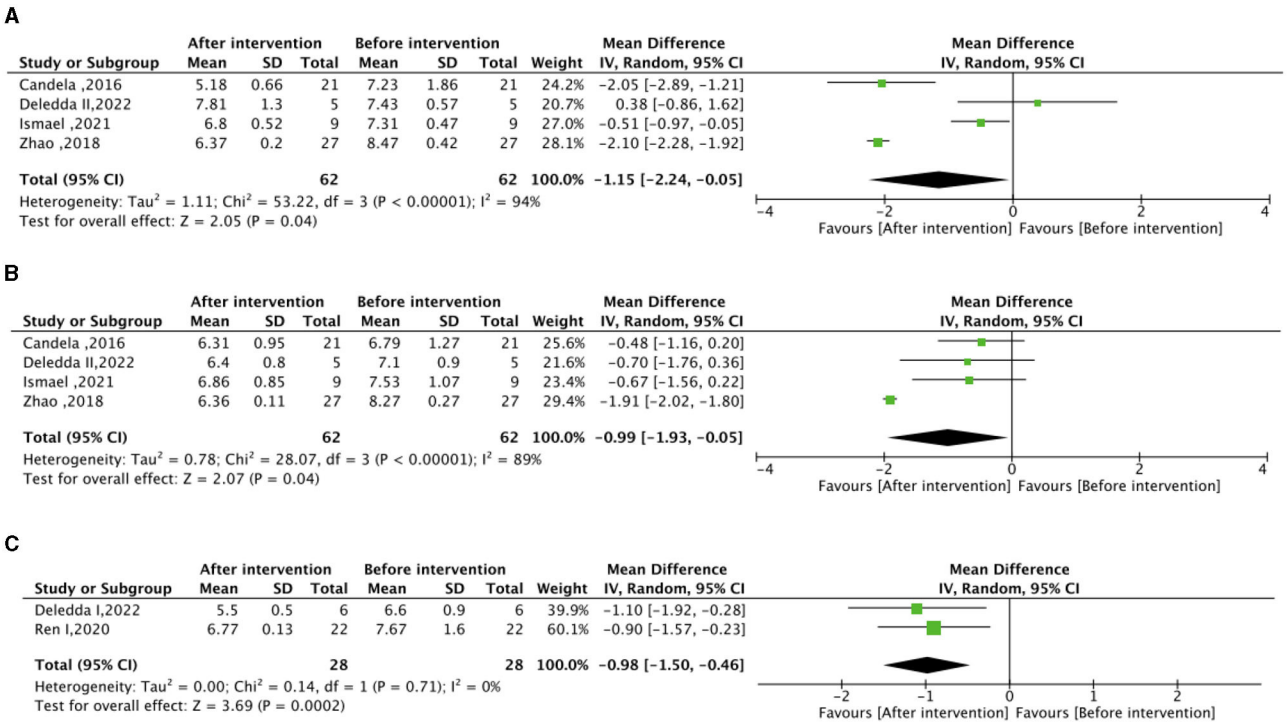

FIGURE 2
Effect of dietary intervention on glycemic control. **(A)** Own control of the effect of high dietary fiber dietary intervention on fasting blood glucose (FBG) (mmol/L). **(B)** Own control of the effect of high dietary fiber dietary intervention on glycated hemoglobin (HbA1c) (%). **(C)** Own control for the effect of high-fat low-carbohydrate dietary intervention on (HbA1c) (%).

yielded even more substantial and significant reductions in weight (-14.3 ± 13.91 kg, $P < 0.0001$), body mass index (-5.3 ± 3.93 kg/m^2, $P < 0.0001$), and waist circumference (-12.3 ± 7.27 cm, $P < 0.0002$). The low–energy diet designed by Jian C et al. demonstrated significant decreases in BMI compared to baseline (-3.9 ± 1.14 kg/m^2, $P<0.001$), as did the low–carbohydrate diet implemented by Ren et al. (-0.51 ± 2.39 kg/m^2, $P = 0.034$). Similarly, the individualized diet by Meleshko et al. led to a significant reduction in BMI during the intervention period (-4.03 ± 10.62 kg/m^2).

Regarding the meta-analysis of BMI, no significant disparity in BMI was observed between the experimental group (intervention diet) and the control group (recommended diet for type 2 diabetic patients), as depicted in Supplementary Figure S2.

5 Discussion

This review included a total of 12 dietary intervention trials. In the comprehensive meta-analysis, alterations in glucose metabolism and BMI within the experimental group (adhering to nutrient intake adjusted according to the recommended diet for individuals with T2D) did not demonstrate statistical significance when compared to the control group (following the recommended diet for T2D patients). However, the experimental group exhibited noteworthy reductions in both total cholesterol and LDL-C levels. In subgroup analyses, it was observed that FBG, HbA1c, and total cholesterol were significantly lower following interventions involving high dietary fiber diets, such as the Ma-PI[2] diet, high-fiber diet, and Mediterranean diet, when contrasted with pre-intervention levels. Furthermore, HbA1c exhibited a significant decrease after high-fat, low-carbohydrate diet interventions, as seen in the ketogenic diet and almond-based low-carbohydrate diet. Notably, the low-fat and low-carbohydrate diet, i.e., a low-energy diet, significantly enhanced glucose metabolism levels (FBG and HOMA-IR), as well as general and central obesity, as measured by BMI and waist circumference, in overweight and obese T2D patients.Furthermore, a personalized diet tailored to the individual's gut microbiota, immune system, and biochemical parameters demonstrated superior efficacy in glycemic control among T2D patients, leading to a more diverse population of beneficial gut bacteria than the specific diets previously mentioned.

The present review appears to provide further evidence of an earlier study by Houghton et al. (27), a systematic review evaluating the effectiveness of dietary interventions on the gut microbiota and glycemic control in adults with type 2 diabetes mellitus. Houghton et al. found a significant reduction in HbA1c and no significant changes in FBG or HOMA-IR in patients after dietary intervention. In terms of the gut microbiota, there were significant changes in diversity matrices (α and β) and the Firmicutes: Bacteroidetes ratios, but no significant changes in the relative abundance of Bifidobacterium spp. However, the present review builds on that study by updating the intervention studies of the last few years and analyzing subgroups according to nutritional characteristics, culminating in further results on the gut microbiota.

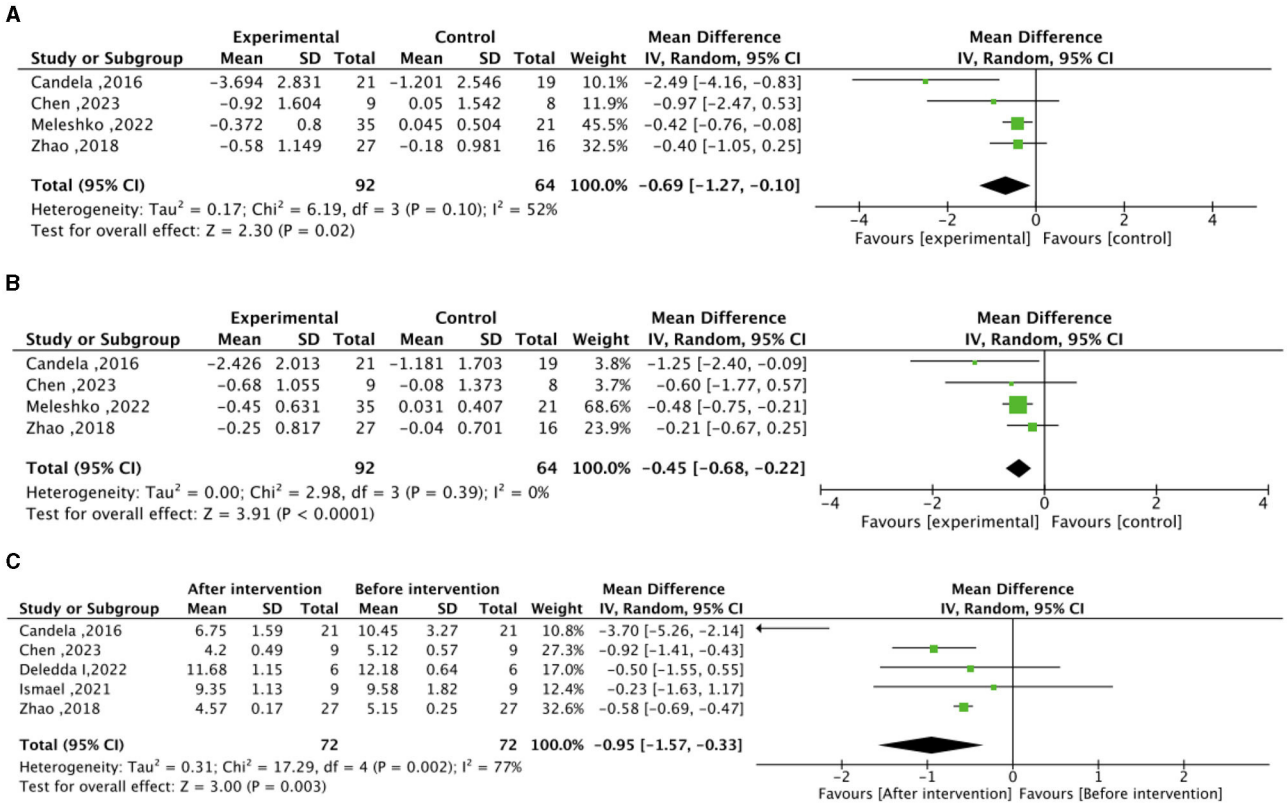

FIGURE 3
Effect of dietary intervention on lipid levels. **(A)** Effect of dietary intervention on total cholesterol (mmol/L) in the experimental group compared to the recommended diet for type 2 diabetic patients. **(B)** Effect of dietary intervention on low-density lipoprotein cholesterol (LDL-C) (mmol/L) in the experimental group compared to the recommended diet for type 2 diabetic patients. **(C)** Self-control of the effect of high dietary fiber dietary intervention on total cholesterol (mmol/L).

In comparison to the healthy population, patients with T2DM present a diminished abundance and diversity of gut microbiota, specifically lacking in butyrate-producing bacteria (e.g., *Ruminococcus*, Subdoligranulum, *Eubacterium*, *Faecalibacterium prausnitzii*, and *Roseburia*) and bacteria inversely associated with inflammation (*Bacteroides*, *Prevotella*, *Akkermansia*, and *Bifidobacterium*) (28). Noteworthy, changes in the gut microbiota appear to precede alterations in the standard biomarker of type 2 diabetes, HbA1c (17). The consumption of a Western-style diet, characterized by elevated levels of refined sugars, carbohydrates, saturated fatty acids, and animal proteins, coupled with a low dietary fiber intake, correlates with inflammation, metabolic disease, and T2DM (29). Remarkable traits of the Western-style diet-associated gut microbiota include an upsurge in protein-metabolizing bacteria (e.g., Bacillus and Aspergillus), saturated fat-metabolizing bacteria (e.g., Bacillus spp.), and a substantial reduction in fiber-degrading bacteria (e.g., *Faecalibacterium* and *Lachnospira*) (30, 31). Following the consumption of red meat, the gut microbiota ferments its constituent choline, carnitine, betaine, and lecithin, resulting in the synthesis of trimethylamine (TMA). Subsequently, the liver further metabolizes TMA to trimethylamine-N-oxide (TMAO) (32). A study demonstrated that TMAO impairs glucose tolerance, elevates HOMA-IR and fasting insulin levels in mice fed a high-fat diet, inducing adipose tissue inflammation and blocking insulin signaling (33). Furthermore, a case-control study revealed a positive correlation between increased plasma TMAO levels and heightened risk of T2DM (34). Therefore, adopting a rational and effective dietary pattern stands as a powerful means to augment gut microbiota diversity, while balancing its composition and metabolism in patients with T2DM.

Dietary interventions examined in this review exhibited a significant impact on the diversity of the gut microbiota. Various interventions, such as low-energy, low-carbohydrate, low-fat, and high-fiber Ma-Pi 2 diets, were found to notably increase alpha diversity. Moreover, the Mediterranean diet and ketogenic diet demonstrated a significant increase in beta diversity of the gut microbiota. Notably, several studies indicated a strong association between bacterial fluctuations resulting from dietary interventions and improved metabolic pathways, including the degradation pathways of limonene and ethylbenzene, glycosaminoglycan, and unsaturated fatty acid biosynthesis (12, 16, 18).

Importantly, the majority of dietary interventions significantly modify the composition of the gut microbiota, with some of the altered flora closely linked to human metabolic function. Specifically, high-fiber Ma-Pi 2 diets, high dietary fiber diet, high-fiber low-energy diet, and Mediterranean diet, all belonging to high dietary fiber categories, upregulated the relative abundance of *Bacteroides*, *Faecalibacterium prausnitzii*, *Akkermansia*, and

Bifidobacterium. *Faecalibacterium prausnitzii*, a major SCFA (such as acetic and butyric acid) producer in the human intestine, notably enhances insulin sensitivity and ameliorates T2DM (35). Additionally, numerous *Firmicutes* members, including *Lachnospira*, *Pseudobutyrivibrio*, *Clostridium leptum*, *Roseburia*, and *Faecalibacterium*, possess robust SCFA-producing capabilities (36). SCFA stimulates insulin secretion from pancreatic β-cells by stimulating the release of glucagon-like peptide (GLP-1) and casein (PPY) from intestinal L-cells and reduces inflammation levels by inhibiting indole- and hydrogen sulfide-producing bacteria (13, 37, 38).

However, the majority of bacterial strains commonly found in today's probiotic supplements do not possess the ability to produce butyrate. This limitation stems from the fact that most butyrate-producing bacteria are highly anaerobic and perish rapidly upon exposure to oxygen. In contrast, direct administration of butyrate can be absorbed by the stomach (36). While it is not possible to directly supplement butyrate-producing bacteria or butyrate itself, it is feasible to nourish butyric acid bacteria within the gut through dietary intake. This indirect approach facilitates an increase in the abundance of butyrate-producing bacteria and stimulates their substantial production of short-chain fatty acids (SCFAs). The consumption of dietary fiber represents the optimal means of augmenting SCFA-producing bacteria. Dietary fiber predominantly encompasses cellulose, resistant starch, pectin, inulin, and oligosaccharides, with whole grains, legumes, nuts, vegetables, and fruits constituting major food sources.

Secondly, *Akkermansia muciniphila* (*A. Muciniphila*) has been the subject of increasing research due to its diminished abundance in patients with diabetes, cardiovascular disease, inflammatory bowel disease, and neurological disorders (39, 40). *A. Muciniphila* may stimulate increased levels of glucagon-like peptide-1 (GLP-1) through protein P9 on the outer membrane, thereby promoting insulin secretion from pancreatic β-cells and suppressing appetite, ultimately improving T2DM and obesity (41). The most effective approach to enhancing *A. Muciniphila* abundance in the gut involves consuming foods rich in polyphenols and fish oil, alongside a dietary fiber intake (42). Notably, polyphenols act as antioxidants, combating oxidative stress and chronic inflammation, while also improving insulin resistance. Foods such as flax seeds, rye bread, walnuts, cranberries, blueberries, and green tea are abundant sources of dietary polyphenols (43). Additionally, fish such as sardines and salmon not only provide fish oil (DHA) but also serve as excellent sources of high-quality protein (44). Our study revealed that a high dietary, low-energy regimen enriched with polyphenols and plant proteins, as designed by Medina-Vera et al. (15), resulted in a 125% increase in *A. Muciniphila* abundance, a 65% decrease in its endotoxin (LSP) concentration, and a significant enhancement of plasma antioxidant activity. Furthermore, *Bifidobacterium* is extensively utilized in fermented dairy products as one of the most prevalent probiotics for promoting healthy intestinal function in humans (45), while *Bacteroides fragilis* exhibits a robust capacity for the extensive breakdown of dietary fiber polysaccharides and host glycans (15).

We observed a decline in *Prevotella* abundance in two high dietary fiber-based diets (14, 15), with a 13% reduction in *Prevotella copri* specifically in the high dietary fiber low-energy diet. Similarly, an animal study (46) and a population intervention trial (47) demonstrated an association between *Prevotella copri* and insulin resistance. Conversely, numerous clinical trials have consistently reported an elevation in *Prevotella* abundance following high dietary fiber interventions (48–51). Moreover, one study revealed the potential benefits of *Prevotella copri* in host metabolism, suggesting its utility as an indicator of postprandial glucose metabolism (52). Nevertheless, the precise effects of *Prevotella* on human health and its underlying mechanisms remain unclear. Potential factors contributing to this discrepancy include inter-individual variability in species and strain-level composition of *Prevotella* within the gut and variations in dietary patterns (53). Thus, the prevailing explanation is that higher diversity of *Prevotella spp*. species corresponds to a greater fermentative capacity, yielding greater benefits for human health (53).

In addition to dietary fiber and polyphenols, the gut microbiota play a vital role in lipid metabolism, encompassing lipid conversion, synthesis, breakdown of dietary lipids, and generation of host-regulated secondary metabolites (54). The Mediterranean diet, rich in n-3 polyunsaturated fatty acids (n-3 PUFA) abundant in fish, is strongly linked to improved T2DM outcomes (55). n-3 PUFA exhibit anti-inflammatory properties by reducing the *Lachnospira*ceae/*Firmicutes* ratio and enhancing *Lachnospira*ceae, thereby interacting with the gut microbiota to suppress inflammation. These effects are particularly attributed to the reduction of lipopolysaccharide (LPS)-producing bacteria and the increase in short-chain fatty acid (SCFA)-producing bacteria (56, 57). However, the three studies (17, 18, 20) included in this paper, which explored dietary interventions enriched in n-3 PUFA (Mediterranean diet and sardine diet), did not consistently demonstrate alterations in gut microbiota composition, suggesting incomplete correlation with reported results of gut microbiota changes.

Regarding the reduced branched-chain amino acid (BCAA) diet, this paper only includes one study (21). The findings indicate that compared to a full BCAA diet, reduced BCAA intake leads to increased postprandial insulin sensitivity, improved gut microbiota composition, and enhanced white adipose tissue metabolism. BCAA, an essential amino acid synthesized by the gut microbiota, has emerged as a biomarker for insulin resistance (58). However, a two-way Mendelian randomization study demonstrated a causal association between insulin resistance and higher BCAA levels, whereas higher BCAA levels were not causally associated with insulin resistance (59).

In summary, the gut microbiota and their metabolites serve as potential mediators between diet and T2DM metabolism (Figure 4). This pivotal connection was also demonstrated in the context of a mediation analysis pertaining to personalized dietary interventions (23), where alterations in the gut microbiome composition elucidated 12.25% of the variations observed in serum metabolites. A Western diet, positively associated with T2DM, promotes impaired glucose tolerance by fostering the growth of saturated fatty acid-metabolizing bacteria and triggering the secretion of trimethylamine N-oxide (TMAO), ultimately elevating the risk of T2DM. Conversely, diets negatively associated with T2DM promotes insulin secretion and improves insulin resistance

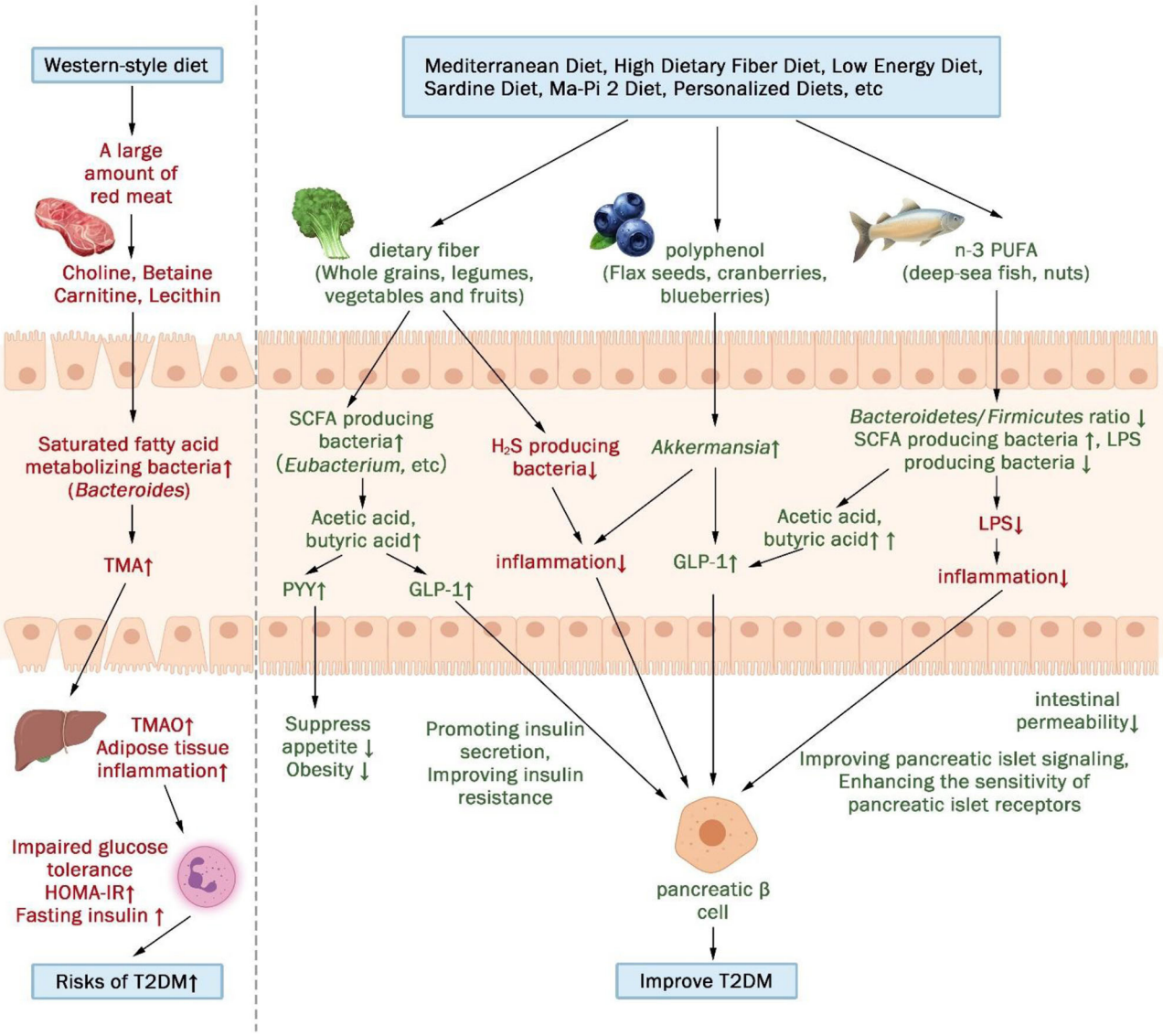

FIGURE 4
The gut microbiota and its metabolites may act as a mediator between diet and metabolism in patients with T2DM. (↑indicates increase, ↓indicates decrease).

by increasing SCFA-producing bacteria and decreasing H2S- and LPS-producing bacteria.

The human gut microbiota is intricately associated with dietary patterns and influenced by confounding factors such as age, race, gender, geography, and socioeconomic variables (60). Furthermore, it can be substantially impacted by different disease stages and treatment medications (60, 61). A Meta-analysis that included 27 studies (62) showed that age is an important factor influencing the diversity, composition and functional characteristics of the gut microbiota, and in particular, beta diversity varies significantly across developmental stages.

The growing recognition of the gut microbiome's predictive capacity for human health necessitates its incorporation as a foundation for personalized health management indicators (63). Moreover, diet-microbiota interactions underpin the advancement of precision nutrition, where the gut microbiota composition emerges as a critical factor in determining responses to dietary interventions (64). Therefore, by integrating gut microbiomics and metabolomics with robust data analysis, machine learning algorithms can aid in devising personalized diets that offer more effective guidance for the prevention and management of T2DM through nutritional interventions (65). This paper includes a study featuring a personalized diet intervention designed by Meleshko et al. (22), which tailored the diet based on the individuals' gut microbiota status, immune responses, and biochemical parameters among T2DM patients. The results showed significant improvements in blood glucose levels, lipid profiles, and inflammatory markers, along with notable reductions in

metabolism-related detrimental bacteria (e.g., *Enterococcus faecalis, Escherichia coli, lac+*, and *Candida spp.*). Shoer et al. (23) found that personalized diets had greater beneficial effects on glycemic control, gut microbiota and metabolites than the Mediterranean diet.

Nevertheless, there are several current challenges and issues associated with employing precision nutrition for disease prevention and management. These include the high cost of histological techniques, the complexity of study design methods, the analysis and interpretation of high-dimensional data, and result reproducibility (66, 67). In the face of these challenges, future research efforts should prioritize rigorous and rational study designs, cost reduction of histological technique analyses, and the development of algorithms capable of handling high-dimensional big data from diverse sources (67).

6 Advantages and limitations

To our knowledge, this is the inaugural meta-analysis and systematic review designed to categorize dietary interventions based on their nutritional attributes, aiming to evaluate their efficacy in ameliorating the gut microbiota and metabolic profiles of individuals with T2DM. This approach holds broader instructive value for individuals in their daily dietary choices. Additionally, we elucidate the intermediary role of the gut microbiota and its metabolites in bridging dietary patterns and T2DM. In this context, as different diets influence the gut microbiota, they subsequently leverage various pathways to enhance the metabolic status of T2DM patients. We should note that our scope of interventions excludes isolated nutritional supplements and probiotic interventions. The inclusion of probiotics, considered beneficial bacteria, could directly interfere with the impact of dietary interventions on the gut microbiota. Moreover, nutritional supplements are not commonly integrated into individuals' daily dietary habits.

However, it is imperative to acknowledge that our meta-analysis incorporated a substantial degree of heterogeneity, primarily attributed to the variance in the methodologies employed for dietary interventions. Additionally, patient age, variations in disease progression, intervention duration combined with medication usage, and the limited sample sizes within the studies could potentially obscure the genuine alterations in gut microbiota and metabolic markers. It is pertinent to mention that the relatively limited number of meta-analyses focusing on gut microbiota is due to the scarcity of specific gut microbiota-related variables accessible for complete experimental and control groups. In certain instances, studies solely compared changes between the intervention and baseline levels, further contributing to this scarcity.

7 Conclusion and prospect

Regarding specific dietary patterns, a high dietary fiber regimen led to a noteworthy reduction in FBG, HbA1c, and total cholesterol levels while augmenting the prevalence of short-chain fatty acid-producing bacteria. The high-fat and low-carbohydrate diet was particularly effective in lowering HbA1c levels, and a low-fat, low-carbohydrate diet exhibited significant reductions in FBG, HOMA-IR, BMI, and waist circumference. Notably, individualized dietary strategies demonstrated superior efficacy in blood glucose management among T2D patients when compared to predefined dietary patterns. This approach also fostered a greater diversity of beneficial gut bacteria.

In summary, diverse dietary interventions can enhance metabolic profiles by positively influencing the gut microbiota, consequently leading to improvements in metabolic parameters among individuals with T2D.

Consequently, future clinical investigations are warranted to comprehensively explore the effects of dietary modalities on the gut microbiota of T2DM patients, as well as establish connections between alterations in the gut microbiota and changes in T2DM-associated biochemical markers. In forthcoming studies, it is imperative to consider appropriate sample sizes, extend intervention durations, and continuously monitor the dynamics of the gut microbiota and its metabolites. Additionally, it is essential to move beyond bacterial classification and perform functional group analyses of functionally similar microorganisms when studying the gut microbiota. Regarding dietary interventions, apart from commonly studied patterns such as the Mediterranean diet, high-fiber diets, and low-energy diets, future investigations should embrace personalized and tailored approaches based on individual variations in gut microbiota characteristics, immune and biochemical indicators, disease stage, and drug response diversity.

Data availability statement

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

Author contributions

Conceptualization: XX and HX. Methodology: XX, FZ, JR, HZ, CJ, MW, and YJ. Validation: XX, FZ, and JR. Formal analysis and writing—original draft preparation: XX. Writing—review and editing: XX, HX, FZ, JR, HZ, CJ, MW, and YJ. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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

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Supplementary material

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Ultra-processed food consumption and metabolic disease risk: an umbrella review of systematic reviews with meta-analyses of observational studies

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Background and aims: There is an ongoing debate on whether to advocate reducing ultra-processed food (UPF) in dietary guidelines to control metabolic disease (such as obesity and type 2 diabetes mellitus [T2DM]). We aimed to summarize the evidence from systematic reviews with meta-analyses between UPF consumption and metabolic diseases risk, assess the credibility, and verify the robustness of these associations.

Methods: We systematically searched PubMed, Web of Science, Embase, and Cochrane Library databases from their inception to July 15, 2023, to identify relevant systematic reviews with meta-analyses. We used the random-effects model to evaluate the summary effect size, along with 95% confidence interval and prediction interval. We also assessed heterogeneity, evidence of small-study effects and excess significance bias, and categorized the credibility of each association based on quantitative umbrella review criteria. Additionally, we conducted subgroup and sensitivity analyses to assess the robustness of associations based on continents, study design, dietary assessment methods, definition methods of UPF, population, and units of UPF consumption.

Results: Overall, 6 systematic reviews with 13 meta-analyses were included. Three (23.08%) meta-analyses were classified as highly suggestive evidence for meeting the criteria that associations were significant at $p < 10^{-6}$, had more than 1,000 cases, and presented the largest study with significance at $p < 0.05$. Among them, the highest UPF consumption quantile was associated with an increased risk of obesity (OR = 1.55, 95% CI: 1.36–1.77) when compared with the lowest UPF consumption quantile. The highest UPF consumption quantile was associated with an increased risk of T2DM (RR = 1.40, 95% CI: 1.23–1.59) when compared with the lowest UPF consumption quantile, and a 10% increase in UPF consumption (% g/d) was associated with an increased risk of T2DM (RR = 1.12, 95% CI: 1.10–1.13). Meanwhile, the robustness of these associations was verified by a series of subgroup and sensitivity analyses.

Conclusion: UPF consumption may be a risk factor for several metabolic diseases. However, well-designed studies are still needed to verify our findings in the future.

KEYWORDS

ultra-processed food, metabolic diseases, meta-analysis, umbrella review, observational study

Introduction

Metabolic disease is a metabolic disorder of organs, tissues, or cells caused by abnormal synthesis and decomposition of certain substances during metabolism, such as obesity and type 2 diabetes mellitus (T2DM) (1). It has become a serious burden on human society due to its rapidly increasing incidence worldwide (2–4). Currently, the precise etiology of metabolic disease is not fully understood, both genetic and environmental factors play crucial roles in the occurrence and development of disease (5, 6). Among them, as one of the most important modifiable environmental factors, the role of diet factors on metabolic disease has received extensive attention (7, 8).

Traditional methods to improve health focused on nutrients as the key determinants of a healthful diet (9). However, this classical nutrient-centric view has been challenged by the NOVA classification system, which is proposed as a novel way to classify foods based on the degree of processing rather than nutritional components (9, 10). According to the NOVA classification system, ultra-processed food (UPF) is a group of foods defined as industrial formulations created mostly or entirely from substances extracted from foods, with additives and with little if any intact food, such as fast foods, savory snacks, cakes, soft and/or sweetened drinks, and sausages (10). In developed countries, UPF has become an important source of energy intake, and the percentage of total energy from UPF could even be more than 50% (11, 12). Several systematic reviews with meta-analyses suggested that UPF consumption was associated with various metabolic disease, such as obesity, T2DM, hypertension, non-alcoholic fatty liver disease (NAFLD), and metabolic syndrome (MetS) (13–18). Therefore, there is a view that it is necessary to advocate the reduction of UPF in dietary guidelines to optimize health and policies (19). However, there is also an opposite view questioning the significance of UPF (20), suggesting that the concept and investigations of UPF are vague, and the association between UPF consumption and metabolic disease such as obesity remain uncertain due to the existence of potential biases, therefore, the mention of UPF in dietary guidelines can add little to existing nutrient profiling systems (21).

The contradictory views may cause confusion for clinicians and public health policymakers to make decisions. Therefore, we conducted an umbrella review (UR) across published systematic reviews with meta-analyses to evaluate the credibility as well as verify the robustness of associations between UPF consumption and metabolic disease.

Methods

The UR is an approach used to provide an overview of published systematic reviews with meta-analyses on the same topic and evaluate the credibility of associations (22–24). In this UR, we strictly followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses reporting guideline (Supplementary Table S1) and meta-analysis of Observational Studies in Epidemiology guidelines (Supplementary Table S2) (25, 26), and the protocol was registered in the International Prospective Register of Systematic Reviews (CRD42023427297).

Search strategy

Two investigators (J-LL and Y-FW) systematically searched PubMed, Web of Science, Embase, and Cochrane Library databases from their inception to July 15, 2023, to identify systematic reviews with meta-analyses that evaluated the associations between UPF consumption and metabolic disease risk. The search strategy is shown in Supplementary Table S3. No language restrictions were used when selecting eligible articles. Furthermore, included studies were backward snowballed manually and forward snowballed using the Web of Science citation tracking feature to identify additional eligible studies (27, 28).

Inclusion and exclusion criteria

Articles were included if they met the following PI[E]COS (Population, Intervention or Exposure, Comparison, Outcome, Study design) criteria: (1) Population: participants of different ages; (2) Intervention/Exposure: including UPF which was defined according to the NOVA classification system; (3) Comparison: highest/moderate vs. lowest, or dose–response analysis, etc.; (4) Outcome: metabolic disease risk (e.g., obesity, T2DM, or MetS); and (5) Study design: UR of systematic reviews with meta-analysis of observational studies (cohort, nested case–control, case–control, or cross-sectional studies, etc.).

Exclusion criteria: (1) genetic polymorphisms, laboratory, and animal studies; (2) systematic reviews without quantitative evaluations; (3) studies that could not obtain study-specific data, including effect sizes (odds ratio [OR], relative risk [RR], or hazard ratio [HR], etc.), 95% confidence interval (CI), and the number of cases or total population; and (4) studies that included less than three original studies. When more than one meta-analysis on the same association was eligible, only the latest meta-analysis was included (29). Of note, any comparison of exposure could be included and treated as a unique meta-analysis, such as highest/moderate vs. lowest, and dose–response analysis (30–32). Moreover, when a systematic review reported meta-analyses on more than one eligible outcome,

they were all included and assessed separately (22). Two investigators (J-LL and Y-FW) independently screened the titles and abstracts of identified records and selected eligible articles by scrutinizing the full text. Any disagreement in the results comparison process was resolved through consensus with a third investigator (Q-JW).

Data extraction

Two investigators (J-NS and Y-CS) independently conducted data extraction, and any disagreement was resolved by consensus with the third investigator (Q-JW). From each eligible meta-analysis, we recorded the first author, year of publication, journal name, outcomes, number of studies included, and comparison (highest/moderate vs. lowest, or dose–response analysis, etc.). Regarding comparison, "lowest" was defined as the lowest UPF consumption quantile, "moderate" was defined as the first exposure quantile, and "highest" was defined as the highest UPF consumption quantile. For each original study, we extracted the first author, year of publication, country, study design (cohort, nested case–control, case–control, or cross-sectional studies, etc.), follow-up year, Newcastle-Ottawa Scale (NOS) score, diagnostic criteria for disease, dietary assessment methods (food frequency questionnaire [FFQ], 24-h dietary recall, or food record, etc.), definition methods (NOVA or non-NOVA) and units (% kcal/d, % g/d, or g/d, etc.) of UPF consumption, number of cases and participants, risk estimate (OR, RR, or HR) and 95% CI from the multivariable model, and covariates used for adjustment.

Statistical analyses

For each association, we calculated the summary effect size and 95% CI using random effects methods (33). The 95% prediction interval (PI) was also calculated to account for between-study heterogeneity and to evaluate the possible range of the effect size in a new study addressing the same association (34). Between-study heterogeneity was evaluated by tau^2 and I^2 statistic (35). An I^2 value <50, 50% ≤ to ≤75%, and > 75% were considered to represent not large, large, and very large heterogeneity, respectively (35). Egger's regression asymmetry test was used to identify whether there was evidence for small-study effects (SSE) (i.e., whether smaller studies tend to report larger effect size than larger studies) (36). A p-value <0.10 with more conservative effects in the largest study (i.e., the study with the smallest standard error) than in random effects meta-analysis was considered to be evidence of SSE (37). We applied the excess statistical significance test to assess whether the number of observed studies (O) with statistically significant results was larger than the expected number of positive studies (E) (38). In each meta-analysis, we calculated E by the sum of the statistical power estimates for each component study (38). We evaluated the power of each component study using the effect size of the largest study, while a non-central t distribution was used to calculate the statistical power of each study (39). Excess significance bias (ESB) for each meta-analysis was denoted at $p < 0.10$ and $O > E$ (38). Cohen's kappa statistic was employed to evaluate the consistency of different procedures between the two investigators. The degree of consistency was explained by the kappa value (40).

In addition, subgroup analyses were performed to evaluate the robustness of results according to continents (America, Asia, or Europe), study design (prospective cohort, case–control, or cross-sectional studies), dietary assessment methods (FFQ, 24-h dietary recall, or food record), and definition methods (NOVA or non-NOVA) of UPF. Furthermore, sensitivity analyses were conducted in our UR. First, only one meta-analysis involved adolescent participants (15), therefore we excluded adolescent participants due to their limited representation in the included meta-analyses which mostly focused on adults. Second, in the meta-analyses that defined UPF entirely based on the NOVA classification system, we further considered the potential impact of UPF units and reanalyzed associations by excluding original studies that differed from the UPF units used in most original studies. All analyses were performed using STATA version 16 (StataCrop, College Station, TX, United States) and IBM SPSS Statistics version 21 (IBM Corp, Armonk, NY, United States).

Grading the evidence

We applied quantitative criteria to categorize the credibility of each association into convincing, highly suggestive, suggestive, or weak according to previously published UR (Table 1) (23, 41, 42).

Quality assessment of evidence and methods

Two investigators (J-LL and M-HS) independently employed the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) to assess the quality of each association (43). According to GRADE, the quality of each association was classified as high, moderate, low, or very low (43). For observational studies, the quality of evidence was initially classified as low, and then could be downgraded based on five factors including study limitations, imprecision, inconsistency, indirectness, and publication bias, and upgraded based on three factors including a large magnitude of effect,

TABLE 1 The criteria to categorize the credibility of evidence in umbrella review.

Evidence class	Criteria
Convincing (Class I)	• $p < 10^{-6}$ under the random-effects model • Number of cases >1,000 • $p < 0.05$ of the largest study in the meta-analysis • $I^2 < 50\%$ • 95% prediction interval that excluded the null value • No evidence of small-study effects • No evidence of excess significance bias
Highly suggestive (Class II)	• $p < 10^{-6}$ under the random-effects model • Number of cases >1,000 • $p < 0.05$ of the largest study in the meta-analysis
Suggestive (Class III)	• $p < 10^{-3}$ under the random-effects model • Number of cases >1,000
Weak (Class IV)	• $p < 0.05$ under the random-effects model
Not significant	• $p \geq 0.05$ under the random-effects model

a dose–response gradient, and attenuation by plausible confounding (43).

In addition, two reviewers (J-LL and M-HS) independently used the Assessment of Multiple Systematic Reviews (AMSTAR) tool to assess the methodological quality of each included systematic review (44). AMSTAR evaluates 11 questions, with a maximum score of 11. Each meta-analysis was categorized as high, moderate, or low quality for scores of ≥8 points, 4–7 points, and ≤3 points, respectively (45).

Results

Literature review

Overall, we initially identified 776 records, 315 records were excluded for duplication, and 429 records were excluded after screening titles and abstracts. Finally, we reviewed 32 full-text articles, and 6 systematic reviews with 13 meta-analyses met the inclusion criteria (Figure 1). Details of the 26 excluded articles are shown in Supplementary Table S4. The two investigators showed a high consistency in terms of data screening and selection, with a kappa value of 0.86.

Characteristics of the included meta-analyses

The characteristics of 13 meta-analyses corresponding to 97 original studies are presented in Supplementary Table S5. Of the 13 meta-analyses, the median number of original studies was 7 (range from 3 to 18), that of participants was 66,235 (range from 15,152 to 992,242), and that of cases was 15,000 (range from 4,302 to 34,924). Regarding exposure, all meta-analyses involved UPF defined by the NOVA classification system, of which 3 (23.08%) meta-analyses also involved specific types of foods belonging to UPF. Data on UPF consumption were mainly collected using FFQ, 24-h dietary recall, and 4-day food records. In addition, the commonly used units of UPF consumption included % kcal/d and % g/d. Regarding outcome, we investigated a total of 6 outcomes, including abdominal obesity (13), hypertension (14), MetS (15), NAFLD (16), obesity (13), overweight (13), overweight and obesity (13), and T2DM (17, 18).

UPF consumption and overweight and/or obesity risk

Six (46.15%) meta-analyses that evaluated the association between UPF consumption and overweight and/or obesity risk were all significant at $p < 0.05$. Among them, one meta-analysis was still significant at $p < 10^{-6}$, had more than 1,000 cases, presented the largest study with significance at $p < 0.05$, and reported large heterogeneity, 95% PI excluding the null value, and evidence of ESB. According to the quantitative UR criteria, the above meta-analysis was classified as highly suggestive evidence, indicating that the highest UPF consumption quantile was associated with an increased risk of obesity (OR = 1.55, 95% CI: 1.36–1.77) when compared with the lowest UPF consumption quantile. In addition, according to criteria that the associations were still significant at $p < 10^{-3}$ and had more than 1,000 cases, two meta-analyses were classified as suggestive evidence, demonstrating that the highest UPF consumption quantile was associated with an increased risk of abdominal obesity (OR = 1.41, 95% CI: 1.18–1.68) when compared with the lowest UPF consumption quantile, and a 10% increase in UPF consumption (% kcal/d) was associated with an increased risk of abdominal obesity (OR = 1.05, 95% CI: 1.02–1.07). The other three meta-analyses were classified as weak evidence, showing that the highest UPF consumption quantile was associated with an increased risk of overweight (OR = 1.36, 95% CI: 1.14–1.63) when compared with the lowest UPF consumption quantile, and a 10% increase in UPF consumption (% kcal/d) was associated with an increased risk of overweight and obesity (OR = 1.03, 95% CI: 1.01–1.06) as well as obesity (OR = 1.07, 95% CI: 1.03–1.11) (Figures 2, 3 and Table 2).

UPF consumption and T2DM risk

Three (23.08%) meta-analyses that evaluated the association between UPF consumption and T2DM risk were all significant at $p < 10^{-3}$. Among them, two meta-analyses were still significant at $p < 10^{-6}$, had more than 1,000 cases, and presented the largest study with significance at $p < 0.05$. According to the quantitative umbrella review criteria, they were classified as highly suggestive evidence, indicating that the highest UPF consumption quantile was associated with an increased risk of T2DM (RR = 1.40, 95% CI: 1.23–1.59) when compared with the lowest UPF consumption quantile, and a 10% increase in UPF consumption (% g/d) was associated with an increased risk of T2DM (RR = 1.12, 95% CI: 1.10–1.13). In addition, one meta-analysis was classified as suggestive evidence due to meeting the criteria of cases more than 1,000 simultaneously, demonstrating that moderate UPF consumption quantile was associated with an increased risk of T2DM (RR = 1.12, 95% CI: 1.06–1.17) when compared with the lowest UPF consumption quantile (Figures 2, 3 and Table 2).

UPF consumption and NAFLD risk

Two (15.38%) meta-analyses that evaluated the association between UPF consumption and T2DM risk were all significant at $p < 0.05$. One meta-analysis was still significant at $p < 10^{-3}$ and had more than 1,000 cases, classifying as suggestive evidence and demonstrating that the highest UPF consumption quantile was associated with an increased risk of NAFLD (RR = 1.39, 95% CI: 1.21–1.60) when compared with the lowest UPF consumption quantile. The other meta-analysis was classified as weak evidence, suggesting that moderate UPF consumption quantile was associated with an increased risk of NAFLD (RR = 1.04, 95% CI: 1.00–1.07) when compared with the lowest UPF consumption quantile (Figures 2, 3 and Table 2).

UPF consumption and hypertension risk

One (7.69%) meta-analysis was classified as suggestive evidence according to $p < 10^{-3}$ and cases more than 1,000, demonstrating that the highest UPF consumption quantile was associated with an increased risk of hypertension (OR = 1.23, 95% CI: 1.11–1.37) when

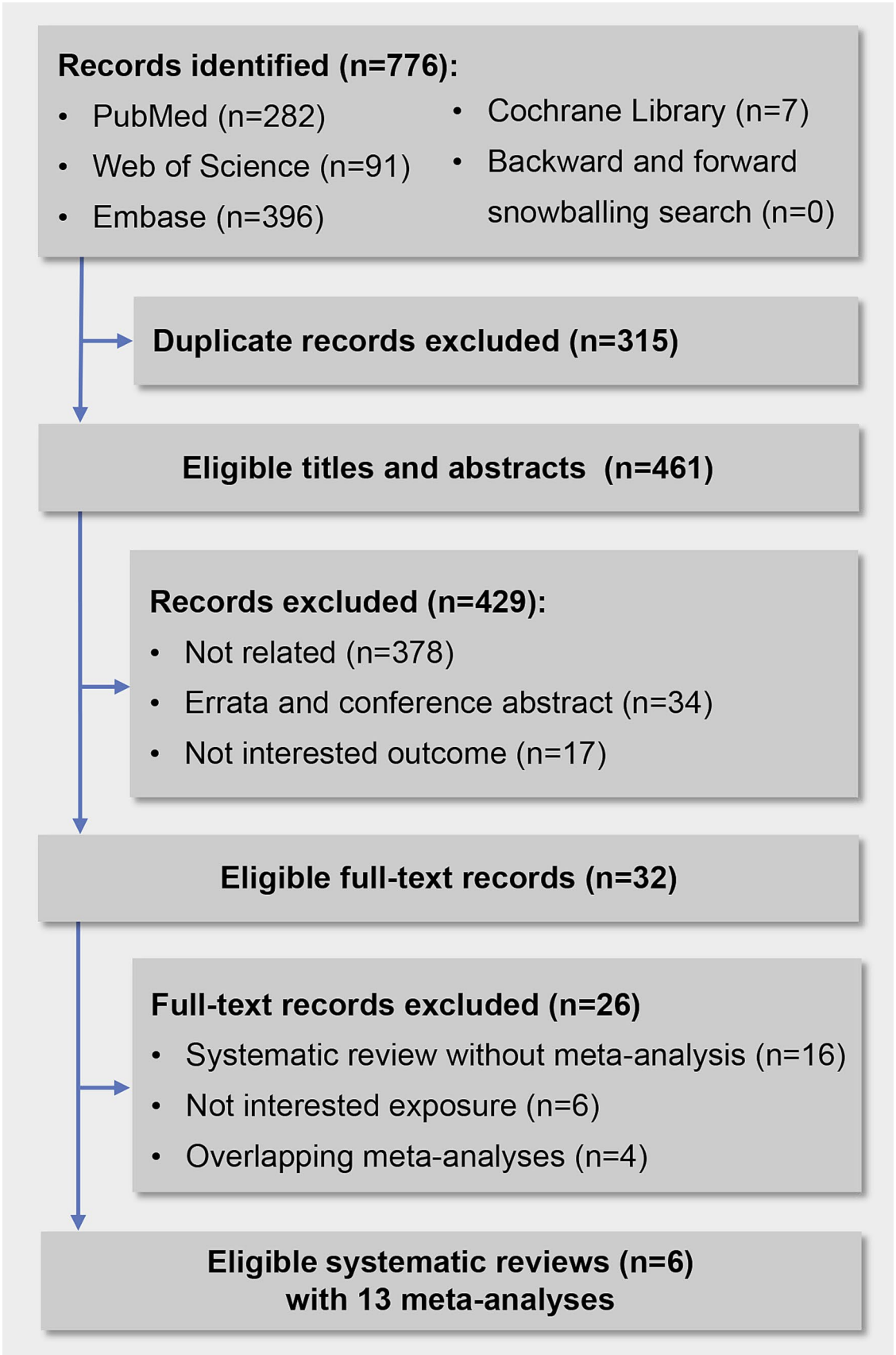

FIGURE 1
Flow diagram of the study selection process.

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Outcomes (Reference)/ Author Year	No. of studies	Comparison[a]	Effect metrics	Random effect estimate (95% CI)	Random effect estimate (95% CI)
Abdominal obesity (14)					
Moradi 2023	4	Highest vs. lowest	OR		1.41 (1.18-1.68)
Moradi 2023	6	A 10% increase[b]	OR		1.05 (1.02-1.07)
Hypertension (15)					
Wang 2022	9	Highest vs. lowest	OR		1.23 (1.11-1.37)
MetS (16)					
Shu 2023	9	Highest vs. lowest	RR		1.25 (1.09-1.42)
NAFLD (17)					
Henney 2023	7	Moderate vs. lowest	RR		1.04 (1.00-1.07)
Henney 2023	9	Highest vs. lowest	RR		1.39 (1.21-1.60)
Obesity (14)					
Moradi 2023	7	Highest vs. lowest	OR		1.55 (1.36-1.77)
Moradi 2023	7	A 10% increase[b]	OR		1.07 (1.03-1.11)
Overweight (14)					
Moradi 2023	4	Highest vs. lowest	OR		1.36 (1.14-1.63)
Overweight and obesity (14)					
Moradi 2023	3	A 10% increase[b]	OR		1.03 (1.01-1.06)
T2DM (18,19)					
Delpino 2022	18	Moderate vs. lowest	RR		1.12 (1.06-1.17)
Chen 2023	7	Highest vs. lowest	RR		1.40 (1.23-1.59)
Chen 2023	7	A 10% increase[c]	RR		1.12 (1.10-1.13)

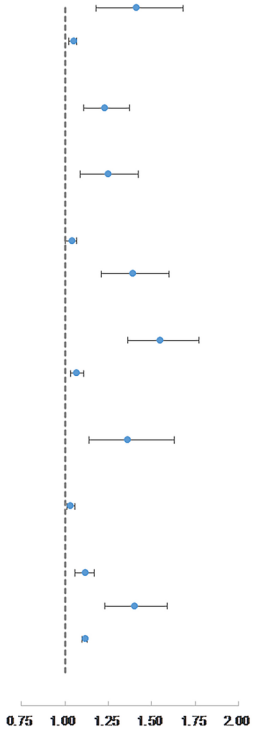

FIGURE 2
Summary random effect estimate with 95% confidence interval from 13 meta-analyses evaluating the association between ultra-processed food consumption and metabolic disease risk. CI, confidence interval, MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; RR, risk ratio; T2DM, type 2 diabetes mellitus. [a] "lowest" was defined as the lowest UPF consumption quantile, "moderate" was defined as the first exposure quantile, and "highest" was defined as the highest UPF consumption quantile. [b] The unit of UPF consumption was % kcal/d. [c] The unit of UPF consumption was % g/d.

compared with the lowest UPF consumption quantile (Figures 2, 3 and Table 2).

UPF consumption and MetS risk

One (7.69%) meta-analysis was classified as weak evidence according to $p < 0.05$, suggesting that the highest UPF consumption quantile was associated with an increased risk of MetS (RR = 1.25, 95% CI: 1.09–1.42) when compared with the lowest UPF consumption quantile (Figures 2, 3 and Table 2).

Findings of subgroup and sensitivity analyses

The findings of subgroup analyses are displayed in Figures 4, 5 and Table 3. In subgroup analyses, the majority of subgroups exhibited consistent direction and significance with the main analysis. Regarding credibility, the credibility of the association between UPF consumption and obesity risk was upgraded or unchanged when compared with the main analysis. The credibility of the association between UPF consumption and T2DM risk was unchanged in the majority of highest vs. lowest and dose–response meta-analyses, while it was degraded in several moderate vs. lowest meta-analyses. In addition, the credibility of associations between UPF consumption and hypertension and NAFLD risk was degraded in the majority of subgroups. Notably, the credibility of the association between UPF consumption and MetS risk was still weak in the majority of subgroups, but it was degraded to not significant in prospective cohort studies.

The results of sensitivity analyses are shown in Figure 6 and Table 4. In our study, only the association between UPF consumption and MetS risk included adolescents. After excluding adolescent participants, the positive association between UPF consumption and MetS risk was still classified as weak evidence. In addition, five meta-analyses could be further analyzed by excluding original studies with inconsistent units, and the results showed the direction and significance of these associations were all unchanged. However, the credibility of the association between UPF consumption and hypertension was degraded from suggestive evidence to weak evidence, and the credibility of the association between UPF consumption and T2DM (highest vs. lowest analysis) was degraded from highly suggestive evidence to suggestive evidence.

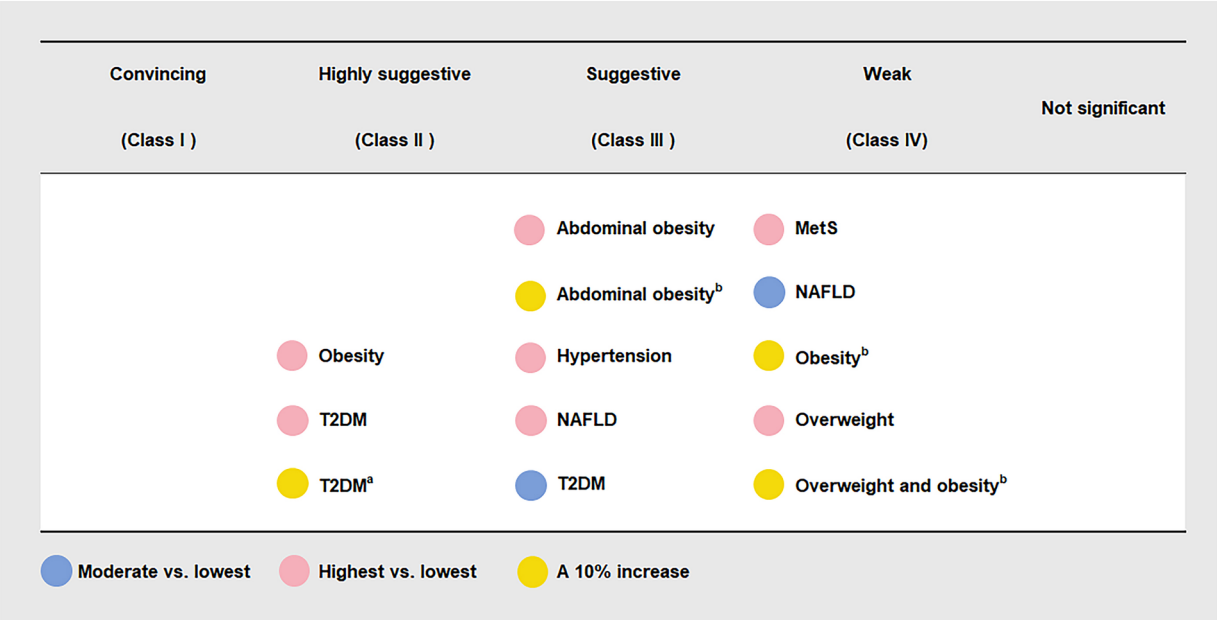

FIGURE 3
Credible assessment of 13 meta-analyses evaluating the association between ultra-processed food consumption and metabolic disease risk. MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus. "Lowest" was defined as the lowest UPF consumption quantile, "moderate" was defined as the first exposure quantile, and "highest" was defined as the highest UPF consumption quantile. [a] The unit of UPF consumption was % g/d. [b] The unit of UPF consumption was % kcal/d.

Quality assessment of evidence and methods

The quality of 13 associations is shown in Supplementary Table S6. According to the GRADE, two (15.38%) associations were classified as moderate evidence, including the dose–response meta-analysis of overweight and obesity as well as T2DM. Four (30.77%) associations were classified as low evidence, including the dose–response meta-analysis of abdominal obesity and obesity, as well as the moderate vs. lowest meta-analysis of NAFLD and T2DM. Seven (53.85%) associations were classified as very low evidence, including the highest vs. lowest meta-analysis of abdominal obesity, hypertension, MetS, NAFLD, obesity, overweight, and T2DM.

The methodological quality of 6 systematic reviews is displayed in Supplementary Figure S1. According to the AMSTAR tool, four (66.67%) systematic reviews were categorized as high quality and the outcomes involved abdominal obesity, MetS, NAFLD, obesity, overweight, overweight and obesity, and T2DM (moderate vs. lowest meta-analysis). Two (33.33%) systematic reviews were categorized as moderate quality, and the outcomes involved hypertension and T2DM (highest vs. lowest and dose–response meta-analyses).

The two investigators showed a high consistency in quality assessment of evidence and methods, with a kappa value of 0.72 and 1.00, respectively.

Discussion

In this UR, we performed a comprehensive overview of 13 meta-analyses to critically assess the credibility of associations between UPF consumption and metabolic disease risk. Overall, we observed significant positive associations in all 13 meta-analyses, with highly suggestive evidence supporting the association between UPF consumption and obesity and T2DM risk. Our findings demonstrated that UPF consumption might contribute to metabolic disease.

Principal findings and possible explanations

In this study, the association between the highest UPF consumption and a 1.55-fold increased risk of obesity was supported by highly suggestive evidence. Although the above association was summarized from 7 cross-sectional studies, a similar trend was also observed in several prospective cohort studies (46, 47). For example, a prospective cohort study of 22,659 adults in the UK Biobank demonstrated that UPF consumption was associated with a 1.79-fold increased risk of obesity (46). In addition, another prospective cohort study of 17,310 adults in South Korea found that there was a significant positive association between UPF consumption and obesity risk (47). Meanwhile, a randomized controlled trial (RCT) study demonstrated that a diet with a large proportion of UPF could cause excess calorie intake and weight gain (48); in contrast, eliminating UPF from the diet could decrease energy intake and lead to weight loss (48). Therefore, UPF consumption may be a risk factor for obesity, and reducing UPF consumption may decrease the risk of obesity. However, of note, our study found evidence of ESB in the association between the highest UPF consumption and obesity risk, suggesting that the harmful association between UPF and obesity might have been exaggerated. Similarly, another dose–response meta-analysis included in our study suggested that UPF consumption was only associated with a 1.07-fold increased risk of obesity. Nevertheless, considering the high prevalence of obesity

TABLE 2 Credible assessment of 13 meta-analyses evaluating the association between ultra-processed food consumption and metabolic disease risk.

Outcomes (Reference)/ Author Year	No. of studies	Comparison[a]	Random *p*-value	No. of cases	95% PI	I^2 (95% CI) (%)	Tau^2	Largest study effect (95% CI)	SSE/ ESB	Evidence class
Abdominal obesity (13)										
Moradi 2023	4	Highest vs. lowest	2.00×10^{-4}	13,928	0.69–2.87	62 (0–87)	0.02	1.21 (1.10–1.46)	No/Yes	Suggestive
Moradi 2023	6	A 10% increase[b]	5.36×10^{-5}	17,011	0.98–1.12	77 (47–89)	0.00	1.02 (1.01–1.03)	No/No	Suggestive
Hypertension (14)										
Wang 2022	9	Highest vs. lowest	1.37×10^{-4}	13,375	0.92–1.64	52 (0–77)	0.01	1.21 (1.06–1.37)	No/Yes	Suggestive
MetS (15)										
Shu 2023	9	Highest vs. lowest	1.00×10^{-3}	8,649	0.84–1.85	85 (73–92)	0.02	1.00 (0.99–1.01)	Yes/No	Weak
NAFLD (16)										
Henney 2023	7	Moderate vs. lowest	0.03	12,367	0.99–1.08	0 (0–71)	0.00	1.03 (0.99–1.07)	No/No	Weak
Henney 2023	9	Highest vs. lowest	2.65×10^{-6}	12,977	0.90–2.17	89 (82–94)	0.03	1.05 (1.02–1.09)	Yes/No	Suggestive
Obesity (13)										
Moradi 2023	7	Highest vs. lowest	1.17×10^{-10}	21,149	1.06–2.26	55 (0–81)	0.02	1.53 (1.29–1.81)	No/Yes	Highly suggestive
Moradi 2023	7	A 10% increase[b]	0.001	15,000	0.95–1.21	88 (79–94)	0.00	1.00 (0.99–1.01)	Yes/No	Weak
Overweight (13)										
Moradi 2023	4	Highest vs. lowest	1.00×10^{-3}	16,131	0.65–2.87	73 (22–90)	0.02	1.13 (1.08–1.41)	No/No	Weak
Overweight and obesity (13)										
Moradi 2023	3	A 10% increase[b]	5.00×10^{-3}	4,302	0.84–1.27	39 (0–81)	0.00	1.02 (1.00–1.04)	No/Yes	Weak
T2DM (17, 18)										
Delpino 2022	18	Moderate vs. lowest	7.63×10^{-6}	34,924	1.00–1.25	24 (0–57)	0.00	1.21 (1.12–1.31)	No/No	Suggestive
Chen 2023	7	Highest vs. lowest	3.15×10^{-7}	21,932	0.91–2.13	88 (78–94)	0.00	1.12 (1.04–1.20)	No/No	Highly suggestive
Chen 2023	7	A 10% increase[c]	5.44×10^{-79}	21,932	1.10–1.14	2 (0–71)	0.02	1.13 (1.11–1.15)	No/Yes	Highly suggestive

CI, confidence interval; ESB, excess significance bias; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; PI, prediction interval; SSE, small-study effects; T2DM, type 2 diabetes mellitus.

[a]"Lowest" was defined as the lowest UPF consumption quantile, "moderate" was defined as the first exposure quantile, and "highest" was defined as the highest UPF consumption quantile.

[b]The unit of UPF consumption was % kcal/d.

[c]The unit of UPF consumption was % g/d.

worldwide (49), a smaller effect size of UPF consumption may have important public health implications. Therefore, when formulating dietary guidelines in the future, it may be necessary to advocate reducing UPF consumption to decrease the incidence of obesity (19).

In addition, two meta-analyses based on prospective cohort studies were supported by highly suggestive evidence, indicating that UPF consumption was associated with T2DM in the highest vs. lowest and dose–response meta-analyses. In addition, in line with our findings, another meta-analysis excluded due to overlap also showed that there was a significant positive association between UPF consumption and T2DM risk (50). In addition, a UR evaluating the role of diet in T2DM demonstrated that several specific types of UPF, such as processed meat and sugar-sweetened beverages, could increase the incidence of T2DM (51). Therefore, the above evidence hints that UPF consumption may be a risk factor for T2DM. Of note, our study also suggests that dose may be an important factor influencing the effects of UPF consumption. In the moderate vs. lowest meta-analysis, the association between UPF consumption and T2DM risk was only supported by suggestive or weak evidence. In addition, the above-mentioned association was even not significant in the subgroups of the Asian population. This imply that the association between UPF consumption and T2DM risk may vary across different populations. Nevertheless, our findings should still be interpreted with caution. Although significant results were observed in the American and European populations and no significant findings were found in the Asian population, this discrepancy may be related to the level of UPF consumption being much lower among Asians than other populations. Meanwhile, only three studies were included in the association of the Asian population, with a relatively small sample size and short follow-up time. Furthermore, of note, the majority of original studies included in the moderate vs. lowest meta-analysis of the association between UPF consumption and T2DM risk did not define UPF based on the NOVA classification system. Further subgroup analyses showed that although the above-mentioned association was still significant in the non-NOVA group, the results were not significant in the NOVA group. Not defining UPF based on the NOVA classification system may affect the results due to potential misclassification bias, therefore more studies are needed to further explore the above-mentioned association.

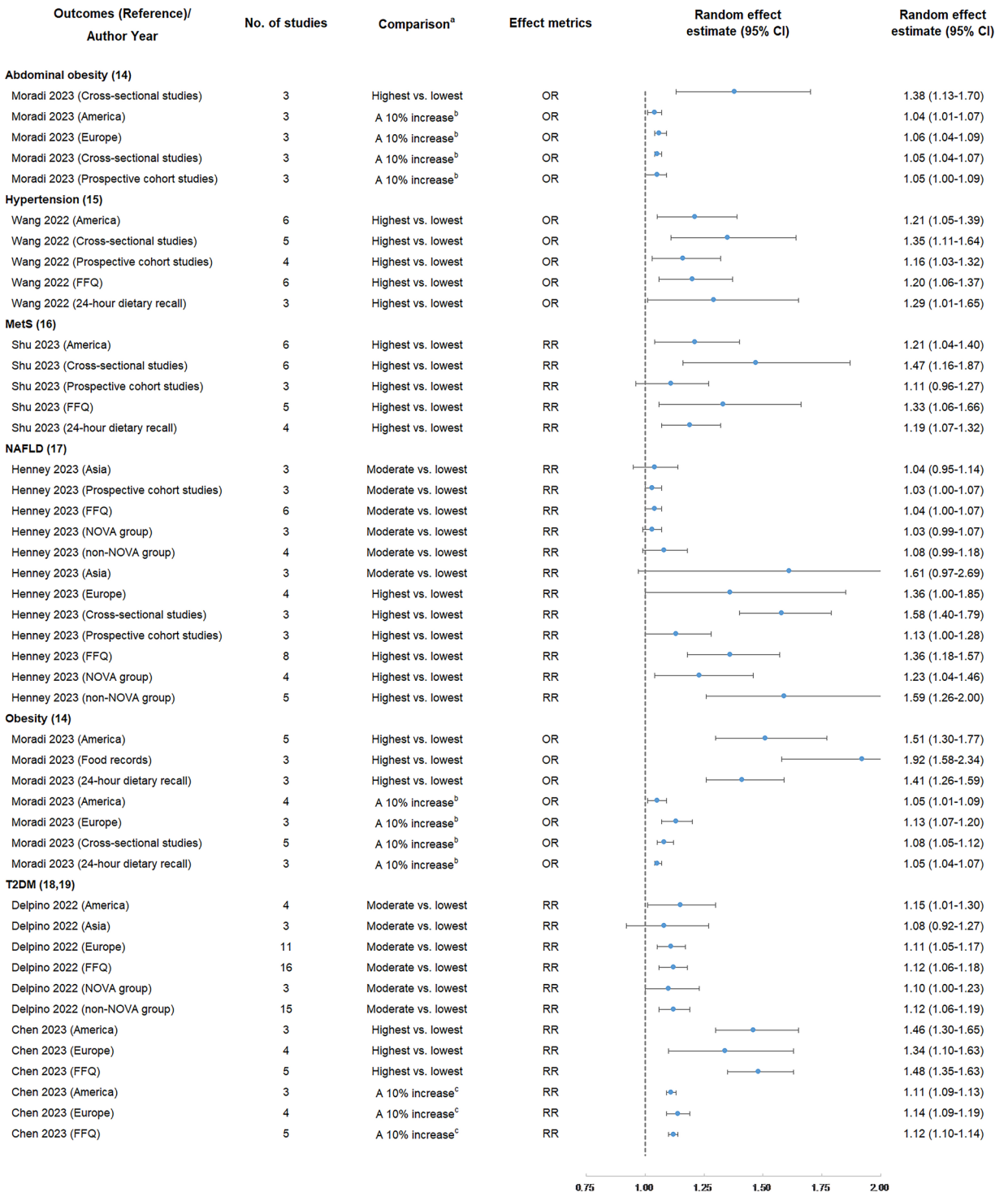

FIGURE 4
Subgroup analyses of summary random effect estimate with 95% confidence interval from meta-analyses evaluating the association between ultra-processed food consumption and metabolic disease risk. CI, confidence interval; FFQ, food frequency questionnaire; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; RR, risk ratio; T2DM, type 2 diabetes mellitus. [a] "lowest" was defined as the lowest UPF consumption quantile, "moderate" was defined as the first exposure quantile, and "highest" was defined as the highest UPF consumption quantile. [b] The unit of UPF consumption was % kcal/d. [c] The unit of UPF consumption was % g/d.

Furthermore, we found that UPF consumption was associated with increased risks of hypertension, NAFLD, and MetS, and these associations were supported by suggestive or weak evidence. The credibility of associations between UPF consumption and hypertension and NAFLD did not seem to be robust as the grade of evidence was degraded in the majority of subgroups. Of note, the association between UPF consumption and NAFLD risk included more than half of the original studies that did not define UPF based on the NOVA classification system. However, the results of subgroup analyses showed that whether to define UPF based on the NOVA

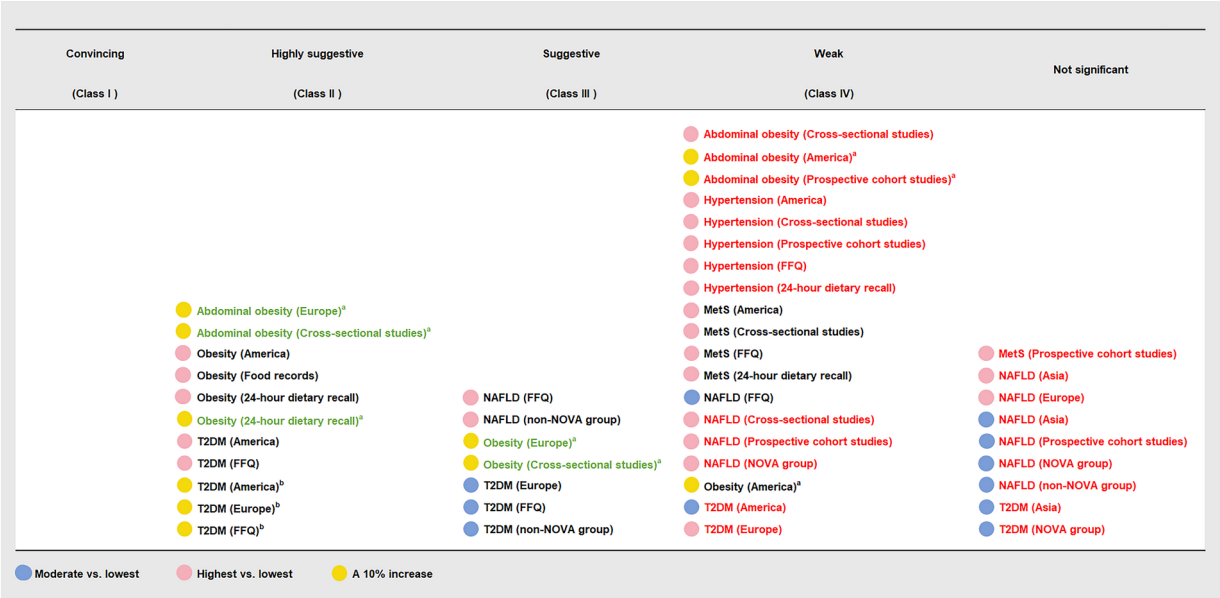

FIGURE 5
Subgroup analyses of credible assessment of meta-analyses evaluating the association between ultra-processed food consumption and metabolic disease risk. FFQ, food frequency questionnaire; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus. "Lowest" was defined as the lowest UPF consumption quantile, "moderate" was defined as the first exposure quantile, and "highest" was defined as the highest UPF consumption quantile. The color of the text reflected the change of credibility compared with the main analyses: black, unchanged; red, degraded; green, upgraded. [a] The unit of UPF consumption was % kcal/d. [b] The unit of UPF consumption was % g/d.

classification system may have an impact on the credibility of the association. The credibility of the association between UPF consumption and MetS risk was still classified as weak in the majority of subgroups, but became not significant when limited to prospective cohort studies only. Therefore, further studies are needed to explore the association between UPF consumption and MetS risk as prospective cohort studies are generally considered to provide higher levels of evidence than other observational studies (31).

The biological mechanisms of the association between UPF consumption and metabolic disease risk are still unclear, but there are several scenarios to explain the above-mentioned association. First, at the nutritional level, UPF usually has a poor nutritional profile and tends to be rich in added sugars, saturated fats, and sodium, as well as poor in fiber and micronutrients (52, 53). Evidence suggests that the poor nutritional profile can increase chronic and low-grade systemic inflammation, and then increase the risk of obesity and related metabolic disease such as T2DM and NAFLD (54). In addition, at the food level, UPF consumption can replace unprocessed or minimally processed food consumption (55). According to the NOVA classification system, unprocessed or minimally processed food is fresh or processed by industrial processes such as removal of unwanted or inedible parts, boiling, drying, roasting, freezing, and refrigeration (10). None of these processes add salt, sugar, oils or fats, or other food substances to the original food, and examples include fresh vegetables and fruits, grains, legumes, pasteurized milk, yogurt without added sugar, nuts and seeds without added sugar, etc. (10). Of note, unprocessed or minimally processed food may exert a protective effect against metabolic disease (56–58). On the one hand, this may be associated with health-promoting components. For example, fresh vegetables and fruits provide antioxidants which may help to prevent inflammation and oxidative stress in metabolic disease (59). On the other hand, beyond single foods or nutrients, the overall dietary pattern may also explain the association between diet and health (60). For instance, Greater adherence to the prudent pattern rich in vegetables and fruits, whole grains, and legumes can predict lower risks of MetS (61).

The aforementioned evidence suggests that nutritional factors can explain the association between UPF consumption and metabolic disease risk. Additionally, food processing factors may also play an important role during this process (57). At the food processing level, the loss of physical and structural characteristics of the food matrix is linked with a lower satiety potential (62), combined with the non-nutritional features of UPF such as delicious and ready-to-eat, UPF may lead to continuous and unconscious eating behaviors and then further increase the risk of metabolic disease (13, 63). In addition, food additives may create an environment in the gut that favors the selection of microbes that promote inflammation-related disease (64). Furthermore, neo-formed compounds resulting from food processing (e.g., advanced glycation end products) are associated with an increased risk of metabolic disease (65). Similarly, chemical contaminants released from food packaging (e.g., phthalates and bisphenol A) are known as endocrine-disrupting chemicals and are involved in the pathophysiological processes of various diseases (66).

Strengths and limitations

To our knowledge, this is the first UR to provide a comprehensive summary of the associations between UPF consumption and metabolic disease from published systematic reviews with meta-analyses and to further analyze the robustness of these associations through a series of subgroup and sensitivity analyses. According to standard criteria, we comprehensively assessed the credibility and quality of each meta-analysis. Our findings demonstrated that UPF consumption might be a risk factor for metabolic disease, providing a

TABLE 3 Subgroup analyses of credible assessment of meta-analyses evaluating the association between ultra-processed food consumption and metabolic disease risk.

Outcomes (Reference)/ Author Year (Subgroups)	No. of studies	Comparison[a]	Random *p*-value	No. of cases	95% PI	I^2 (95% CI) (%)	Tau^2	Largest study effect (95% CI)	SSE/ESB	Evidence class[d]
Abdominal obesity (13)										
Moradi 2023 (Cross-sectional studies)	3	Highest vs. lowest	2.00×10^{-3}	13,751	0.13–14.61	74 (11–92)	0.02	1.21 (1.10–1.46)	No/Yes	Weak
Moradi 2023 (America)	3	A 10% increase[b]	5.00×10^{-3}	12,434	0.75–1.44	85 (55–95)	0.00	1.02 (1.01–1.03)	No/Yes	Weak
Moradi 2023 (Europe)	3	A 10% increase[b]	1.11×10^{-7}	4,577	0.92–1.23	0 (0–90)	0.00	1.06 (1.03–1.08)	No/Yes	Highly suggestive
Moradi 2023 (Cross-sectional studies)	3	A 10% increase[b]	1.83×10^{-11}	13,751	0.95–1.17	0 (0–90)	0.00	1.06 (1.04–1.08)	No/Yes	Highly suggestive
Moradi 2023 (Prospective cohort studies)	3	A 10% increase[b]	0.03	3,260	0.67–1.62	82 (46–94)	0.00	1.02 (1.01–1.03)	No/Yes	Weak
Hypertension (14)										
Wang 2022 (America)	6	Highest vs. lowest	7.00×10^{-3}	11,093	0.80–1.84	63 (10–85)	0.02	1.19 (1.03–1.38)	No/Yes	Weak
Wang 2022 (Cross-sectional studies)	5	Highest vs. lowest	3.00×10^{-3}	6,239	0.78–2.33	44 (0–80)	0.02	1.19 (1.03–1.38)	No/No	Weak
Wang 2022 (Prospective cohort studies)	4	Highest vs. lowest	0.02	7,136	0.72–1.89	55 (0–85)	0.01	1.21 (1.06–1.37)	No/Yes	Weak
Wang 2022 (FFQ)	6	Highest vs. lowest	5.00×10^{-3}	7,716	0.85–1.70	51 (0–81)	0.01	1.21 (1.06–1.37)	No/Yes	Weak
Wang 2022 (24-h dietary recall)	3	Highest vs. lowest	0.04	5,659	0.09–17.72	62 (0–89)	0.03	1.19 (1.03–1.38)	No/No	Weak
MetS (15)										
Shu 2023 (America)	6	Highest vs. lowest	0.01	6,393	0.76–1.91	87 (73–93)	0.02	1.00 (0.99–1.01)	Yes/No	Weak
Shu 2023 (Cross-sectional studies)	6	Highest vs. lowest	2.00×10^{-3}	4,112	0.73–2.95	69 (28–87)	0.05	1.20 (1.07–1.35)	No/No	Weak
Shu 2023 (Prospective cohort studies)	3	Highest vs. lowest	0.15	4,537	0.21–5.82	87 (61–95)	0.01	1.00 (0.99–1.01)	No/No	Not significant
Shu 2023 (FFQ)	5	Highest vs. lowest	0.01	3,383	0.65–2.72	88 (75–94)	0.04	1.00 (0.99–1.01)	Yes/No	Weak
Shu 2023 (24-h dietary recall)	4	Highest vs. lowest	1.00×10^{-3}	5,266	0.86–1.64	26 (0–72)	0.00	1.20 (1.07–1.35)	No/Yes	Weak
NAFLD (16)										
Henney 2023 (Asia)	3	Moderate vs. lowest	0.41	4,091	0.54–2.01	2 (0–90)	0.00	1.03 (0.95–1.11)	No/No	Not significant
Henney 2023 (Prospective cohort studies)	3	Moderate vs. lowest	0.09	8,988	0.82–1.29	0 (0–90)	0.00	1.03 (1.00–1.07)	No/No	Not significant
Henney 2023 (FFQ)	6	Moderate vs. lowest	0.03	12,065	0.99–1.08	0 (0–75)	0.00	1.03 (0.99–1.07)	No/No	Weak
Henney 2023 (NOVA group)	3	Moderate vs. lowest	0.10	8,752	0.82–1.29	0 (0–90)	0.00	1.03 (0.99–1.07)	No/No	Not significant
Henney 2023 (non-NOVA group)	4	Moderate vs. lowest	0.09	3,615	0.89–1.32	0 (0–85)	0.00	1.08 (0.98–1.19)	No/No	Not significant
Henney 2023 (Asia)	3	Highest vs. lowest	0.07	4,091	0.00–900.52	89 (72–96)	0.18	1.11 (1.03–1.21)	No/No	Not significant
Henney 2023 (Europe)	4	Highest vs. lowest	0.05	5,610	0.34–5.44	93 (84–97)	0.08	1.05 (1.02–1.09)	No/No	Not significant
Henney 2023 (Cross-sectional studies)	3	Highest vs. lowest	5.22×10^{-13}	676	0.71–3.53	0 (0–90)	0.00	1.71 (1.43–2.03)	No/Yes	Weak
Henney 2023 (Prospective cohort studies)	3	Highest vs. lowest	0.04	8,988	0.29–4.34	80 (37–94)	0.01	1.05 (1.02–1.09)	Yes/Yes	Weak
Henney 2023 (FFQ)	8	Highest vs. lowest	1.71×10^{-5}	12,675	0.87–2.12	90 (82–94)	0.03	1.05 (1.02–1.09)	Yes/No	Suggestive
Henney 2023 (NOVA group)	4	Highest vs. lowest	0.02	9,057	0.59–2.57	90 (77–96)	0.02	1.05 (1.02–1.09)	No/No	Weak

(Continued)

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TABLE 3 (Continued)

Outcomes (Reference)/ Author Year (Subgroups)	No. of studies	Comparison[a]	Random *p*-value	No. of cases	95% PI	I^2 (95% CI) (%)	Tau^2	Largest study effect (95% CI)	SSE/ESB	Evidence class[d]
Henney 2023 (non-NOVA group)	5	Highest vs. lowest	7.07×10^{-5}	3,920	0.72–3.49	79 (49–91)	0.05	1.21 (1.10–1.33)	Yes/Yes	Suggestive
Obesity (13)										
Moradi 2023 (America)	5	Highest vs. lowest	2.02×10^{-7}	18,918	0.90–2.55	65 (7–87)	0.02	1.53 (1.29–1.81)	No/Yes	Highly suggestive
Moradi 2023 (Food records)	3	Highest vs. lowest	4.28×10^{-11}	5,769	0.41–8.97	16 (0–91)	0.00	2.17 (1.64–2.70)	No/Yes	Highly suggestive
Moradi 2023 (24-h dietary recall)	3	Highest vs. lowest	5.91×10^{-9}	13,612	0.66–3.01	0 (0–90)	0.00	1.53 (1.29–1.81)	No/Yes	Highly suggestive
Moradi 2023 (America)	4	A 10% increase[b]	0.02	11,822	0.87–1.26	92 (81–96)	0.00	1.00 (0.99–1.01)	No/No	Weak
Moradi 2023 (Europe)	3	A 10% increase[b]	8.67×10^{-6}	3,178	0.79–1.62	0 (0–90)	0.00	1.18 (1.08–1.28)	No/No	Suggestive
Moradi 2023 (Cross-sectional studies)	5	A 10% increase[b]	9.92×10^{-6}	13,305	0.97–1.21	61 (0–85)	0.00	1.05 (1.03–1.07)	Yes/Yes	Suggestive
Moradi 2023 (24-h dietary recall)	3	A 10% increase[b]	3.58×10^{-9}	10,253	0.94–1.18	0 (0–90)	0.00	1.05 (1.03–1.07)	No/Yes	Highly suggestive
T2DM (17, 18)										
Delpino 2022 (America)	4	Moderate vs. lowest	0.04	11,129	0.70–1.88	54 (0–85)	0.01	1.21 (1.12–1.31)	No/No	Weak
Delpino 2022 (Asia)	3	Moderate vs. lowest	0.34	3,818	0.20–5.80	56 (0–88)	0.01	1.25 (1.06–1.47)	No/No	Not significant
Delpino 2022 (Europe)	11	Moderate vs. lowest	2.14×10^{-4}	19,977	1.04–1.18	0 (0–60)	0.00	1.08 (0.98–1.19)	No/No	Suggestive
Delpino 2022 (FFQ)	16	Moderate vs. lowest	8.60×10^{-5}	33,798	0.97–1.29	31 (0–62)	0.00	1.21 (1.12–1.31)	No/No	Suggestive
Delpino 2022 (NOVA group)	3	Moderate vs. lowest	0.06	1,301	0.56–2.17	0 (0–90)	0.00	1.13 (1.01–1.27)	No/No	Not significant
Delpino 2022 (non-NOVA group)	15	Moderate vs. lowest	9.45×10^{-5}	33,623	0.97–1.30	34 (0–65)	0.00	1.21 (1.12–1.31)	No/No	Suggestive
Chen 2023 (America)	3	Highest vs. lowest	3.93×10^{-10}	19,503	0.35–6.15	82 (45–94)	0.01	1.36 (1.26–1.46)	No/Yes	Highly suggestive
Chen 2023 (Europe)	4	Highest vs. lowest	4.00×10^{-3}	2,429	0.58–3.09	75 (30–91)	0.03	1.21 (1.04–1.20)	No/Yes	Weak
Chen 2023 (FFQ)	5	Highest vs. lowest	3.69×10^{-16}	20,806	1.10–1.99	66 (10–87)	0.01	1.36 (1.26–1.46)	No/Yes	Highly suggestive
Chen 2023 (America)	3	A 10% increase[c]	8.58×10^{-31}	19,503	0.92–1.35	54 (0–87)	0.00	1.13 (1.11–1.15)	No/Yes	Highly suggestive
Chen 2023 (Europe)	4	A 10% increase[c]	1.32×10^{-9}	2,429	1.04–1.25	0 (0–85)	0.00	1.12 (1.04–1.20)	No/Yes	Highly suggestive
Chen 2023 (FFQ)	5	A 10% increase[c]	6.94×10^{-39}	20,806	1.07–1.17	34 (0–75)	0.00	1.13 (1.11–1.15)	No/Yes	Highly suggestive

CI, confidence interval; ESB, excess significance bias; FFQ, food frequency questionnaire; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; PI, prediction interval; SSE, small-study effects; T2DM, type 2 diabetes mellitus.

[a]"Lowest" was defined as the lowest UPF consumption quantile, "moderate" was defined as the first exposure quantile, and "highest" was defined as the highest UPF consumption quantile.

[b]The unit of UPF consumption was % kcal/d.

[c]The unit of UPF consumption was % g/d.

[d]The color of the text reflected the change of credibility compared with the main analyses: black, unchanged; red, degraded; green, upgraded.

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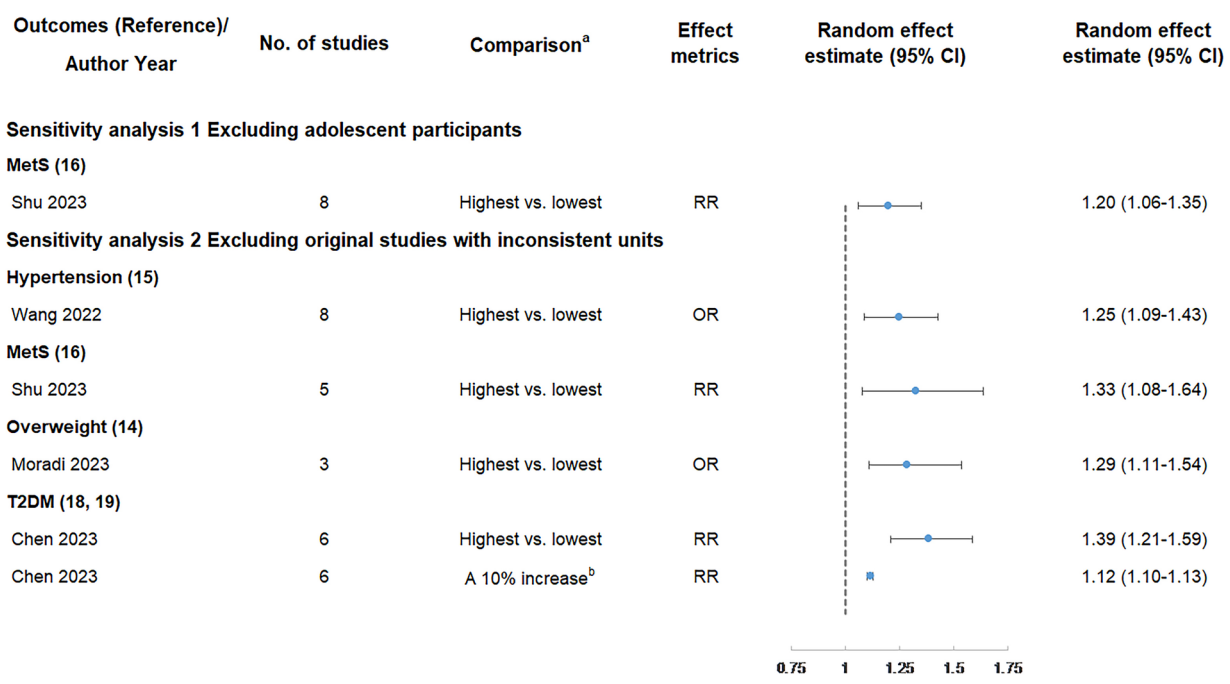

FIGURE 6
Sensitivity analyses of summary random effect estimate with 95% confidence interval from meta-analyses evaluating the association between ultra-processed food consumption and metabolic disease risk. CI, confidence interval; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; RR, risk ratio; T2DM, type 2 diabetes mellitus. [a] "lowest" was defined as the lowest UPF consumption quantile, "moderate" was defined as the first exposure quantile, and "highest" was defined as the highest UPF consumption quantile. [b] The unit of UPF consumption was % g/d.

TABLE 4 Sensitivity analyses of credible assessment of meta-analyses evaluating the association between ultra-processed food consumption and metabolic disease risk.

Outcomes (Reference)/ Author Year	No. of studies	Comparison[a]	Random *p*-value	No. of cases	95% PI	I^2 (95% CI) (%)	Tau^2	Largest study effect (95% CI)	SSE/ ESB	Evidence class[c]
Sensitivity analysis 1 Excluding adolescent participants										
MetS (15)										
Shu 2023	8	Highest vs. lowest	4×10^{-3}	8,635	0.83–1.73	83 (69–91)	0.02	1.00 (0.99–1.01)	Yes/No	Week
Sensitivity analysis 2 Excluding original studies with inconsistent units										
Hypertension (14)										
Wang 2022	8	Highest vs. lowest	1.00×10^{-3}	11,673	0.86–1.80	58 (8–81)	0.02	1.19 (1.03–1.38)	No/Yes	Weak
MetS (15)										
Shu 2023	5	Highest vs. lowest	6.00×10^{-3}	4,098	0.72–2.47	59 (0–85)	0.03	1.20 (1.07–1.35)	No/Yes	Week
Overweight (13)										
Moradi 2023	3	Highest vs. lowest	1.00×10^{-3}	16,041	0.23–7.20	68 (0–91)	0.01	1.13 (1.08–1.41)	No/No	Week
T2DM (17, 18)										
Chen 2023	6	Highest vs. lowest	1.96×10^{-6}	21,757	0.87–2.21	90 (81–95)	0.02	1.12 (1.04–1.20)	No/No	Suggestive
Chen 2023	6	A 10% increase[b]	5.25×10^{-55}	21,757	1.09–1.15	16 (0–79)	0.00	1.13 (1.11–1.15)	No/Yes	Highly suggestive

CI, confidence interval; ESB, excess significance bias; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; PI, prediction interval; SSE, small-study effects; T2DM, type 2 diabetes mellitus.
[a]"Lowest" was defined as the lowest UPF consumption quantile, "moderate" was defined as the first exposure quantile, and "highest" was defined as the highest UPF consumption quantile.
[b]The unit of UPF consumption was % g/d.
[c]The color of the text reflected the change of credibility compared with the main analyses: black, unchanged; red, degraded; green, upgraded.

basis for clinicians and public health policymakers to make decisions. In addition, our study also found the shortcomings of the current research and provided study directions for future analyses.

Nevertheless, our UR also had several limitations. First, as the UR is a method used to summarize the evidence from meta-analyses, the reliability of the UR relies heavily on the included meta-analyses and

their original studies. Potential issues in meta-analysis and original studies may affect the analysis of UR. Second, as the UR only evaluates published meta-analyses with available data, meta-analyses that lack specific data or include less than three original studies as well as individual studies that have not been summarized by meta-analyses may be ignored. Therefore, future meta-analyses should report data in detail and focus on outcomes that had not yet been evaluated by our UR, such as dyslipidemia and hyperuricemia (56, 67, 68). In addition, due to the lack of RCTs, our UR only included meta-analyses of observational studies. Therefore, residual confounding and measurement errors were unavoidable. Meanwhile, due to the nature of observational studies and factors for quality downgrade, the quality of most associations was only classified as low or very low based on the GRADE. Third, the most commonly used dietary assessment method in the included original studies was FFQ, however, there was no study specifically designed FFQ based on the NOVA classification system. In addition, several meta-analyses simultaneously included original studies that defined UPF based on the NOVA classification system and original studies that did not define UPF based on the NOVA classification system (16, 17). These might lead to the misclassification of UPF, thereby leading to biased associations. Furthermore, specific types of UPF may have different effects on the results, but this was not been considered in our study. Fourth, the majority of included original studies divided UPF consumption into tertiles, quartiles, or quintiles, rather than pre-defined cut-off values. These inconsistencies in classification might limit conclusions about how much UPF consumption was needed in the diet to trigger adverse metabolic disease. In addition, the units used to assess UPF consumption were not consistent in some meta-analyses, therefore this might be an important source of heterogeneity and limit the interpretation of the results as well as the comparability of different meta-analyses. Meanwhile, our sensitivity analyses also suggested that the unit of UPF might have an impact on the credibility of the associations. In this case, it is important for original studies to unify the units of UPF. According to our UR, an energy ratio was widely used, but recent studies suggested that a weight ratio might be more appropriate than an energy ratio to assess UPF consumption as it accounted for UPF that did not provide energy (69, 70). Nevertheless, there was a view that the weight ratio was also flawed, and no ideal weighting method exists at present (71). Hence, this issue needs to be explored in future studies. Furthermore, it is very important for original studies to reasonably determine the confounding factors that need to be adjusted. For example, body mass index which might be a potential mediator variable had been adjusted in most of the original studies. In this case, the effect size represented the effect after deducting the influence of body mass index rather than the overall effect. Therefore, we suggest that the causal directed acyclic graphs can be used in future studies to more reasonably determine the confounding factors that need to be adjusted. Last, considering that the existing studies were mainly conducted in Brazil, the United States and several European countries, the extrapolation of the results might be limited.

Conclusion

In conclusion, highly suggestive evidence indicated that UPF consumption was associated with increased risks of obesity and T2DM. The associations between UPF consumption and other metabolic disease need to be further explored. In addition, considering that there are still many limitations in the existing original studies and meta-analyses. Therefore, more well-designed original studies and meta-analysis are needed to verify our findings in the future.

Data availability statement

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

Author contributions

J-LL: Writing – original draft. Y-FW: Writing – original draft. J-NS: Writing – original draft, Data curation. Y-CS: Writing – original draft, Data curation. F-HL: Writing – original draft, Data curation. M-HS: Writing – original draft, Data curation. QC: Writing – review & editing. Q-JW: Writing – review & editing. Y-HZ: Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1306310/full#supplementary-material

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Association and dose–response relationship of plasma magnesium with metabolic syndrome in Chinese adults older than 45 years

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Purpose: Magnesium (Mg) is an essential nutrient for the maintenance of vital physiological functions. Magnesium deficiency is associated with diseases such as obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome (MetS); however, conclusions have been inconsistent, and there is a particular lack of evidence regarding this association in Chinese population older than 45 years. This study aimed to assess the association between plasma magnesium and the risk of MetS and its components, the dose–response relationship, and the threshold effect relationship in a Chinese population involving older than 45 years.

Methods: A total of 2,101 individuals were randomly selected from the China Nutrition and Health Surveillance (CNHS) (2015–2017) by considering monitoring points. We used the joint statement of the International Diabetes Federation (IDF) in 2009 to define participants with MetS. The plasma magnesium was tested by inductively coupled plasma mass spectrometry (ICP-MS). The logistic regression and restricted cubic spline (RCS) models were used to analyze the association and dose–response relationship between plasma Mg and MetS and its components.

Results: Compared with the lowest quintile (Q1) for plasma Mg, the odds ratios (ORs) and 95% confidence intervals (95% CI) for MetS, impaired fasting glucose (IFG), hypertension, and triglyceride (TG) elevation at the highest quintile (Q5) were 0.419 (0.301, 0.583), 0.303 (0.221, 0.415), 0.446 (0.322, 0.618), and 0.526 (0.384, 0.720), respectively, with all $p < 0.05$. However, in the components of decreased high-density lipoprotein cholesterol (HDL-C) and central obesity, no trend toward lowering with higher plasma magnesium was observed ($p = 0.717$, $p = 0.865$). These associations were not altered by further adjustment for potential confounding variables, including age, gender, education, nationality, area, residence, body mass index (BMI), and heart rate. The RCS analysis showed that, when plasma magnesium was lower than 0.85 mmol/L, the curve was leveled off, and then, the curve showed a decreasing trend with the increase in plasma magnesium.

Conclusion: Therefore, plasma Mg was negatively associated with MetS and its components (including IFG, hypertension, and elevated TG) in people older than 45 years. In addition, plasma Mg greater than or equal to 0.85 mmol/L, which is higher than the commonly used threshold of 0.75 mmol/L, may be protective

against MetS and its components (including elevated FPG, elevated blood pressure, and elevated TG). More prospective studies, such as randomized controlled trials, are necessary to confirm the effective impact of Mg on MetS and its components. Plasma Mg levels in the MetS population older than 45 years require attention.

KEYWORDS

magnesium, metabolic syndrome, IFG, hypertension, Chinese adults

1 Introduction

Magnesium (Mg) is one of the essential nutrients for maintaining important physiological functions of the body. It is involved in many fundamental processes, and Mg deficiency is often associated with negative health outcomes (1). Mg is involved in more than 300 enzymatic reactions and significantly influences neurotransmitter release, oxidative stress prevention, bone metabolism, heart rhythm, and vascular tone (2). Mg deficiency is frequent in obese patients, individuals with type 2 diabetes (T2DM), and those with metabolic syndrome (MetS). The symptoms of Mg deficiency are usually non-specific and may be mistaken for an inadequate intake of other nutrients. Typically, according to the Nutrition and Health Status Monitoring Report of Chinese Residents 2010–2013 (3), the prevalence of MetS has increased significantly from the age of 45 years. Although the relationship between plasma Mg and the body Mg content may not be obvious, plasma Mg remains the most widely used indicator of Mg nutrition status, and there is still a lack of reports on Mg nutrition status in Chinese people older than 45 years (4).

The MetS is characterized by a clustering of cardiovascular risk factors, including central obesity, elevated blood pressure, dyslipidemia (high triglycerides), low high-density lipoprotein cholesterol (HDL-C), and fasting hyperglycemia (5). MetS is on the rise globally and is clinically important due to its association with cardiovascular disease, T2DM, and cancer (6). Although the pathogenesis of MetS is not well defined, Mg may play a role in the development of MetS, insulin resistance, and chronic low-grade inflammation due to central obesity, which are the most widely accepted underlying reasons (7). Lifestyle risk factors such as obesity, physical inactivity, smoking, and unhealthy diet are strongly associated with the risk of developing MetS (8). Nikniaz et al. (9) study also demonstrated that a multi-mineral-based dietary pattern including Mg is associated with healthier metabolic factors in the Iranian population. However, studies exploring the relationship between plasma Mg and MetS and its components as well as whether there is a dose-effect or threshold-effect relationship in Chinese population have been relatively limited.

Guerrero-Romero et al. (10) review of clinical evidence from randomized controlled trials assesses the efficacy of Mg supplementation in improving the composition of MetS. Their results suggest that supplementation for people with hypomagnesemia can effectively treat MetS. Champagne (11) review also recognized that adequate Mg intake is beneficial in controlling blood pressure, promoting weight loss, and improving the risk of chronic diseases. While data are not entirely consistent, Sarrafzadegan et al. systematic review and meta-analysis (12) found a negative correlation between Mg intake and MetS. However, this negative correlation is highly heterogeneous and sensitive.

As mentioned above, the nutritional status of Mg may be associated with MetS. Moreover, the surveillance results in China have shown that the prevalence of MetS has begun to increase in the Chinese population older than 45 years, which is a cause for concern. Studies on MetS and plasma Mg, especially in the Chinese population older than 45 years, were scarce. Therefore, we aimed to explore the relationship between plasma Mg and MetS in Chinese adults older than 45 years, especially the dose–response relationship and threshold-effect relationship.

2 Materials and methods

2.1 Study population

The nationally representative China Nutrition and Health Monitoring (2015) (CNHS 2015) was the data source for this study. Protocols for monitoring the sample selection have been published elsewhere (13). According to the design of natural population distribution, a representative sample set of people older than 45 years was selected from approximately 180,000 monitoring populations by the stratified sampling method. The formula for calculating the sample size of this cross-sectional study is $N = deff \frac{u^2 p(1-p)}{d^2}$. According to Wang et al. result, in China, in 2013 the prevalence of diabetes in 2013 was 10.9% (14). The values of the parameters u, p, $deff$, and d were 1.96, 0.109, 1.5, and 4%, respectively. The calculation yields a minimum sample size of 350 for each stratification factor. Taking into account gender and geographic location (east, mid, and west), there were six strata, and the whole cluster was randomly sampled with a population of 2,101 participants based on the distribution of 302 monitoring sites in China. Detailed sampling strategies are available in the previous article. All subjects in this study signed informed consent before the start of the trial. This study is also in line with the Declaration of Helsinki. This study has also been approved by the ethics committee of the Institute of Nutrition and Health, Chinese Center for Disease Control and Prevention, Changping district, Beijing, China (No. 201519-A).

2.2 Criteria for inclusion and diagnosis of MetS

The criteria we use for MetS, according to the joint statement of the International Diabetes Federation (IDF) in 2009 (15), recognize

MetS when any three of the following conditions are present: (1) Elevated plasma glucose: Diagnosed with diabetes, taking hypoglycemic medications, or having fasting blood glucose (FPG) ≥ 5.6 mmol/L. (2) Elevated blood pressure (BP): systolic blood pressure (SBP) ≥130 mmHg, diastolic blood pressure (DBP) ≥85 mmHg, or under treatment with medications for essential hypertension. (3) Central obesity: waist circumference (WC) ≥ 85 cm for men and 80 cm for women. (4) Triglyceride (TG) elevation: ≥ 1.7 mmol/L or receiving treatment. (5) Reduced high-density lipoprotein cholesterol (HDL-C): < 1.0 mmol/L for men and < 1.3 mmol/L for women or receiving relevant medication.

2.3 Basic information and laboratory measurements

Each participant was asked to fast for more than 8 h, and 5 mL of venous blood was collected and centrifuged at 3000 × g for 15 min in separate tubes. Separated plasma was stored in a refrigerator at 80°C pending relevant testing. Training professionals used a nationally standardized questionnaire to collect basic information such as name, gender, ethnicity, education level, smoking, and alcohol consumption. Height, weight, WC, and BP were measured by a trained professional three times for each individual, and the measurements were averaged. Body mass index (BMI) was calculated by dividing weight (kg) by the square of height (m^2). Non-smokers and ex-smokers who have quit smoking were judged to be non-smokers, and non-drinkers and ex-drinkers who have quit drinking were judged to be non-drinkers. The residence was categorized as urban and rural. Geographic location was divided into east, central, and west according to longitude. Educational attainment was divided into elementary school and below, middle school and high school, and college and above.

The study used an automatic biochemical analyzer (Hitachi 7600, Tokyo, Japan) to measure the liver and renal function, including fasting plasma glucose (FPG), plasma lipids, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and plasma uric acid (UA). Glycosylated hemoglobin (HbA1c) was determined by a high-performance lipid chromatography (HPLC) method using Trinity Biotech Premier Hb9210 (Dublin, Ireland). In this study, plasma Mg was determined by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer, NexION 350, Waltham, MA, United States). Plasma Mg was measured with 0.5% (v/v) high-purity nitric acid diluted with plasma in a 1:20 ratio. Standard quality control (QC) assays were performed on every 20 samples. QC consisted of Seronorm (Level 2, Billingstad, Norway) and ClinChek (Level 2, Munich, Germany). The coefficient of variation for Mg was 3.56% between batches and 2.30% within batches.

2.4 Statistical analysis

Quantitative information was expressed in the form of mean ± SD, and qualitative information was expressed in the form of percentage. An analysis of variance (ANOVA) and the chi-squared tests were used to compare the demographic characteristics of individuals with different plasma Mg levels. In this study, multiple logistic regression was applied to explore the relationship between plasma Mg and MetS and its components. Different variables were added to the model to compare the odds ratios (ORs) and 95% confidence intervals (95% CI) with the results of the reference quartile (lowest quartile, Q1) under different components in the model. We also used restricted cubic spline (RCS) to test for non-linear relationships and to explore the shape of the dose–response relationship between plasma Mg and MetS and their different components. The statistical analysis was conducted using SAS version 9.4 software (SAS Institute Inc., Cary, NC, United States). All *p*-values were two-sided, and the differences were considered statistically significant with the *p*-values less than or equal to 0.05.

3 Results

3.1 Basic characteristics of 2,101 participants

The mean plasma Mg was 0.88 ± 0.10 mmol/L. Based on the plasma Mg levels, 2,101 subjects were equally divided into five parts to compare the various influences on different plasma Mg levels in Table 1. As plasma Mg levels increased, the percentage of obese, WC, TC, TG, LDL-C, FPG, HbA1c, systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate showed a tendency to decrease, and the difference was statistically significant ($p < 0.05$). Other variables including age, gender, nationality, education, region, residence, smoking or drinking status, HDL-C, and UA did not show statistically significant differences between the different plasma Mg groups ($p > 0.05$).

3.2 Multivariate logistic regression analysis of plasma Mg and MetS and their components

Table 2 summarizes the results of the multivariate logistic analysis of the relationship between plasma Mg and MetS and its components. In the context of MetS as a whole, there was a gradual and statistically significant decrease in ORs with increasing plasma Mg levels, regardless of correction for other possible confounders. Model 1 was an uncorrected model, model 2 corrected for age, gender, education, nationality, area, and residence, and model 3 further corrected for BMI and heart rate. Its components, including impaired fasting glucose (IFG), hypertension, and elevated TG, also showed a tendency to decrease with elevated plasma Mg levels. Compared with Q1 for plasma Mg, the ORs (95% CI) for MetS, IFG, hypertension, and TG elevation at Q5 were 0.419 (0.301, 0.583), 0.303 (0.221, 0.415), 0.446 (0.322, 0.618), and 0.526 (0.384, 0.720), respectively, with all the *p-values* less than 0.05. However, in the components of decreased HDL and central obesity, no trend toward lowering with higher plasma Mg was observed ($p = 0.717$, $p = 0.865$).

3.3 Dose–response relationship between plasma Mg and MetS and its components

The results of the RCS analysis are shown in Figure 1. When plasma Mg levels were greater than 0.85 mmol/L, there was a

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TABLE 1 Characteristics of 2,101 participants according to the quintiles of plasma Mg.

Variables	Quintile of plasma Mg (mmol/L)					*p*-value
	Q1(≤0.805)	Q2(≤0.852)	Q3(≤0.895)	Q4(≤0.950)	Q5(>0.950)	
N	419	419	420	424	419	
Gender						0.825
Man	51.10%	49.40%	51.00%	47.40%	50.10%	
Woman	48.90%	50.60%	49.00%	52.60%	49.90%	
Nationality						0.481
Han	86.60%	88.50%	89.00%	89.60%	90.50%	
Ethnic minorities	13.40%	11.50%	11.00%	10.40%	9.50%	
Age group						0.361
45<age ≤ 55	23.20%	29.10%	24.00%	25.90%	28.20%	
55<age ≤ 65	36.30%	32.90%	35.50%	29.00%	33.20%	
65<age ≤ 75	19.60%	19.60%	18.60%	22.40%	17.70%	
age>75	21.00%	18.40%	21.90%	22.60%	21.00%	
BMI group						0.033
<18.5	4.30%	2.90%	3.10%	5.20%	5.00%	
18.5–23.9	44.40%	45.80%	47.10%	46.00%	54.90%	
24–27.9	33.90%	34.40%	35.20%	35.60%	29.10%	
≥28	17.40%	16.90%	14.50%	13.20%	11.00%	
Education						0.088
Elementary school and blow	65.40%	57.80%	57.90%	56.60%	59.70%	
Middle school and high school	33.70%	39.60%	40.50%	41.70%	39.60%	
College and above	1.00%	2.60%	1.70%	1.70%	0.70%	
Area						0.252
East	31.50%	33.90%	36.00%	38.70%	37.00%	
Mid	32.20%	28.40%	27.10%	30.20%	30.80%	
West	36.30%	37.70%	36.90%	31.10%	32.20%	
Residence						0.357
City	60.40%	57.30%	57.10%	60.80%	63.00%	
Rural area	39.60%	42.70%	42.90%	39.20%	37.00%	
Smoke						0.158
Yes	29.60%	23.60%	26.70%	22.90%	24.30%	
No	70.40%	76.40%	73.30%	77.10%	75.70%	
Drink						0.949
Yes	32.90%	32.50%	34.00%	31.60%	33.70%	
No	67.10%	67.50%	66.00%	68.40%	66.30%	
BMI	24.25 ± 3.75	24.46 ± 3.56	24.30 ± 3.60	23.97 ± 3.55	23.60 ± 3.33	0.004
Waist (cm)	83.84 ± 11.25	84.11 ± 10.51	83.78 ± 11.26	82.78 ± 10.59	82.19 ± 10.46	0.048
TC (mmol/L)	4.96 ± 1.08	4.81 ± 0.98	4.79 ± 0.99	4.93 ± 1.01	4.69 ± 0.91	<0.001
TG (mmol/L)	1.66 ± 1.19	1.53 ± 1.26	1.45 ± 0.98	1.60 ± 1.30	1.33 ± 0.87	<0.001
LDL-C (mmol/L)	3.12 ± 0.93	3.01 ± 0.84	2.97 ± 0.87	3.05 ± 0.88	2.88 ± 0.80	0.002
HDL-C (mmol/L)	1.30 ± 0.34	1.27 ± 0.32	1.31 ± 0.33	1.30 ± 0.33	1.32 ± 0.30	0.269
SBP (mmHg)	144.35 ± 23.89	140.29 ± 22.65	141.61 ± 22.61	140.91 ± 21.34	135.61 ± 20.34	<0.001

(Continued)

TABLE 1 (Continued)

Variables	Quintile of plasma Mg (mmol/L)					*p*-value
	Q1(≤0.805)	Q2(≤0.852)	Q3(≤0.895)	Q4(≤0.950)	Q5(>0.950)	
DBP (mmHg)	81.19 ± 12.41	79.76 ± 10.93	79.67 ± 11.60	79.29 ± 11.02	77.19 ± 10.53	<0.001
FPG (mmol/L)	6.57 ± 2.80	5.84 ± 1.75	5.64 ± 1.30	5.60 ± 1.36	5.22 ± 0.88	<0.001
HbA1c (%)	5.66 ± 1.66	5.25 ± 1.06	5.17 ± 0.87	5.12 ± 0.87	4.95 ± 0.57	<0.001
UA	321.17 ± 96.36	311.98 ± 85.05	316.13 ± 83.41	307.40 ± 78.59	308.01 ± 86.17	0.108
Heart rate	80.11 ± 35.78	77.68 ± 19.42	76.17 ± 19.07	75.37 ± 10.93	76.09 ± 18.18	0.016

BMI, Body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; UA, uric acid.

TABLE 2 Multivariate logistic regression analysis of plasma Mg and MetS and its components [OR (95%CI)].

	Quintile of plasma Mg (mmol/L)					*p*-value
	Q1(≤0.805)	Q2(≤0.852)	Q3(≤0.895)	Q4(≤0.950)	Q5(>0.950)	
MetS						
Model 1	1(ref)	0.918 (0.700, 1.203)	0.753 (0.574, 0.989)	0.741 (0.565, 0.973)	0.426 (0.321, 0.566)	<0.001
Model 2	1(ref)	0.909 (0.689, 1.200)	0.732 (0.554, 0.967)	0.682 (0.516, 0.901)	0.396 (0.296, 0.529)	<0.001
Model 3	1(ref)	0.855 (0.622, 1.176)	0.678 (0.491, 0.936)	0.713 (0.517, 0.982)	0.419 (0.301, 0.583)	<0.001
IFG						
Model 1	1(ref)	0.834 (0.636, 1.094)	0.677 (0.515, 0.889)	0.556 (0.422, 0.733)	0.296 (0.220, 0.398)	<0.001
Model 2	1(ref)	0.814 (0.618, 1.072)	0.652 (0.494, 0.861)	0.518 (0.391, 0.686)	0.274 (0.203, 0.371)	<0.001
Model 3	1(ref)	0.807 (0.603, 1.078)	0.675 (0.504, 0.904)	0.564 (0.420, 0.758)	0.303 (0.221, 0.415)	<0.001
Hypertension						
Model 1	1(ref)	0.885 (0.651, 1.203)	0.799 (0.590, 1.083)	0.810 (0.598, 1.097)	0.454 (0.339, 0.609)	<0.001
Model 2	1(ref)	0.950 (0.686, 1.316)	0.796 (0.577, 1.100)	0.773 (0.560, 1.069)	0.428 (0.313, 0.585)	<0.001
Model 3	1(ref)	0.902 (0.644, 1.265)	0.756 (0.541, 1.057)	0.780 (0.558, 1.090)	0.446 (0.322, 0.618)	<0.001
Hyperlipidemia—increased TG						
Model 1	1(ref)	0.709 (0.532, 0.944)	0.798 (0.601, 1.058)	0.486 (0.360, 0.657)	0.612 (0.502, 0.745)	<0.001
Model 2	1(ref)	0.654 (0.488, 0.875)	0.690 (0.516, 0.921)	0.763 (0.573, 1.015)	0.472 (0.348, 0.639)	<0.001
Model 3	1(ref)	0.630 (0.465, 0.852)	0.683 (0.505, 0.923)	0.817 (0.607, 1.100)	0.526 (0.384, 0.720)	0.001
Dyslipidemia—decreased HDL-C						
Model 1	1(ref)	1.021 (0.771, 1.351)	0.996 (0.753, 1.319)	0.942 (0.711, 1.247)	0.758 (0.569, 1.009)	0.238
Model 2	1(ref)	1.005 (0.748, 1.350)	0.994 (0.740, 1.335)	0.878 (0.653, 1.180)	0.722 (0.534, 0.976)	0.149
Model 3	1(ref)	0.992 (0.730, 1.348)	0.992 (0.728, 1.350)	0.932 (0.685, 1.269)	0.823 (0.602, 1.126)	0.717
Central obesity						
Model 1	1(ref)	0.894 (0.681, 1.175)	0.884 (0.674, 1.161)	0.721 (0.550, 0.947)	1.328 (1.094, 1.611)	0.147
Model 2	1(ref)	0.960 (0.726, 1.269)	0.884 (0.669, 1.167)	0.835 (0.633, 1.103)	0.686 (0.519, 0.906)	0.069
Model 3	1(ref)	0.857 (0.584, 1.258)	0.819 (0.558, 1.201)	0.937 (0.640, 1.373)	0.881 (0.605, 1.283)	0.865

IFG, impaired fasting glucose; model 1 unadjusted; model 2 further added age, gender, education, nationality, area, and residence; model 3 further added BMI and heart rate.

significant protective effect against elevated MetS (a), FPG (b), increased blood pressure (c), and elevated TG (d), with no significant effect on the reduction of HDL-C (e), or the increase in waist circumference (f). MetS showed a flattening and then a decreasing trend with increasing plasma Mg, with a cutoff point of approximately 0.85 mmol/L, which is similar to the relationship between blood pressure and plasma Mg. FPG tended to decrease as plasma Mg increased and was not statistically significant until 0.85 mmol/L. There was a statistically significant tendency for blood glucose to decrease when plasma Mg was greater than 0.85 mmol/L, which is similar to the relationship between TG and plasma Mg.

4 Discussion

In this study, we analyzed the association and dose–response relationship between plasma Mg and MetS in adults older than 45 years using the nationally representative data from China. The

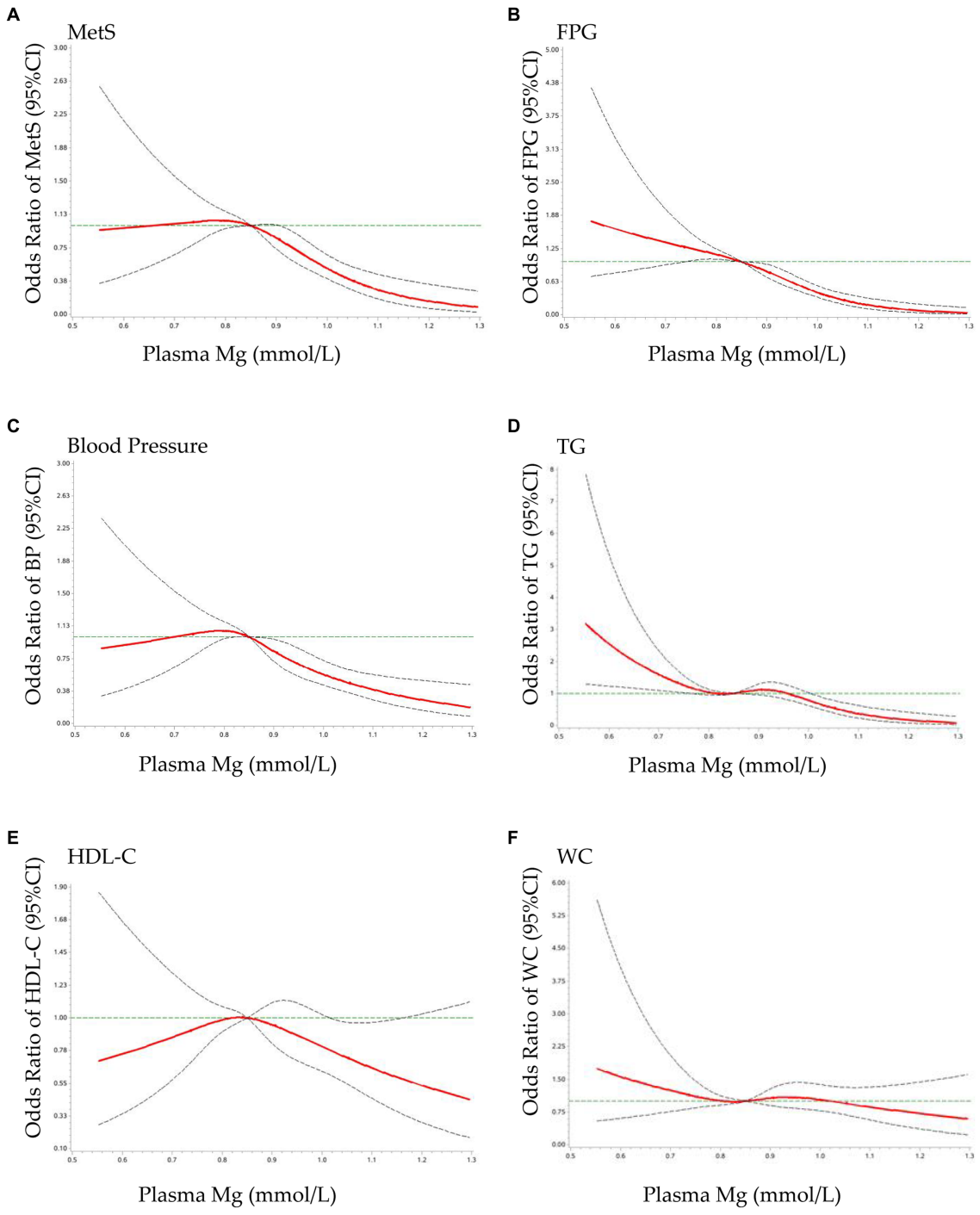

FIGURE 1
Dose–response relationship of plasma Mg with MetS and its components OR: The ORs (red solid lines) and 95% CIs (dashed lines) of plasma Mg are indicated by straight lines based on restrictive cubic splines (RCS). Gender, area, age, education, residence, SBP, DBP, BMI, WC, TG, LDL-C, and UA were adjusted. **(A)** MetS, metabolic syndrome; **(B)** FPG, fasting plasma glucose; **(C)** blood pressure; **(D)** TG, triglyceride; **(E)** HDL-C, high-density lipoprotein cholesterol; **(F)** WC, waist circumference.

results showed that, when the plasma Mg concentration was greater than 0.85 mmol/L, the OR values of the increased MetS, FPG, BP, and TG were significantly lower than 1 but had no significant effect on the decrease of HDL and WC.

The present study obtained a significantly negative correlation between overall MetS and plasma Mg levels. Huang et al. (16) recruited 1,277 adults to evaluate the relationship between metal mixture exposure and the prevalence of MetS in Chinese middle-aged and elderly populations. They also used ICP-MS and RCS methods to detect the plasma levels of 13 metals and the dose–response relationships of plasma metals with MetS, respectively. The results showed that the concentrations of Mg were lower in the MetS group

($p < 0.05$), and the adjusted OR (95% CI) in the highest quartile was 0.44 (0.35, 0.76) compared with the lowest quartile. It was demonstrated that Mg and Mo were the major contributors to the combined effect, and elevated plasma Mg levels were associated with a reduced prevalence of MetS. Except for Huang's cross-sectional research, Afitska et al. RCT study also confirms this. Afitska et al. (17) recruited 50 participants with normal plasma Mg status and randomly assigned them to 400 mg/day Mg citrate or placebo for 12 weeks. Compared with the placebo group, Mg supplementation resulted in a statistically significant reduction in SBP and DBP (145 ± 10 vs. 121 ± 5 mmHg and 85 ± 3 vs. 79 ± 3 mmHg) along with a statistically significant reduction in HbA1c (6.43 ± 0.64% vs. 6.15 ± 0.55%). Thus, this study confirms that oral Mg citrate supplementation even when given to individuals with normal plasma Mg with MetS reduces components of MetS such as HbA1c and increased blood pressure.

The negative correlation between elevated TG and plasma Mg found in this study has consistent results in other studies. Rayssiguier et al. (18) review showed that Mg deficiency leads to stress effects and increased susceptibility to stress-generated physiological damage. Inflammation occurring during experimental Mg deficiency induces hypertriglyceridemia and primary atherosclerosis changes in lipoprotein metabolism. Guerrero-Romero et al. (19) cross-sectional study enrolled 427 men and non-pregnant women aged 20–65 years and also showed that hypomagnesemia is strongly associated with hypertriglyceridemia and insulin resistance in obese individuals.

In addition to the negative correlation with MetS, this study also found a negative correlation between plasma Mg levels and FPG. Del Gobbo et al. (20) included 16 prospective studies comprising 313,041 individuals with 11,995 cardiovascular disease (CVD), 7,534 ischemic heart disease (IHD), and 2,686 fatal IHD events. The results found that each 0.2 mmol/L increase in plasma Mg was associated with a 30% reduction in the risk of CVD (RR: 0.70; 95% CI: 0.56, 0.80), a lower risk of IHD (RR: 0.83; 95% CI: 0.75, 1.05), and lethal IHD (RR: 0.61; 95% CI: 0.37, 1.00). Increasing dietary Mg by 200 mg/day was not significantly associated with cardiovascular disease (RR: 0.89; 95% CI: 0.75, 1.05) but was associated with a 22% lower risk of IHD (RR: 0.78; 95% CI: 0.67, 0.92). Dietary Mg was non-linear ($p = 0.001$) and negatively associated with lethal IHD compared to those with lower intakes, with an observed threshold of 250 mg/day (RR: 0.73; 95% CI: 0.62, 0.86). In conclusion, this meta-analysis study found that circulating and dietary Mg were inversely associated with the risk of cardiovascular disease. Zhang et al. (21) recruited 254 patients with T2DM to determine the relationship between serum Mg and the peripheral nerve function in patients with T2DM. The results showed that serum Mg levels were significantly lower in patients with diabetic peripheral neuropathy (DPN). The percentage of DPN was lower in T2DM patients with higher serum Mg levels, which suggests a correlation between plasma Mg levels and diabetic complications of DPN. Albaker et al. (22) conducted an RCT clinical trial to evaluate the effect of adding Mg chloride supplements to desalinated water consumed by T2DM patients on blood glucose, metabolic parameters, and insulin sensitivity indices. A total of 102 T2DM patients completed the trial, and the results showed that adding a dose (50 mg/L in this study) of Mg to drinking water improves long-term glycemic control indices and reduces insulin resistance in T2DM patients. Das (23) also suggests that Mg promotes the metabolism of essential fatty acids (EFAs), which, in turn, protects pancreatic b-cells, improves insulin sensitivity, and suppresses inflammation, which leads to the protective role of Mg against T2DM.

This study also found a negative correlation between blood pressure (BP) and plasma Mg. Several intervention studies also demonstrated that oral Mg supplementation lowered BP in patients with mild-to-moderate hypertension. Zhang et al. (24) meta-analysis included 34 RCTs and involved 2,028 participants to quantify the effect of oral Mg supplementation on BP. Their meta-analysis showed that a 3-month Mg supplementation of 368 mg/day significantly reduced SBP and DBP by 2.00 mmHg and 1.78 mmHg, respectively. The RCS analysis showed that the addition of 300 mg/day for 1 month was sufficient to raise serum Mg and lower blood pressure. Furthermore, serum Mg was negatively correlated with DBP and not significantly correlated with SBP ($p < 0.05$). Kass et al. (25) meta-analysis included 141 articles, which also certified that Mg supplementation can achieve a small but clinically significant reduction in BP. Witteman et al. (26) recruited 91 middle-aged and elderly women with mild-to-moderate hypertension who were randomized to 6 months of treatment with aspartate-Mg hydrochloride (20 mmol/d) or placebo with the same appearance. The results showed that the SBP and DBP of Mg-supplemented group decreased by 2.7 mmHg and 3.4 mmHg, respectively.

No dose–response relationship between plasma Mg and blood HDL-C and WC was found in this study. However, contrary to our findings, Salehidoost et al. (27) conducted a 12-week RCT, which recruited 86 prediabetic patients who were given Mg oxide 250 mg/day versus placebo, and the results showed that Mg supplementation increased HDL-C levels in patients with prediabetes. However, Mg supplementation did not improve other cardiometabolic indices, such as HOMA-IR index, TC, LDL-C, TG, UA, and C-reactive protein (CRP). Some studies are consistent with our findings, such as Simental-Mendía et al. (28) research. LUIS performed a meta-analysis of 18 RCTs to evaluate the effect of oral Mg supplementation on lipids in diabetic and non-diabetic patients. The results of this meta-analysis suggest that Mg supplementation does not have a significant effect on lipid profiles, including TC ($p = 0.671$), LDL-C ($p = 0.903$), HDL-C ($p = 0.076$), and TG concentrations ($p = 0.149$) in diabetic or non-diabetic individuals.

This study also found that BMI tended to decrease with increasing plasma Mg levels, and the difference was statistically significant. Singh et al. (29) research also found that serum Mg/insulin ratio is negatively associated with high body fat percentage. They randomly selected 850 men aged 25–64 years to determine the relationship between high body fat percentage and serum Mg levels in an urban Indian population. The results showed that Mg deficiency (OR: 1.02) was a risk factor for high body fat rate and central obesity. Hosseini et al. (30) reviewed 31 articles, which also demonstrated a negative correlation between plasma Mg and obesity. At the same time, contrary studies, such as Asbaghi et al. study, are showing that plasma Mg is not associated with obesity, OMID summarized five randomized controlled trials that showed that Mg supplementation did not affect weight (WMD: −0.01 kg), BMI (WMD: −0.07), and WC (WMD: 0.12) (31).

The study also identified 0.85 mmol/L as a critical inflection point for MetS and its three components: elevated FPG, elevated TG, and elevated blood pressure. Other research groups, including those in the US (32) and German (33), proposed that the lower cutoff value of the Mg deficiency should be 0.85 mmol/L. They all agreed that the range of reference values usually found for serum Mg (0.75–0.95 mmol/L), especially the lower reference of 0.75 mmol/L, is no longer universally

applicable. This is because subclinical Mg deficiency may still exist despite the normal performance within the current serum Mg reference. According to the current data, serum Mg values below 0.85 mmol/L are associated with increased health risks. Therefore, the lower limit of the reference range should be raised to 0.85 mmol/L. Rosanoff et al. (34) consensus also suggests an updated standardization of serum Mg reference ranges. Andrea argued that it would be more appropriate to standardize the lower reference value for serum Mg to 0.85 mmol/L (2.07 mg/dL; 1.7 mg eq/L) for proper diagnosis, awareness, and management of Mg status. Our study also supports 0.85 mmol/L as a reasonably low value for disease prevention.

5 Strengths and limitations

This study has several advantages. First, the sample was a representative sample randomly selected from nationally representative data (CNHS 2015) with a complete quality control system. Second, we used the more recognized ICP-MS method for plasma Mg with comparable experimental results. Third, we synthesized the relationship between plasma Mg and MetS and its components using an RCS approach and obtained a meaningful inflection point value of 0.85 mmol/L.

This study also has some disadvantages that need to be mentioned. First, due to the use of cross-sectional data, causal associations could not be determined. Second, as the data used were nationally monitored and included only some common physiological and biochemical factors, there may be other confounding factors that are not measured and controlled for, causing the possibility of error and bias.

6 Conclusion

In conclusion, plasma Mg was negatively associated with MetS and its components (including IFG, hypertension, and elevated TG) in people older than 45 years. In addition, plasma Mg greater than or equal to 0.85 mmol/L, which is higher than the commonly used threshold of 0.75 mmol/L, may be protective against MetS and its components (including elevated FPG, elevated blood pressure, and elevated TG). More prospective studies, such as randomized controlled trials, are necessary to confirm the effective impact of Mg on MetS and its components. The plasma Mg levels in the MetS population older than 45 years require attention.

Data availability statement

The data set presented in this article is not readily available as it belongs to China Nutrition and Health Surveillance (2015) (CNHS 2015). The database is not publicly available. Requests regarding the datasets should be directed to 243671926@qq.com.

Ethics statement

The studies involving humans were approved by the National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

JY: Writing – original draft. YC: Investigation, Writing – review & editing. HZ: Methodology, Writing – review & editing. YH: Investigation, Writing – review & editing. JL: Investigation, Writing – review & editing. RW: Investigation, Writing – review & editing. JF: Formal analysis, Writing – review & editing. LY: Funding acquisition, Writing – review & editing.

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Conflict of interest

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

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The impacts of dietary sphingomyelin supplementation on metabolic parameters of healthy adults: a systematic review and meta-analysis of randomized controlled trials

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Background: Studies have shown that sphingomyelin (SM) and its metabolites play signaling roles in the regulation of human health. Endogenous SM is involved in metabolic syndrome (MetS), while dietary SM supplementation may maintain lipid metabolism and prevent or alleviate MetS. Therefore, we hypothesized that dietary SM supplementation is beneficial for human health.

Aims: In order to examine the impacts of dietary SM on metabolic indexes in adults without MetS, we performed a meta-analysis to test our hypothesis.

Methods: A comprehensive search was performed to retrieve randomized controlled trials that were conducted between 2003 and 2023 to examine the effects of dietary SM supplementation on metabolic parameters in the Cochrane Library, PubMed, Web of Science, Embase, and ClinicalTrials.gov databases. RevMan 5.4 and Stata 14.0 software were used for meta-analysis, a sensitivity analysis, the risk of bias, and the overall quality of the resulted evidence.

Results: Eventually, 10 articles were included in this meta-analysis. Dietary SM supplementation did not affect the endline blood SM level. When compared to the control, SM supplementation reduced the blood total cholesterol level [MD: −12.97, 95% CI: (−14.57, −11.38), $p < 0.00001$], low-density lipoprotein cholesterol level [MD: −6.62, 95% CI: (−10.74, −2.49), $p = 0.002$], and diastolic blood pressure [MD: −3.31; 95% CI (−4.03, −2.58), $p < 0.00001$] in adults without MetS. The supplementation also increased high-density lipoprotein level [MD:1.41, 95% CI: (0.94, 1.88), $p < 0.00001$] and muscle fiber conduction velocity [MD: 95% 1.21 CI (0.53, 1.88), $p = 0.0005$]. The intake of SM had no effect on the blood phospholipids and lyso-phosphatidylcholine, but slightly decreased phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol concentrations. Dietary SM supplementation reduced insulin level [MD: −0.63; 95% CI (−0.96, −0.31), $p = 0.0001$] and HOMA-IR [MD: −0.23; 95% CI (−0.31, −0.16), $p < 0.00001$] without affecting blood levels of glucose and inflammatory cytokines.

Conclusion: Overall, dietary SM supplementation had a protective effect on blood lipid profiles and insulin level, but had limited impacts on other metabolic parameters in adults without MetS. More clinical trials and basic research are required.

Systematic review registration: PROSPERO, identifier CRD42023438460.

KEYWORDS

sphingomyelin, metabolic parameters, randomized controlled trials, meta-analysis, protective effect

1 Introduction

Metabolic syndrome (MetS), also known as syndrome X, is a pathological condition characterized by abdominal obesity, insulin resistance, hypertension, and hyperlipidemia (1). Approximately 20 to 25% of adults in the world are affected by MetS (2). In the meantime, the prevalence of MetS resulting from obesity in children and adolescents is also on the rise (3, 4). MetS has the potential to induce metabolic disorders in the body, which is a risk factor not only for cardiovascular diseases (CVDs), type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD) and other chronic metabolic diseases, but also for cancer and all-cause mortality (5–7). As CVDs are the primary cause of mortality on a global scale, it has become imperative to investigate the impact of MetS in order to alleviate the substantial burden of CVDs (8). In conjunction with genetic and epigenetic influences, some lifestyle and environmental variables, such as excessive caloric intake and sedentary behavior, have been recognized as significant determinants in the pathogenesis of MetS (8, 9). Identifying clinical high-risk factors for MetS and finding early intervention methods to prevent the occurrence and progression of severe complications have significant public health implications. According to World Health Organization, European group for study of insulin resistance, International Diabetes Federation, American Heart Association, and National Cholesterol Education Program Adult Treatment Panel III, the main diagnostic criteria of MetS are central obesity (elevated waist circumference), elevated blood glucose level, elevated triglyceride (TG) and total cholesterol (TC) levels, reduced high density lipoprotein cholesterol (HDL-C) level, and elevated blood pressure (8). The prevention and treatment of MetS often involve individualized therapies targeting dyslipidemia, hyperglycemia, and hypertension, with food restriction and regular exercise (10).

Blood lipid profiles such as levels of TG, cholesterol, phospholipids (PLs) and fatty acids, particularly those related to cholesterol homeostasis, are the essential indexes for controlling MetS (11). The plasma low density lipoprotein cholesterol (LDL-C) is maintained though the intestinal cholesterol absorption, endogenous cholesterol synthesis, and cholesterol clearance (12). Sphingomyelin (SM) is a sphingolipid found in animal tissues, which consists of a phosphorylcholine head group, a long-chain fatty acyl group and a sphingosine (13). It predominantly colocalizes with cholesterol on the outer leaflet of the plasma membrane, lysosomal and Golgi membranes, as well as in lipoproteins (14, 15). SM and its metabolites, including sphingoid bases, ceramide (Cer), ceramide-1-phosphate (C1P), and sphingosine-1-phosphate (S1P), play an important role in human health (10). Several investigations have shown that the endogenous SM and its metabolites are involved in the pathological processes associated with obesity, diabetes, and atherosclerosis (16–18). The amount of endogenous SM in the plasma is associated with atherosclerosis, which is considered a risk factor for CVDs (19). It has been shown that atherosclerotic LDL contains 10–50 times more Cer than the control ones (20). Patients with coronary heart disease showed higher plasma SM levels than the control subjects in case–control studies and multi-ethnic cohort studies (14, 21, 22). SM and Cer species containing palmitate exhibited the strongest positive correlation with cardiovascular and total mortality (23). Circulating C16-SM and C16-Cer, the potential biomarkers, have been associated with CVDs in people with T2DM (24, 25). However, one study shows that in adults with T2DM, SM containing a very long chain saturated fatty acid is associated with a reduced risk of CVDs (25). Nevertheless, Yeboah et al. discovered that plasma SM levels did not serve as a predictor of incidence of coronary heart disease events after a 5-year follow-up study (26).

However, the potential impact of dietary SM on the regulation of cholesterol homeostasis, lipid metabolism, and the mitigation of symptoms associated with obesity, diabetes, and atherosclerosis has not been revealed and is of significant interest. Studies on animals have shown that the dietary SM supplementation prevents atherosclerosis through the inhibition of cholesterol absorption (27–30), modification of plasma and hepatic cholesterol and TG metabolism (31, 32), formation of lipoproteins and intestinal mucosal development (33). Dietary SM derived from milk and egg yolk diminished the high-fat diet (HFD)-induced hepatic steatosis by controlling lipid absorption and metabolism, and reduced blood lipopolysaccharide via bifidogenic effects and changes in the distal gut microbiota (34, 35). Then, whether dietary SM has a positive impact on human health is still an open question. The average Western diet provides humans with 300 mg to 400 mg of sphingolipids daily, and the majority of them is SM found in meat, milk, egg products and fish (36). The extent to which dietary SM influences the endogenous sphingolipidome is still unknown. Ohlsson et al. first designed a parallel study to examine the effects of the consumption of buttermilk fortified with SM on plasma lipids over 4 weeks, and did not observe the lipid-lowering benefits of SM-enriched milk polar lipids. However, the results suggest that 700 mg/day of dietary SM mitigated the rise in TG and LDL levels associated with an increase in calorie and fat consumption (37). According to Ramprasath et al., with the exception of an increase in HDL, the addition of 1 g/day of SM to the diet has no effect on cholesterol absorption, synthesis, and the blood lipid profile in a crossover randomized controlled trial (RCT) (38). No clinically significant changes in body mass index (BMI), glucose, blood lipid profile and liver function of healthy adults after the dietary SM supplementation were observed in subsequent RCTs (39–42). Therefore, we assume that dietary SM intake is beneficial to human

body. In order to test this hypothesis, we conducted a meta-analysis using data retrieved from 10 RCT studies to obtain a more definite conclusion about the effect of dietary SM supplementation on relevant biomarkers of MetS.

2 Methods

2.1 Scheme and registration

The present systematic review was filed in PROSPERO (CRD42023438460) and carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (43). For the current study, ethical approval was not required.

2.2 Search strategy

The search strategy took into account three primary concepts, SM, PLs, and adults. For each concept, Medical Subject Headings and keywords were mapped. Subsequently, a search was conducted in the PubMed database to get a broader range of search terms, which included SM, PLs, gangliosides, sphingolipids, Cer, sphingosine, milk, egg, along with adults. RCTs on the effects of dietary SM on adults were searched in PubMed, Web of Science, Embase, ClinicalTrials.gov, and Cochrane Library databases from January 2003 to October 2023, with a language restriction to English. The search process and the related results were shown in Supplementary material 1. To ensure the comprehensiveness of reference lists, the cited references of the included relevant studies were also carefully reviewed. Four team members were divided into two groups: LMW and HYD, and CZL and CXZ. These two groups independently conducted reference screening in pairs and later compared the titles and/or abstracts of the publications that they retrieved. Subsequently, a comprehensive assessment was conducted on the whole texts of possibly eligible research in order to identify papers that satisfied the predetermined criteria for inclusion and exclusion. To ensure the integrity of the research selection process, a calibration exercise was first conducted. A consensus was achieved either via the resolution of conflicts or with the aid of another reviewer (FY).

2.3 Inclusion and exclusion criteria

The following inclusion criteria were used to ensure that the studies were appropriately selected. (1) The effects of SM supplementation on metabolic parameters of adults without MetS were studied. (2) RCTs studies should have compared SM supplementation with a placebo. (3) At least one MetS component (anthropometric parameters, blood pressure measurement, blood lipid and glycemic profile) was investigated in RCTs. (4) Only RCTs lasting at least 2 weeks were included to ensure that the interventions had enough time to have an effect. (5) The data presented in the selected studies should be continuous measures, as the mean ± standard deviation (SD) of the baseline and final value, and 95% confidence interval (CI) of the indicators must also be provided. (6) The language was limited to English. Articles that did not provide information on the amount of SM consumed and specific physical indicators were excluded. Studies were excluded if their subjects are minors, elderly people, unhealthy people with MetS, atherosclerosis, CVDs, diabetes and other chronic metabolic diseases, or acute diseases such as appendicitis, myocardial infarction, and cerebral hemorrhage. Furthermore, studies with an intervention duration of less than 2 weeks or longer than 13 weeks should be precluded, as the duration of the intervention is decisive. Finally, studies that were published as letters, meeting abstracts, meta-analyses, or reviews were excluded. If there were duplicate studies, we included only the most recent or complete one.

2.4 Data extraction

The following extracted data were collected from all eligible studies, including fundamental characteristics (the first author's name, year of publication, country, type of RCT, representation of population and sources of funding), diagnosis criteria of MetS, sample size, the age, gender, and conditions of the subjects, baseline data of participants, control and intervention (the dosage of SM and duration time), outcome measures, and records used for assessing bias risk. The outcome indicators included: (1) blood SM, (2) anthropometric parameters including BMI, body fat percentage (BF%), knee extension, muscle fiber conduction velocity (MFCV), systolic blood pressure (SBP) and diastolic blood pressure (DBP), (3) blood lipid profile including TC, TG, LDL-C, HDL-C and LDL-C/HDL-C, apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1), (4) blood PL profile including PLs, phosphatidylcholine (PC), lyso-phosphatidyl choline (Lyso-PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI), (5) blood glycemic indexes including glucose, insulin and homeostasis model assessment of insulin resistance (HOMA-IR), (6) inflammatory response factors including aspartate transaminase (AST), alanine transaminase (ALT) and C-reactive protein (CRP). To determine if any data were missing or inadequate, we attempted to contact the authors of the listed papers. Prior to validating the data extraction process, a calibration procedure was performed. Data were cross-checked, and any discrepancies were resolved through discussion with the two authors (FY and GXC).

2.5 Quality assessment of studies

Two researchers (CZL, LMW) assessed the risk of bias in the included RCTs independently and consistently using the Cochrane criteria (44). Any controversy regarding literature deviation risk was resolved through discussion and consultation with the two authors (FY and GXC). The risk of bias in the included RCTs was assessed, including random sequence generation, allocation concealment, blindness of participants and personnel, blindness of outcome assessment, incomplete outcome data, selective reporting bias, and other biases. Grading of Recommendations Assessment, Development, and Evaluation (GRADE) methodology was used to assess the overall quality of the evidence generated by the meta-analysis, taking into account high risk of bias, imprecision, indirectness, heterogeneity, and

publication bias.[1] Supplementary material 2 shows that the overall certainty of evidence was rated as "very low," "low," "moderate" or "high."

2.6 Data synthesis and statistical analysis

To extract the baseline and final values of the indicators mentioned above, unifying the index nomenclature and units in the involved articles was needed. Using the following conversion factors to unify the units of the indicators: 1 μg/100 ul = 0.01 mg/mL for SM, 1 mmo/L = 38.66 mg/dL for TC, HDL-C, and LDL-C, 1 mmol/L = 88.6 mg/dL for TG, 1 mmol/L = 18 mg/dL for glucose, 1 pmol/L = 0.167 mIU/L for insulin, and 1 mg/dL = 10 mg/L for CRP. For PLs, PC, PE, PI and Lyso-PC, 1 μg/100ul = 0.01 mg/mL. The statistical analyses to perform a meta-analysis and the funnel plots to evaluate publication bias were performed by RevMan software (version 5.4; Cochrane, London, United Kingdom) and Stata software (version 14.0; StataCorp, Texas, United States). The effects of SM supplementation were described by the mean difference (MD) with 95% CI. $p < 0.05$ was considered as statistically significant. Before stratifying the indicators by dose, statistical heterogeneity across the included studies was assessed by using the I^2 value with 50% or higher regarded as high (45). In consideration of the heterogeneity of the included studies, effects models were chosen. The random-effects model was utilized to aggregate the data if $I^2 > 50\%$ and $p < 0.05$. Otherwise, the fixed-effects model was applied. In order to elucidate the underlying factors contributing to the observed heterogeneity, a subgroup analysis was performed based on the dosage of SM supplementation. Prescribed by stringent criteria for inclusion and exclusion, and based on the average daily intake of 300 mg to 400 mg sphingolipids (36), the subjects were divided into high and low dose SM intervention groups. The high dose group had a dose greater than 400 mg/day, while the low dose group had a dose less than or equal to 400 mg/day. Sensitivity analysis was used to determine the robustness and stability of the meta-analysis results by excluding (1) studies with a high risk of bias and (2) numerical outliers. Evidence of publication bias was assessed with the Egger's test using Stata if ten or more studies were included in each meta-analysis.

3 Results

3.1 Search results

The flowchart of retrieval process is shown in Figure 1, a total of 968 articles were retrieved from the 5 databases mentioned above, no more articles were found when searching the references' list. In all, 546 articles were examined after the removal of duplicates and records marked as ineligible by automation tools. Based on the titles and abstracts, an additional 508 studies were excluded as they were non-SM ($n = 234$), non-clinical ($n = 147$), no clear dosage ($n = 27$) and inconsistent with the aim of this research ($n = 100$). In addition, there were 3 articles with incomplete retrieval reports. Eventually, 35 full text articles left, which 25 of them were excluded based on the lack of analyzable indicators ($n = 10$), infant participants ($n = 5$), patients with acute cancer ($n = 4$), and incomplete indicators ($n = 6$). Ultimately, a total of 10 RCT articles (37, 39–42, 46–50) were included in the final systematic review and meta-analysis with a total of 458 study subjects.

3.2 Quality assessment of the studies

According to Cochrane's criteria, the risk assessment of the included studies is shown in Figure 2. Overall, the quality of the articles is good. Only 1 article did not specify whether randomization grouping was used (47). For group concealment, 9 articles used the group concealment method in the experimental process, while only 1 article did not use it (47). Similarly, only 1 article in the included studies did not use the double-blind method (37). We also assessed whether there was bias in the study results. The quality of the studies was evaluated in terms of completeness of results, reporting bias, and information bias. All the studies had complete outcome data. Only 4 studies did not clearly describe the blinding of outcome assessment (39, 40, 46, 48). Five studies found that there were no reporting bias from various aspects (39, 41, 47, 49, 50), while 3 studies identified potential biases (41, 49, 50). These three articles are considered to pose other bias, such as information bias and measure bias. The GRADE evidence quality of the included studies is shown in Supplementary material 2. Among the 35 outcome indicators in the total, low and high dose subgroups of the same indicator, 20 indicators (SM, BF%, knee extension, DBP, TC-high dose subgroup, LDL-C-high dose subgroup, HDL-C-high dose subgroup, LDL-C/HDL-C, LDL-C/HDL-C low dose subgroup, LDL-C/HDL-C high dose subgroup, ApoA, ApoB, PL, PC, Lyso-PC, insulin, HOMA-IR, AST, ALT and CRP) were of high quality and 14 indicators (BMI, MFCV, SBP, TC, TC-low dose subgroup, TG, TG low dose subgroup, TG high dose subgroup, LDL-C, LDL-C low dose subgroup, HDL-C, HDL-C low dose subgroup, PE, PI and glucose) were of medium quality. Taking the results of Cochrane's criteria and GRADE evidence together, the overall quality of this meta-analysis was of high quality.

3.3 Characteristics results of the included RCTs

The basic characteristics of the included studies with RCT design are shown in Table 1. A total of 448 participants were included in this meta-analysis and were published between 2003 and 2023. Among the 10 studies included, one survey was conducted in Switzerland, four in Japan, one in Canada, two in Germany, and two in France. Four of these investigations were reported from Asian countries, the others reports were from North America and European countries. All studies were conducted in developed countries. The SM supplementation was orally administered, without other conversion forms. The experimental group was given different dosages of milk SM, while the control group was given placebo with the same durations time. Confounding factors including age and gender were controlled in 10 studies. The summary of outcomes and results obtained from the 10 included RCTs are detailed in Table 2.

1 https://gradepro.org/

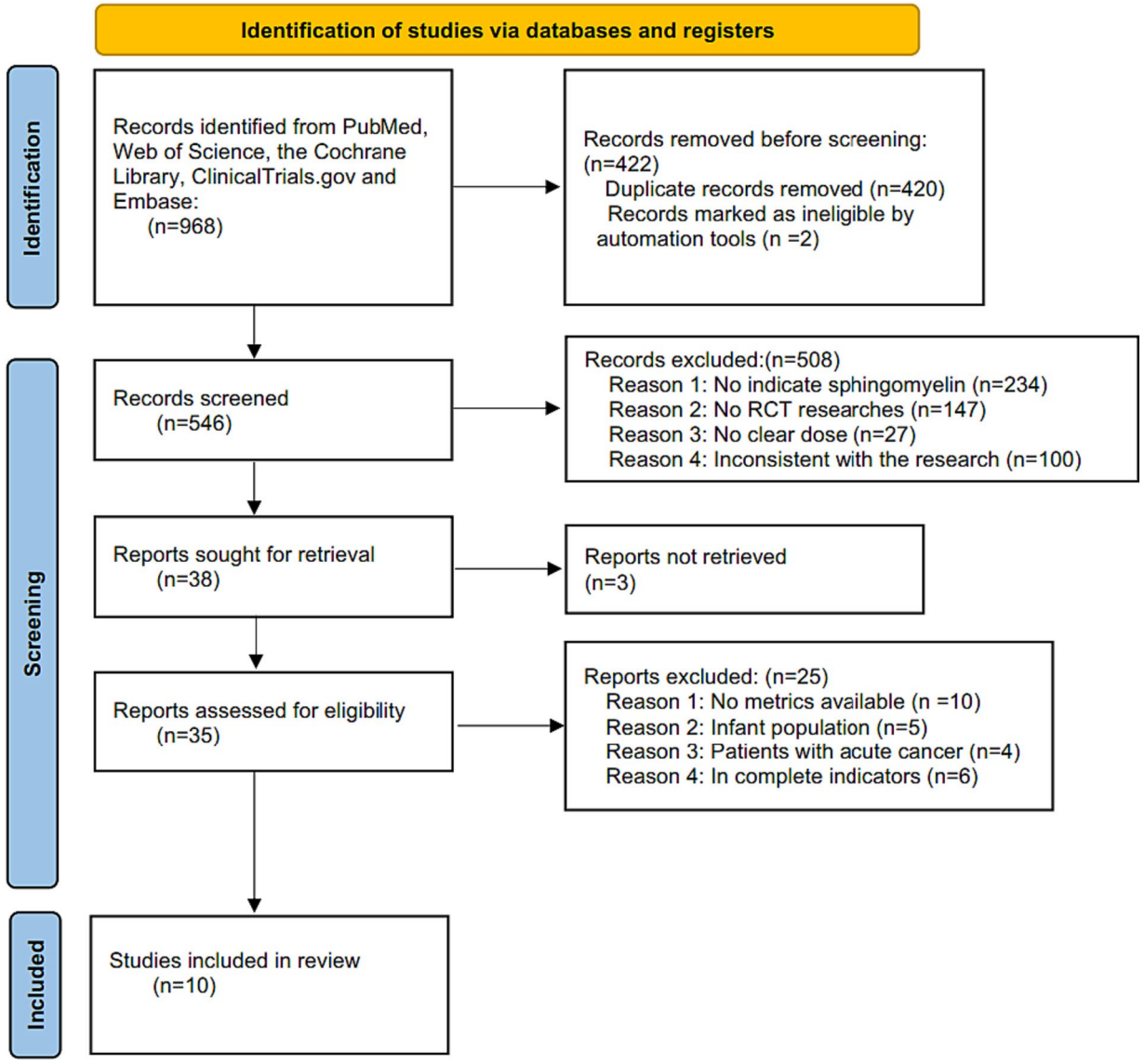


FIGURE 1
Flow chart of retrieval process included in meta-analysis.

3.4 Results of the meta-analyses

3.4.1 Impact of dietary SM on blood SM level

For the daily dietary SM intervention-based meta-analysis, Figure 3 displays the forest plot results for the MD in serum SM levels among adults without MetS from five RCTs. Squares represent the MD for RCTs, while line segments crossing the squares, which are aligned parallel to the X-axis, depict the 95% CIs. The pooled MD is represented by diamonds indicating the effect size and confidence interval of multiple studies combined. The result showed that the dietary SM intervention did not affect the blood SM level in healthy adults [MD: 0.01, 95% CI (−0.01, 0.02), $p = 0.34$].

3.4.2 Impact of dietary SM on anthropometric parameters and blood pressure

The effects of SM consumption on anthropometric parameters and blood pressure in adults without MetS are illustrated in Figures 4A–F. These parameters include BMI, BF%, knee extension, MFCV, SBP and DBP. According to the findings, SM consumption significantly decreased DBP [MD: −3.31, 95% CI (−4.03, −2.58), $p < 0.00001$], while significantly increased MFCV [MD: 1.21, 95% CI (0.53, 1.88), $p = 0.0005$] when compared with the control group. It is noteworthy to mention that the DBP remained within the range of 70–80 mmHg even after the fall. There were no significant changes in BMI, BF%, knee extension and SBP.

3.4.3 Impact of dietary SM on blood lipid profile

Overall, dietary SM had little effect on blood lipid profile in adults without MetS, including TC, TG, LDL-C, HDL-C, LDL-C/HDL-C, ApoB, and ApoA1, as shown in Figures 5A–G. The overall effects of dietary SM supplementation significantly decreased the blood TC [MD: −12.97, 95% CI: (−14.57, −11.38), $p < 0.00001$] and LDL-C [MD: −6.62, 95% CI: (−10.74, −2.49), $p = 0.002$] levels, and increased HDL-C level [MD:1.41, 95% CI: (0.94, 1.88), $p < 0.00001$]. Upon examining various concentrations, the

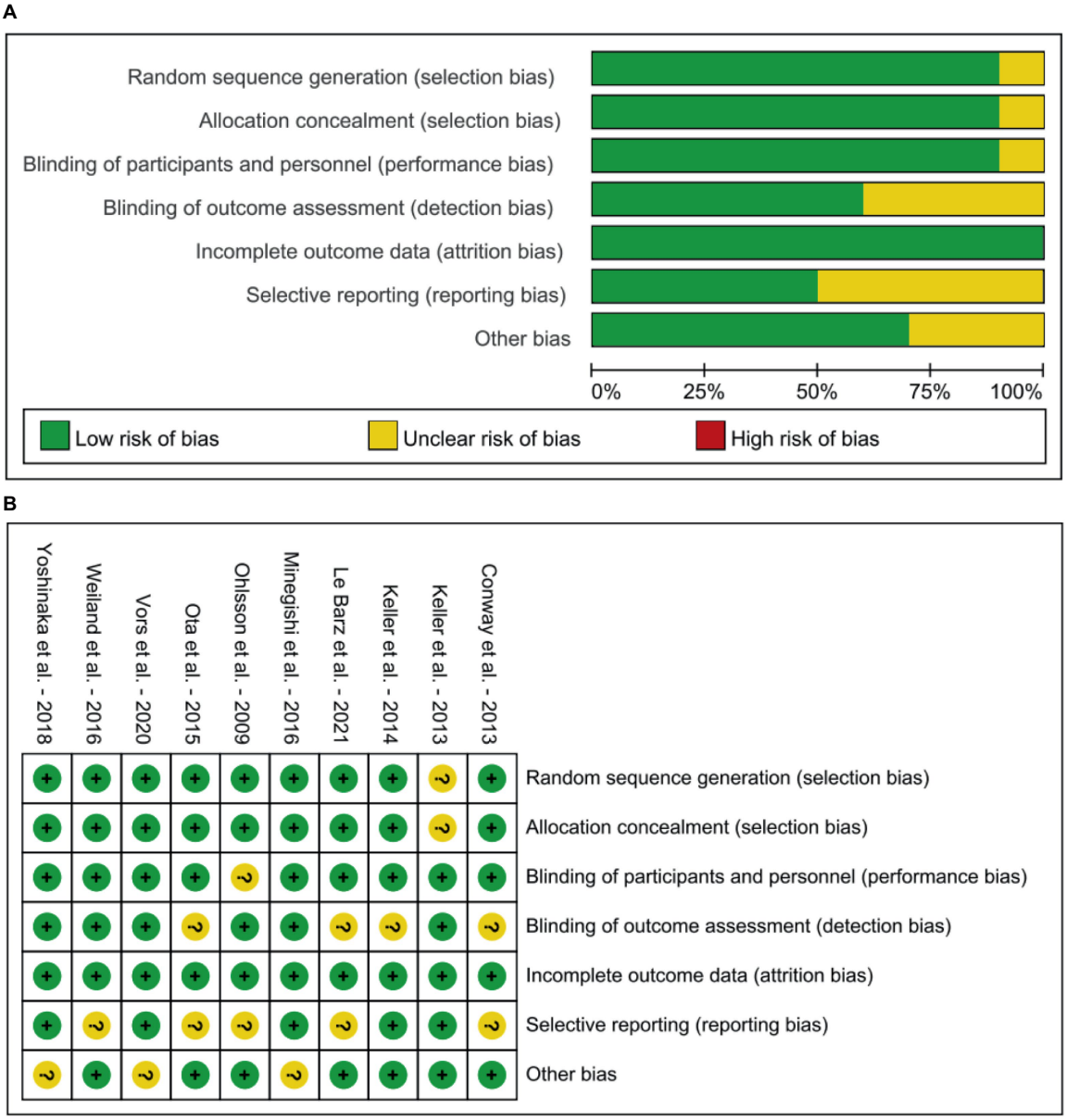


FIGURE 2
Risk of bias graph of included studies **(A)** risk of bias graph and **(B)** risk of bias summary.

meta-analysis results indicated that low dose dietary SM intervention (≤ 400 mg/day) significantly decreased in blood TC [MD: −13.37, 95% CI: (−15.01, −11.73), $p < 0.00001$] and LDL-C [MD: −7.78, 95% CI: (−13.55, −2.01), $p = 0.008$] levels, increased in HDL-C [MD:1.48, 95% CI: (1.01, 1.95), $p < 0.00001$] level, while the other blood lipid indicators were not affected. However, the high dose dietary SM treatment did not change in any parameter of the blood lipid profile.

3.4.4 Impact of dietary SM on blood phospholipid profile

The impacts of dietary SM supplementation on blood PLs, PC, lyso-PC, PE, and PI levels in adults without MetS are depicted in Figures 6A–E. Dietary SM intervention did not change the levels of PLs and Lyso-PC. The intake of SM slightly decreased the blood PC [MD: −0.05; 95% CI (−0.09, −0.01), $p = 0.008$], PE [MD: −0.03; 95% CI (−0.03, −0.03), $p < 0.0001$] and PI [MD: −0.02; 95% CI (−0.03, −0.02), $p < 0.0001$] levels.

3.4.5 Impact of dietary SM on blood glycemic indices

Figures 7A–C shows the meta-analysis results of blood glucose, insulin and HOMA-IR in adults without MetS after dietary SM supplementation. The dietary SM supplementation did not affect the glucose level. On the other hand, SM intervention significantly decreased the insulin level [MD: −0.63; 95% CI (−0.96, −0.31), $p = 0.0001$] and HOMA-IR [MD: −0.23; 95% CI (−0.31, −0.16), $p < 0.00001$].

3.4.6 Impact of dietary SM on liver function biomarkers

Forest plots for the effects of dietary SM intervention on liver function biomarkers, AST, ALT and CRP in adults without MetS are

TABLE 1 Information of included studies.

Included studies	Country	NO. (I/C)	Treatment group	Control group	Daily Dose Equivalent (mg)	Duration Time (d)	Clinical outcomes
Ohlsson et al. (37)	Sweden	29/19	SM	Placebo	700	28 days	③④⑮⑯⑰⑱⑲
Keller et al. (47)	Japan	14/0	SM	Placebo	700 or 1,400	30 days	⑦⑧⑨⑩⑪⑫⑬⑭⑮⑯⑰
Conway et al. (46)	Canada	17/17	SM	Placebo	23.62	56 days	⑬⑭⑮⑯⑰
Keller et al. (39)	German	19/20	SM	Placebo	750	42 days	①②③④⑬⑭ ⑮⑯⑰㉕
Ota et al. (40)	Japan	22/22	SM	Placebo	38.1	70 days	⑤⑥⑬⑭⑱⑳㉓㉔
Minegishi et al. (49)	Japan	11/11	SM	Placebo	80.3	70 days	④⑤⑥
Weiland et al. (42)	German	31/31	SM	Placebo	462	56 days	①②③④⑬⑭⑮⑯⑰⑱⑲⑳㉑ ㉒㉓㉔㉕
Yoshinaka et al. (50)	Japan	36/35	SM	Placebo	38.1	56 days	③④⑤
Vors et al. (41)	France	38/18	SM	Placebo	24 or 65	28 days	①②③⑬⑭⑮ ⑯⑰⑱⑲⑳㉑㉒
Barz et al. (48)	France	39/19	SM	Placebo	750 or 1,250	28 days	⑦⑧⑨⑩⑪⑫

No., numbers of participants; I, intervention group; C, control group; Outcome Indicators: ① SBP, systolic blood pressure; ② DBP, diastolic blood pressure; ③ BMI, body mass index; ④ BF%, body fat percentage; ⑤ Knee extension; ⑥ MFCV, muscle fiber conduction velocity; ⑦ SM, sphingomyelin; ⑧ PLs, total phospholipids; ⑨ Lyso-PC, Lyso-phosphatidyl cholines; ⑩ PC, phosphatidyl cholines; ⑪ PE, phosphatidyl-ethanolamine; ⑫ PI, phosphatidyl-inositol; ⑬ TC, total cholesterol; ⑭ TG, triglyceride; ⑮ LDL-C, low-density lipoprotein cholesterol; ⑯ HDL-C, high-density lipoprotein cholesterol; ⑰ LDL-C/HDL-C; ⑱ ApoA, apolipoprotein A; ⑲ ApoB, apolipoprotein B; ⑳ Glucose; ㉑ insulin; ㉒ HOMA-IR, homeostasis model assessment of insulin resistance; ㉓ AST, aspartate transaminase; ㉔ ALT, alanine transaminease; ㉕ CRP, C-reactive protein.

shown in Figures 8A–C. The dietary SM supplementation did not significantly affect serum AST, ALT, and CRP levels.

3.5 Publication bias

Publication bias was assessed using funnel plots as shown in Figure 9. Funnel plots for serum SM, BF%, knee extension, MFCV, SBP, DBP, TC, TG, LDL-C, HDL-C, LDL-C/HDL-C, ApoB, ApoA1, PL, PC, Lyso-PC, PE, PI, glucose, insulin, HOMA-IR, AST, ALT, CRP are shown, and publication bias may be present. However, due to the small number of literatures included, the results of funnel plot may not be sufficient to fully prove publication bias.

4 Discussion

SM is found in the brain, plasma, skin, as well as in dietary sources such as dairy products, meat, eggs, aquatic products and soybeans (10). Sphingolipid metabolites are regarded as bioactive lipids and are involved in numerous crucial cellular processes, including cell survival, apoptosis, metabolism, immune cell trafficking, autophagy, and mitochondrial function (51). SM and its metabolites Cer, C1P, and S1P are involved in MetS-related pathophysiological processes, including CVDs, T2DM, inflammation, non-alcoholic fatty disease, and cancer (52). Whether exogenous SM positively or negatively affects human health deserved to be evaluated thoroughly. Our systemic review and meta-analyze of the RCT data first systematically examined the effects of dietary SM on metabolic indexes in adults according to PRISMA principles. By employing explicit data extraction techniques and conducting rigorous and comprehensive searches across multiple databases, this meta-analysis is distinguished by its comprehensive indicators in adults.

Our meta-analysis of RCTs showed that dietary SM supplementation did not affect the blood PLs and SM levels, as well as anthropometric markers including BMI and BF% in adults without MetS. Meanwhile, the dietary SM supplementation increased MFCV and improved neuromuscular functions in adults. As reported by Le Barz et al., the intervention group that received 750 or 1,250 mg/d dietary SM from milk showed no significant differences in the concentrations of serum SM, Cer and PLs when compared with the control group (48). One possible reason for this is that the majority of SM and its hydrolysate Cer could not be absorbed in their original form and hence, do not contribute to the chylomicron and plasma SM pools (33, 48). An early study indicated that approximately 40% of ingested SM might be excreted intact as SM, Cer or sphingosine in the feces (53). Large amounts of dietary SM remain in the intestine unabsorbed due to absorption barriers in adults, which is likely co-excreted with cholesterol (54). Dietary SM and its metabolites have been shown to inhibit cholesterol absorption in Caco-2 cells and animal studies (28–30), and substantially alter the metabolism of TG and TC in rats (31, 32). SM possesses a greater interface area and a more effective capacity for hydrogen bond formation in comparison to other PLs, which is critical for facilitating interactions between SM and other lipids within the cell membrane (55). The crucial intermolecular hydrogen bond is established through the interaction between the hydroxyl group of cholesterol and the 2-NH of SM. Additionally, the formation of intermolecular hydrogen bonds and a network by the phosphate oxygen and 3-OH between SM molecules may impede the release of cholesterol from these lipid complexes (56). By virtue of their strong affinity for cholesterol, SM impedes the transfer of micellar lipids to the enterocytes, and inhibits luminal hydrolysis and micellar solubilization (57). A four-week milk

TABLE 2 Outcomes and results of included studies.

First Author, year	Baseline SM	Endline SM	Baseline Outcomes	Endline Outcomes	Conclusion
Ohlsson et al., 2009 (37)			TC: I: male: 187.89 ± 7.73 female: 181.70 ± 11.21 C: male: 173.58 ± 8.89 female: 172.81 ± 8.89	TC: I: male: 189.05 ± 5.41 female: 179.77 ± 5.41 C: male:177.06 ± 15.46 female: 178.59 ± 20.10	Increased daily SM consumption had no discernible effect on fasting plasma lipids or lipoprotein levels.
			TG: I: male: 101.89 ± 13.29 female: 81.51 ± 11.51 C: male: 85.06 ± 10.63 female: 59.36 ± 6.20	TG: I: male: 101.89 ± 7.08 female: 88.68 ± 27.47 C: male: 101.00 ± 35.44 female: 67.96 ± 13.29	
			LDL-C: I: male: 127.96 ± 6.96 female: 119.46 ± 10.44 C: male: 114.05 ± 8.51 female: 103.61 ± 5.80	LDL-C: I: male: 129.51 ± 11.21 female: 115.59 ± 3.87 C: male: 119.07 ± 10.82 female: 107.86 ± 15.07	
			HDL-C: I: male: 51.42 ± 2.71 female: 57.99 ± 3.48 C: male: 51.80 ± 3.09 female: 69.59 ± 7.73	HDL-C: I: male: 51.42 ± 3.48 female: 57.26 ± 4.41 C: male: 52.58 ± 5.41 female: 72.68 ± 10.05	
			LDL-C/HDL-C: I: male: 2.49 ± 2.57 female: 2.06 ± 3.00 C: male: 2.20 ± 2.75 female: 1.49 ± 0.75	LDL-C/HDL-C: I: male: 2.42 ± 3.22 female: 2.03 ± 0.88 C: male: 2.27 ± 1.93 female: 1.48 ± 1.50	
			ApoB: I: male: 0.88 ± 0.05 female: 0.82 ± 0.06 C: male: 0.79 ± 0.05 female: 0.69 ± 0.03	ApoB: I: male: 0.88 ± 0.03 female: 0.78 ± 0.01 C: male: 0.80 ± 0.08 female: 0.72 ± 0.01	
			ApoA 1: I: male:1.39 ± 0.03 female: 1.51 ± 0.07 C: male: 1.37 ± 0.05 femalee:1.60 ± 0.04	ApoA 1: I: male:1.34 ± 0.04 female: 1.48 ± 0.04 C: male: 1.38 ± 0.09 female: 1.58 ± 0.07	
Keller et al., 2013 (47)	C: 0.46 ± 0.08	700 mg/d: 0.45 ± 0.10 1,400 mg/d: 0.48 ± 0.07	TC: C: 197.93 ± 24.36	TC: I: 700 mg/d: 182.47 ± 27.44 I: 1400 mg/d: 196.01 ± 30.54	After SM consumption, there was no significant change in TC, TG, LDL-C concentration and the LDL/HDL ratio compared to baseline.
			TG: C: 93.92 ± 18.60	TG: I: 700 mg/d: 95.68 ± 26.58 I: 1400 mg/d: 98.35 ± 31.89	
			LDL-C: C: 104.76 ± 29.76	LDL-C: I: 700 mg/d: 98.19 ± 26.67 I: 1400 mg/d: 110.56 ± 32.47	

(Continued)

TABLE 2 (Continued)

First Author, year	Baseline SM	Endline SM	Baseline Outcomes	Endline Outcomes	Conclusion
			HDL-C: C: 68.04 ± 18.17	HDL-C: I: 700 mg/d: 62.62 ± 15.46 I: 1400 mg/d: 64.94 ± 16.23	
			LDL-C/HDL-C: C: 1.74 ± 1.02	LDL-C/HDL-C: I: 700 mg/d: 1.72 ± 0.86 I: 1400 mg/d: 1.86 ± 0.93	
			PLs: C: 2.23 ± 0.34	PLs: I: 700 mg/d: 2.27 ± 0.44 I: 1400 mg/d: 2.35 ± 0.36	
			Lyso-PC: C: 0.07 ± 0.04	Lyso-PC: I: 700 mg/d: 0.08 ± 0.03 I: 1400 mg/d: 0.08 ± 0.05	
			PC: C: 1.58 ± 0.24	PC: I: 700 mg/d: 1.59 ± 0.36 I: 1400 mg/d: 1.65 ± 0.30	
			PE: C: 0.09 ± 0.04	PE: I: 700 mg/d: 0.12 ± 0.05 I: 1400 mg/d: 0.11 ± 0.05	
			PI: C: 0.03 ± 0.01	PI: I: 700 mg/d: 0.03 ± 0.01 I: 1400 mg/d: 0.03 ± 0.01	
Conway et al., 2013 (46)			TC: C: 227.32 ± 35.95	TC: I: 221.91 ± 31.31 C: 228.87 ± 36.34	The consumption of dietary SM may potentially lead to. decreased the serum TC, LDL-C and TG levels in both men and women, primarily by impeding the absorption of cholesterol into the intestines
			TG: C: 107.21 ± 52.27	TG: I: 102.78 ± 41.64 C: 115.18 ± 53.16	
			LDL-C: C: 144.98 ± 26.68	LDL-C: I: 138.79 ± 24.74 C: 143.43 ± 27.45	
			HDL-C: C: 60.70 ± 18.56	HDL-C: I: 62.63 ± 16.62 C: 62.63 ± 18.17	
			LDL-C/HDL-C: C: 2.38 ± 1.43	LDL-C/HDL-C: I: 2.21 ± 1.49 C: 2.29 ± 1.51	
Keller et al., 2014 (39)	I: 0.63 ± 0.13 C: 0.61 ± 0.11	I:0.58 ± 0.08 C:0.57 ± 0.10	TC: I: 172.04 ± 21.65 C: 170.10 ± 22.43	TC: I: 172.81 ± 24.74 C: 178.60 ± 23.19	There was no significant difference in plasma SM and lipid profile after the dietary SM consumption.
			TG: I: 93.91 ± 44.3 C: 94.80 ± 43.41	TG: I: 93.03 ± 37.21 C: 92.14 ± 37.21	
			LDL-C: I: 92.78 ± 19.71 C: 90.46 ± 20.10	LDL-C: I: 92.79 ± 17.78 C: 95.49 ± 20.49	
			HDL-C: I: 59.15 ± 14.69 C: 57.99 ± 15.07	HDL-C: I: 59.53 ± 14.30 C: 61.86 ± 14.30	

(Continued)

TABLE 2 (Continued)

First Author, year	Baseline SM	Endline SM	Baseline Outcomes	Endline Outcomes	Conclusion
			LDL-C/HDL-C: I: 1.56 ± 1.34 C: 1.55 ± 1.33	LDL-C/HDL-C: I: 1.56 ± 1.24 C: 1.54 ± 1.43	
			PLs: I: 2.13 ± 0.36 C: 2.12 ± 0.33	PLs: I: 2.09 ± 0.29 C: 2.09 ± 0.35	
			Lyso-PC: I: 0.074 ± 0.017 C: 0.0069 ± 0.015	Lyso-PC: I: 0.076 ± 0.013 C: 0.072 ± 0.015	
			PC: I: 1.28 ± 0.23 C: 1.28 ± 0.21	PC: I: 1.27 ± 0.18 C: 1.28 ± 0.22	
			PE: I: 0.08 ± 0.03 C: 0.09 ± 0.02	PE: I: 0.10 ± 0.02 C: 0.10 ± 0.02	
			PI: I: 0.09 ± 0.02 C: 0.08 ± 0.02	PI: I: 0.09 ± 0.01 C: 0.08 ± 0.02	
			CRP: I: 1.97 ± 2.65 C: 3.26 ± 6.90	CRP: I: 1.50 ± 1.79 C: 1.43 ± 1.48	
Ota et al., 2015 (40)			Knee extension: I: 34.1 ± 3.2 C: 32.2 ± 3.1	Knee extension: I: 37.1 ± 3.8 C: 33.8 ± 3.2	There were no clinically significant changes in BMI, glucose, blood pressure, serum profile and muscle strength after the dietary SM-rich globular membrane protein consumption.
			MFCV: I: 5.77 ± 0.32 C: 5.91 ± 0.39	MFCV: I: 5.58 ± 0.39 C: 4.17 ± 0.45	
			TC: I: 219 ± 9.8 C: 206 ± 9.2	TC: I: 212 ± 7.5 C: 211 ± 9.9	
			TG: I: 104 ± 9.4 C: 132 ± 12.7	TG: I: 95 ± 8.6 C: 109 ± 10.9	
			Glucose: I: 90.4 ± 2.13 C: 93.3 ± 2.01	Glucose: I: 88.6 ± 1.96 C: 90.4 ± 2.22	
			AST: I: 22.1 ± 0.98 C: 24.5 ± 1.82	AST: I: 22.7 ± 1.06 C: 24.1 ± 1.52	
			ALT: I: 20.1 ± 2.02 C: 25.1 ± 4.33	ALT: I: 19.7 ± 2.38 C: 22.9 ± 2.79	

(Continued)

TABLE 2 (Continued)

First Author, year	Baseline SM	Endline SM	Baseline Outcomes	Endline Outcomes	Conclusion
Minegishi et al., 2016 (49)			BF%: I: 25.2 ± 1.8 C: 25.8 ± 2.1	BF%: I: 25.5 ± 1.6 C: 26.4 ± 2.22	There were no overall changes in BF%, while knee extension strength and MFCV markedly increased after the dietary SM-rich globular membrane protein consumption.
			Knee extension: I: 27.9 ± 2.5 C: 28.2 ± 2.0	Knee extension: I: 32.3 ± 2.6 C: 29.6 ± 2.1	
			MFCV: I: 4.72 ± 0.28 C: 4.65 ± 0.28	MFCV: I: 5.55 ± 0.27 C: 4.62 ± 0.32	
Weiland et al., 2016 (42)			BMI: I: 30.7 ± 1.9 C: 30.9 ± 2.9	BMI: I: 30.4 ± 2.0 C: 30.8 ± 2.8	Consumption of SM-rich milk did not affect plasma lipid parameters.
			BF%: I: 31.2 ± 2.6 C: 30.8 ± 2.4	BF%: I: 30.9 ± 2.8 C: 30.3 ± 2.6	
			SBP: I: 139.6 ± 21.6 C: 131.7 ± 17.2	SBP: I: 135.3 ± 19.1 C: 131.7 ± 16.4	
			DBP: I: 86.4 ± 10.6 C: 82.7 ± 8.9	DBP: I: 84.0 ± 11.0 C: 82.6 ± 8.9	
			TC: I: 233.12 ± 31.70 C: 220.36 ± 36.34	TC: I: 228.10 ± 40.21 C: 221.14 ± 39.82	
			TG: I: 129.36 ± 70.88 C: 124.93 ± 43.41	TG: I: 132.90 ± 56.70 C: 150.62 ± 94.80	
			LDL-C: I: 146.13 ± 31.70 C: 138.40 ± 29.38	LDL-C: I: 148.45 ± 34.41 C: 142.66 ± 31.31	
			HDL-C: I: 55.28 ± 12.76 C: 51.03 ± 13.14	HDL-C: I: 54.90 ± 14.30 C: 50.26 ± 10.82	
			LDL-C/HDL-C: I: 2.64 ± 2.48 C: 2.71 ± 2.23	LDL-C/HDL-C: I: 2.70 ± 2.40 C: 2.84 ± 2.89	
			ApoB: I: 1.28 ± 0.27 C: 1.25 ± 0.23	ApoB: I: 1.27 ± 0.25 C: 1.26 ± 0.22	
			ApoA1: I: 1.64 ± 0.21 C: 1.56 ± 0.24	ApoA1: I: 1.61 ± 0.23 C: 1.56 ± 0.22	
			Glucose: I: 98.82 ± 11.88 C: 99.18 ± 8.46	Glucose: I: 97.56 ± 11.34 C: 96.30 ± 7.74	
			Insulin: I: 14.87 ± 8.78 C: 17.40 ± 8.55	Insulin: I: 15.45 ± 9.07 C: 17.97 ± 16.48	
			HOMA-IR: I: 3.20 ± 2.06 C: 3.74 ± 2.03	HOMA-IR: I: 3.30 ± 2.16 C: 3.74 ± 3.72	

(Continued)

TABLE 2 (Continued)

First Author, year	Baseline SM	Endline SM	Baseline Outcomes	Endline Outcomes	Conclusion
Yoshinaka et al., 2018 (50)			ALT: I: 17.5 ± 9.9 C: 18.0 ± 8.6	ALT: I: 17.3 ± 7.8 C: 20.9 ± 10.5	Ingestion of milk fat globular membrane containing SM had no significant changes on BMI, BF% and knee extension.
			AST: I: 25.0 ± 7.1 C: 26.2 ± 7.3	AST: I: 23.5 ± 6.7 C: 25.1 ± 6.2	
			CRP: I: 2.53 ± 3.03 C: 2.19 ± 2.32	CRP: I: 1.75 ± 1.47 C: 1.42 ± 1.30	
			BMI: I: 21.6 ± 3.1 C: 21.8 ± 2.5	BMI: I: 21.5 ± 3.0 C: 21.7 ± 2.4	
			BF%: I: 26.8 ± 7.5 C: 26.7 ± 6.9	BF%: I: 26.3 ± 7.4 C: 27.4 ± 7.5	
			Knee extension: I: 28.6 ± 9.2 C: 28.8 ± 9.4	Knee extension: I: 27.1 ± 7.8 C: 29.0 ± 8.3	
Vors et al., 2020 (41)			BMI: I: 24 mg/d: 29.05 ± 0.58 65 mg/d: 29.18 ± 0.56 C: 30.22 ± 0.76	BMI: I: 24 mg/d: 29.03 ± 0.57 65 mg/d: 29.06 ± 0.66 C: 30.23 ± 0.83	Consuming SM-rich milk can lower cardiovascular lipid levels, and improve heart health by decreasing several lipid cardiovascular markers.
			SBP: I: 24 mg/d: 124.68 ± 4.17 65 mg/d: 124.47 ± 3.94 C: 124.32 ± 2.74	SBP: I: 24 mg/d: 123.05 ± 6.17 65 mg/d: 120.26 ± 6.82 C:119.53 ± 5.02	
			DBP: I: 24 mg/d: 76.21 ± 2.07 65 mg/d: 75.84 ± 1.94 C: 71.68 ± 2.28	DBP: I: 24 mg/d: 74.37 ± 3.94 65 mg/d: 72.95 ± 3.18 C: 72.52 ± 3.97	
			TC: I: 24 mg/d: 215.72 ± 7.73 65 mg/d: 219.58 ± 9.28 C: 216.11 ± 6.57	TC: I: 24 mg/d: 207.60 ± 11.59 65 mg/d: 204.12 ± 12.75 C: 214.56 ± 10.44	
			TG: I: 24 mg/d: 109.86 ± 9.74 65 mg/d: 130.24 ± 11.52 C: 109.86 ± 7.08	TG: I: 24 mg/d: 108.98 ± 17.72 65 mg/d: 156.82 ± 7.08 C: 119.61 ± 12.40	
			LDL-C: I: 24 mg/d: 136.86 ± 6.57 65 mg/d: 140.33 ± 7.34 C: 143.04 ± 5.80	LDL-C: I: 24 mg/d: 130.28 ± 9.66 65 mg/d: 127.19 ± 10.44 C: 141.49 ± 8.89	
			HDL-C: I: 24 mg/d: 47.55 ± 1.93 65 mg/d: 45.23 ± 2.32 C: 44.85 ± 1.93	HDL-C: I: 24 mg/d: 46.01 ± 3.09 65 mg/d: 47.55 ± 3.09 C: 44.07 ± 3.09	
			LDL-C/HDL-C: I: 24 mg/d: 2.88 ± 3.40 65 mg/d: 3.10 ± 3.16 C: 3.18 ± 3.00	LDL-C/HDL-C: I: 24 mg/d: 2.83 ± 3.12 65 mg/d: 2.67 ± 3.38 C: 3.21 ± 2.88	

(Continued)

TABLE 2 (Continued)

First Author, year	Baseline SM	Endline SM	Baseline Outcomes	Endline Outcomes	Conclusion
			ApoB: I: 24 mg/d: 1.01 ± 0.05 65 mg/d: 1.02 ± 0.06 C: 24 mg/d 1.03 ± 0.05	ApoB: I: 24 mg/d: 0.07 ± 0.07 65 mg/d: 0.03 ± 0.09 C: 1.04 ± 0.07	
			ApoA1: I: 24 mg/d: 1.17 ± 0.02 65 mg/d: 1.19 ± 0.03 C: 1.16 ± 0.02	ApoA1: I: 24 mg/d: 1.17 ± 0.04 65 mg/d: 1.18 ± 0.05 C: 1.15 ± 0.04	
			Glucose: I: 24 mg/d: 91.98 ± 1.98 65 mg/d: 92.70 ± 1.8 C: 94.14 ± 1.8	Glucose: I: 24 mg/d: 92.34 ± 3.06 65 mg/d: 90.72 ± 2.88 C: 93.60 ± 3.06	
			Insulin: I: 24 mg/d: 7.11 ± 0.72 65 mg/d: 8.07 ± 1.36 C:7.29 ± 0.99	Insulin: I: 24 mg/d: 7.05 ± 1.33 65 mg/d: 7.66 ± 2.26 C: 7.72 ± 1.62	
			HOMA-IR: I: 24 mg/d: 1.67 ± 0.19 65 mg/d: 1.82 ± 0.28 C: mg/d: 1.74 ± 0.26	HOMA-IR: I: 24 mg/d: 1.64 ± 0.33 65 mg/d: 1.72 ± 0.46 C: 1.91 ± 0.43	
Barz et al., 2021 (48)	I: 750 mg/d: 0.43 ± 0.015 1,250 mg/d: 0.422 ± 0.018 C: 0.436 ± 0.017	I: 750 mg/d: 0.413 ± 0.027 1,250 mg/d: 0.395 ± 0.027 C: 0.407 ± 0.029	PLs: I: 750 mg/d: 2.20 ± 0.05	PLs: I: 750 mg/d: 2.14 ± 0.10	Supplementation with SM-rich milk was associated with a reduction in atherogenic SM and Cer species, which improved cardiovascular risk markers.
			1,250 mg/d: 2.30 ± 0.06 C: 2.28 ± 0.09	1,250 mg/d: 2.12 ± 0.10 C: 2.21 ± 0.14	
			Lyso-PC: I: 750 mg/d: 0.0899 ± 0.047 1,250 mg/d: 0.0938 ± 0.048 C: 0.0957 ± 0.063	Lyso-PC: I: 750 mg/d: 0.089 ± 0.080 1,250 mg/d: 0.086 ± 0.082 C: 0.0925 ± 0.010	
			PC: I: 750 mg/d: 1.44 ± 0.04 1,250 mg/d: 1.54 ± 0.04 C: 1.50 ± 0.05	PC: I: 750 mg/d: 1.40 ± 0.07 1,250 mg/d: 1.42 ± 0.07 C: 1.48 ± 0.09	
			PE: I: 750 mg/d: 0.052 ± 0.0031 1,250 mg/d: 0.054 ± 0.0028 C: 0.049 ± 0.0036	PE: I: 750 mg/d: 0.048 ± 0.0059 1,250 mg/d: 0.052 ± 0.0047 C: 0.048 ± 0.0052	
			PI: I: 750 mg/d: 0.195 ± 0.016 1,250 mg/d: 0.188 ± 0.016 C: 0.192 ± 0.019	PI: I: 750 mg/d: 0.18 ± 0.029 1,250 mg/d: 0. 17 ± 0.032 C: 0.181 ± 0.031	

No, number of participants; I, intervention group; C, control group; Outcome Indicators: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; BF%, body fat percentage; Knee extension; MFCV, muscle fiber conduction velocity; SM, sphingomyelin; PLs, phospholipids; Lyso-PC, Lyso-phosphatidyl cholines; PC, phosphatidyl cholines; PE, phosphatidyl-ethanolamine; PI, phosphatidyl-inositol; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C/HDL-C; Glucose; Insulin; HOMA-IR, homeostasis model assessment of insulin resistance; AST, aspartate transaminase; ALT, alanine transaminease; CRP, C-reactive protein.

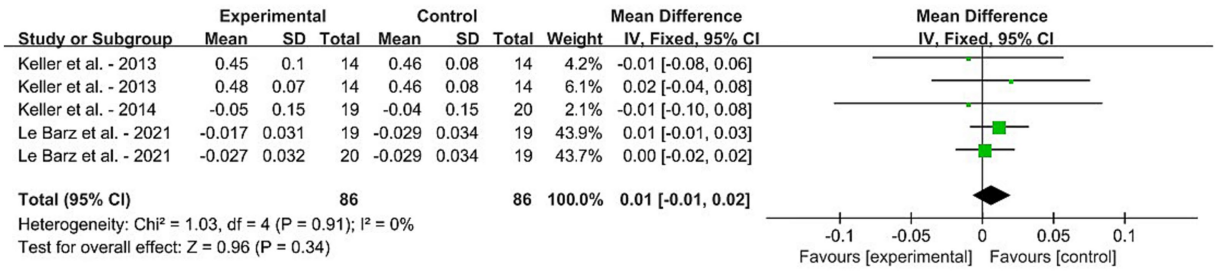


FIGURE 3
Forest plots for the effects of sphingomyelin (SM) on blood SM in adults without metabolic syndrome (MetS). The horizontal bar represents the 95% confidence interval (CI). The magnitude of the rectangle at the center of the horizontal bar is proportional to the weight of the provided study. As indicated by the diamond at the bottom, the pooled mean difference (MD) is present. **(A)** BMI, **(B)** BF%, **(C)** knee extension, **(D)** MFCV, **(E)** SBP, and **(F)** DBP.

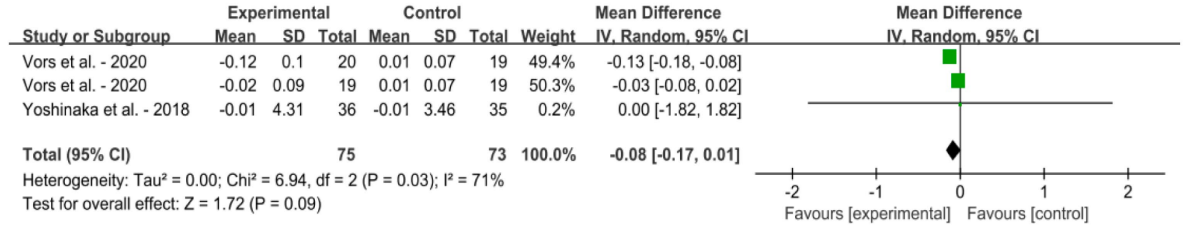
SM intervention resulted in a significant increase in fecal SM and Cer in comparison to the control group (48). It is unknown whether this is the result of decreased SM synthesis in enterocytes or liver following dietary SM intervention. Alternatively, the size of the body's total SM pool is large enough that the absorbed amount of dietary SM is an insignificant fraction of it. Whether any of these hypotheses is true remains to be determined.

The basic and clinical researchers agree that milk SM provides protection against dysfunctional lipid metabolism, intestinal dysbiosis, and inflammation (55). It is noteworthy that the inhibitory effect of SM derived from milk on cholesterol absorption surpasses that of SM derived from eggs. This disparity may be attributed to the presence of longer chain and greater degree of saturation of the fatty acyl group in the milk SM than that in the egg ones (55, 58). Milk SM has substantial proportion of very-long chain fatty acids (C22:0-C24:0) and diverse sphingoid bases (d16:0 to d19:0) (34, 57). Dietary SM is hydrolyzed in the intestinal mucosa by alkaline sphingomyelinase to Cer, and phosphorylcholine. Cer is subsequently hydrolyzed by neutral ceramidase and bile salt-stimulated lipase into sphingosine and fatty acids, which are then absorbed into the enterocytes. The majority of sphingosine is either dephosphorylated to generate S1P by sphingosine kinase, or to a lesser degree, form new Cer and SM (10). Multiple studies have demonstrated that distinct cellular functions can be carried out by various Cer species, contingent upon the fatty acyl chains that they contain (59). During the progression of T2DM and NAFLD, C16-Cer and C18-Cer species are thought to be generated *de novo* and deleterious in the liver and muscle tissues (60–63). On the contrary, Cer denoted as C22:0-, C24:1, and C24:0- are more widely regarded as having beneficial and protective effects on cells, particularly in the liver (62, 64–66). Research has demonstrated that mice with a deficiency in the Cer synthase 2, which is accountable for producing C24:1, C22:0-, and C24:0-Cer, were more susceptible to diet-induced hepatic steatohepatitis and developed insulin resistance (62). Le Barz et al. discovered that the atherogenic C16-SM, C18-SM, and C24:1-Cer species in serum were significantly reduced by the milk SM intervention, which leads to significant increases in the proportions of C22:0-SM, C24:0-SM, C22:0-Cer, and C24:0-Cer species (48). This might be attributed to the beneficial effects of milk SM.

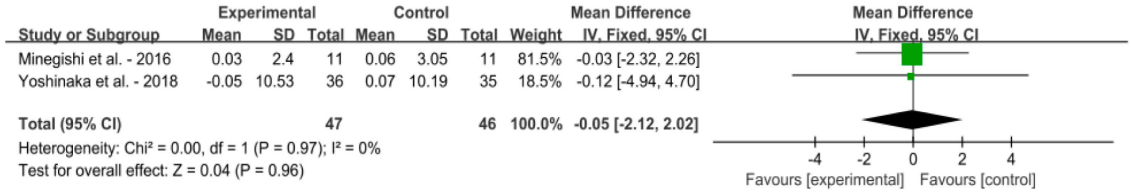
According to Ramprasath et al., with the exception of an elevated HDL-C concentration, the blood lipid profile in humans remains unaffected by the consumption of 1 g/day of dietary SM. Additionally, cholesterol absorption, synthesis, and intraluminal solubilization remain unaffected when compared to the control group (38). However, our meta-analysis results showed that a low dose of dietary SM intervention ($\leq$ 400 mg/day) significantly reduced the blood TC and LDL-C levels, and increased HDL-C level. The levels of TG, LDL-C/HDL-C, ApoB, and ApoA1 were not changed. Interestingly, the high dose dietary SM treatment did not change the lipid profile. Animal experiments have shown that dietary SM supplementation has no effect on atherosclerosis or circulating SM levels in HFD apoE$^{-/-}$ mice, but it inhibits atherosclerosis in chow-fed apoE$^{-/-}$ mice (27). It is possible that the effects of dietary SM derived from milk on the lipid profile depends on other components in the diet. Alternatively, there is an interaction between the dietary SM and dietary energy content (37). The lack of response in the high dietary SM group could be due to a mechanism that induces excretion of SM in the feces or blocks absorptions of sphingosine when more SM is present in the gastrointestine tract. Whether this mechanism exists remains to be tested.

Circulating Cer has been linked to the development of atherosclerosis and CVDs over the past decade (67). The conversion of SM in the blood LDL-C particles to Cer by sphingomyelinase facilitates Cer aggregation. The increases in SM levels were associated with an early onset of atherosclerosis (68–70). Cer may undergo phosphorylation to form C1P, which promotes inflammation via oxidative stress or tumor necrosis factor-α and causes apoptosis and non-alcoholic steatohepatitis (71, 72). Additionally, hydrolysis of Cer can produce sphingosine, which can be phosphorylated to produce S1P (10). Conversely, S1P has been thought to prevent apoptosis and linked to cellular proliferation and growth (73). The elevated concentration of S1P in the bloodstream serves vital homeostatic roles in preserving the integrity of blood vessels, while the S1P gradient is indispensable for the movement of immune cells (51). Research has indicated that impairments of fatty acid oxidation can be attributed to disturbances in sphingolipid metabolism, where Cer and S1P may play a role (74). The dogma is that accumulation of Cer is detrimental, whereas accumulation of S1P is advantageous (74, 75). So far, there is no discernible impact of dietary SM on blood SM and Cer levels. Therefore, more clinical studies are required to monitor its effects on the endogenous S1P and sphingolipidome in the bloodstream and along the gastrointestinal tract in humans.

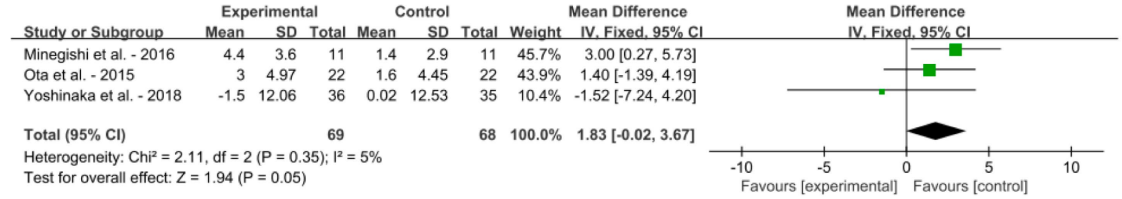
A BMI



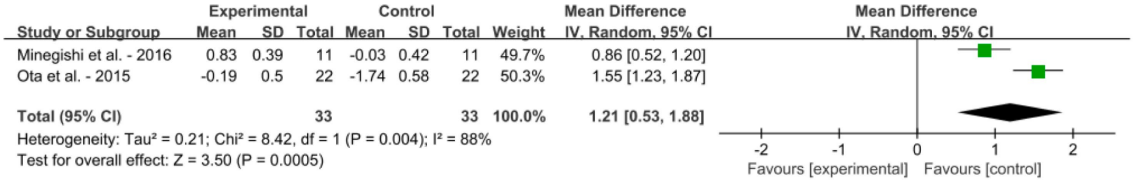
B BF%



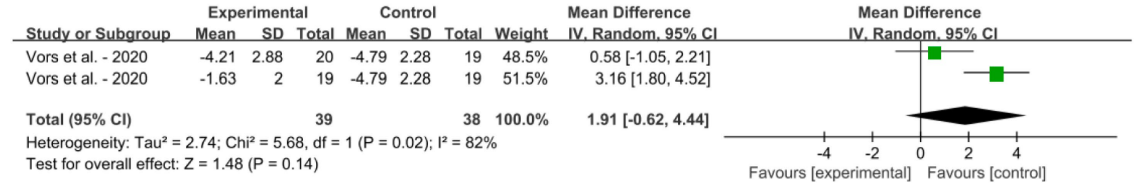
C Knee extension



D MFCV



E SBP



F DBP

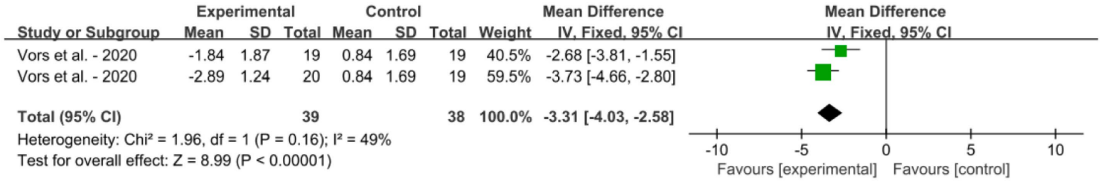


FIGURE 4
Forest plots for the effects of sphingomyelin (SM) on anthropometric parameters and blood pressure in adults without metabolic syndrome (MetS). **(A)** BMI, body mass index; **(B)** BF%, body fat percentage; **(C)** Knee extension; **(D)** MFCV, muscle fiber conduction velocity; **(E)** SBP, systolic blood pressure; **(F)** DBP, diastolic blood pressure. The horizontal bar represents the 95% confidence interval (CI). The magnitude of the rectangle at the center of the horizontal bar is proportional to the weight of the provided study. As indicated by the diamond at the bottom, the pooled mean difference (MD) is present.

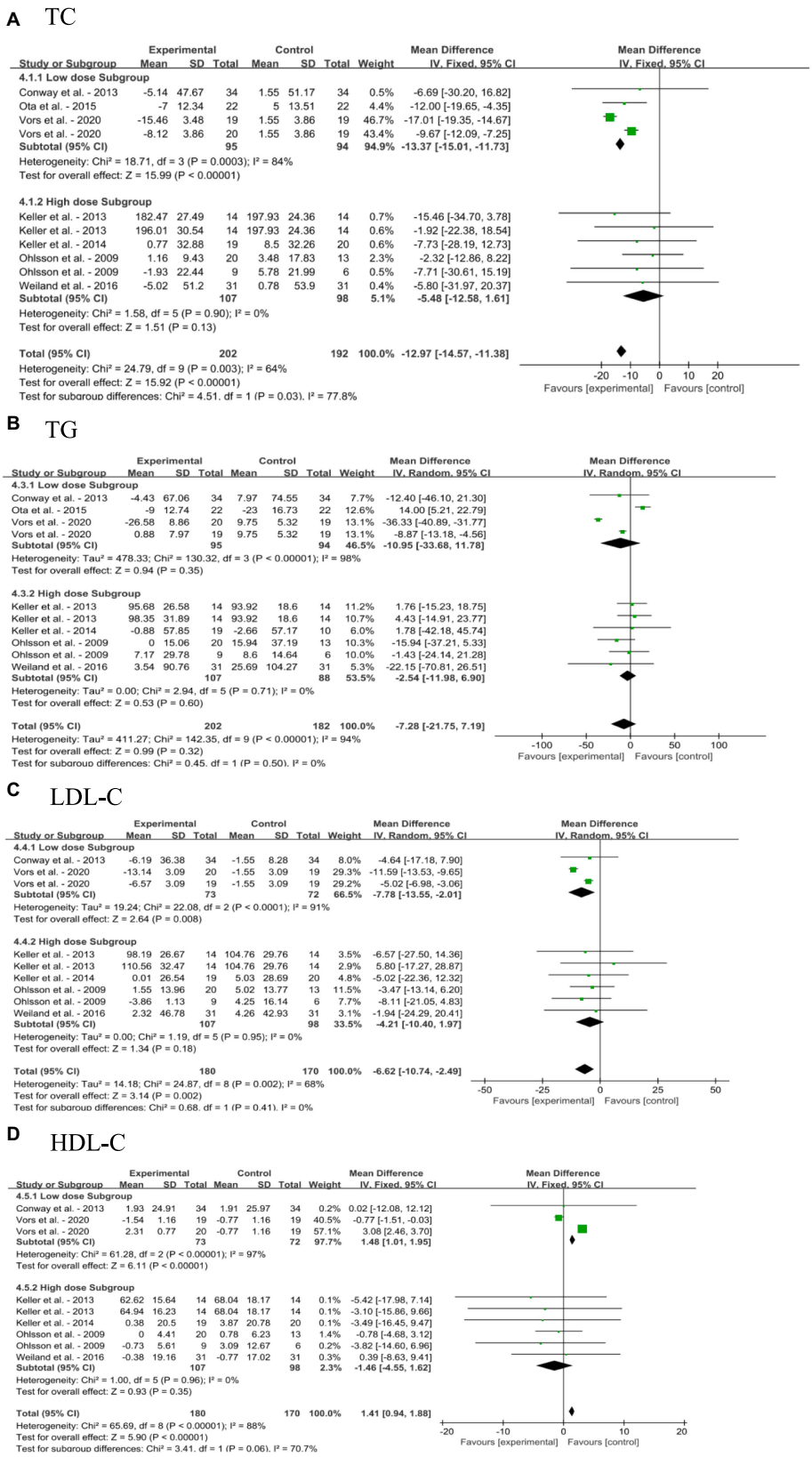


FIGURE 5 (Continued)

Another thing worth noting in this meta-analysis is that dietary SM supplementation reduced insulin levels and HOMA-IR, but did not affect blood glucose or inflammatory response factors. This might be related to the fact that dietary SM does not significantly affect serum SM, Cer, and PL concentrations (48). Congestive complications such as retinopathy, neuropathy, stroke, myocardial infarction, and arteritis of the lower limbs

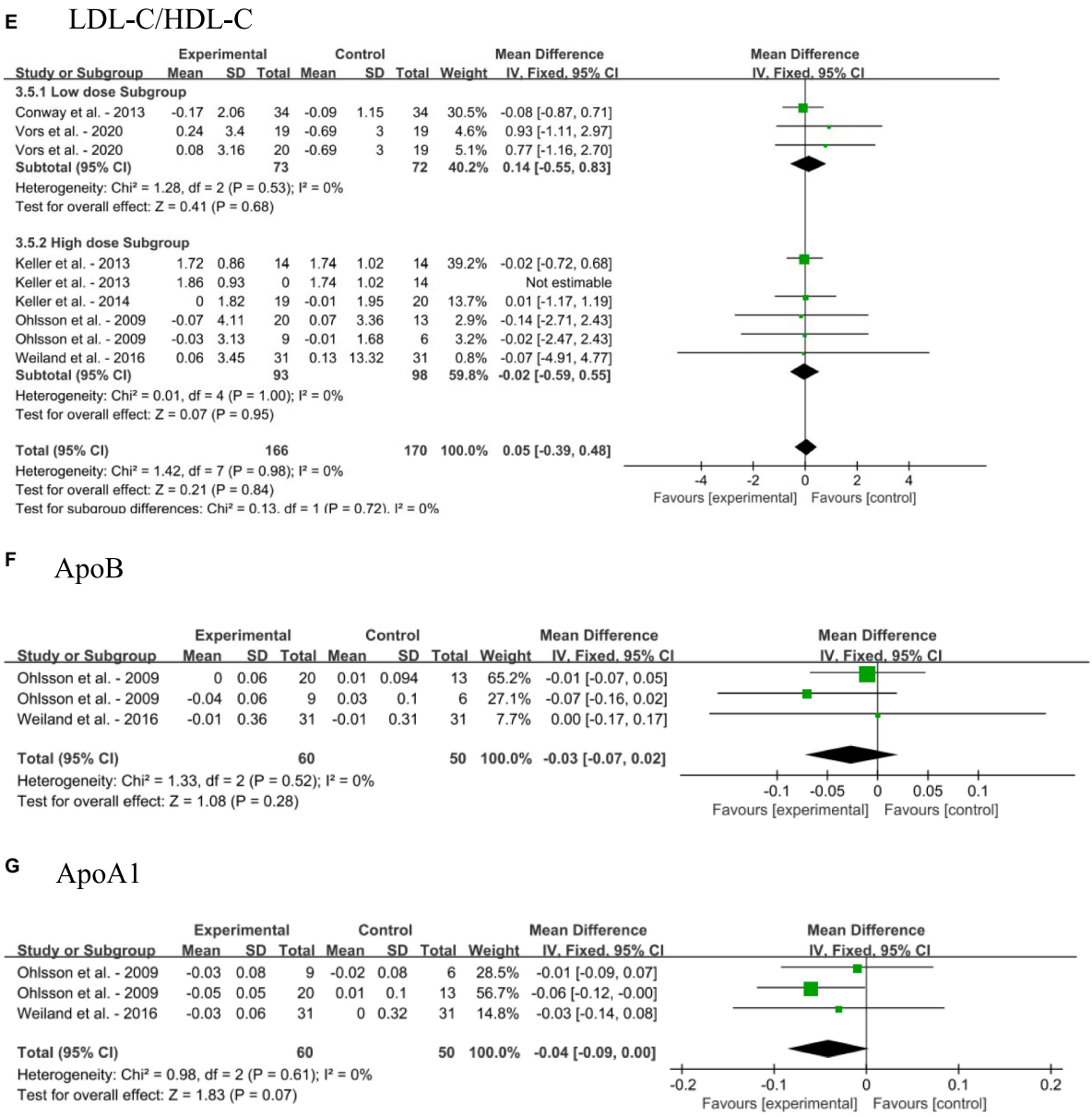


FIGURE 5
Forest plots for the effects of sphingomyelin (SM) on blood lipid profile in adults without metabolic syndrome (MetS). **(A)** TC, total cholesterol; **(B)** TG, triglyceride; **(C)** LDL-C, low-density lipoprotein cholesterol; **(D)** HDL-C, high-density lipoprotein cholesterol; **(E)** LDL-C/HDL-C; **(F)** ApoB, apolipoprotein B; **(G)** ApoA1, apolipoprotein A1. The horizontal bar represents the 95% confidence interval (CI). The magnitude of the rectangle at the center of the horizontal bar is proportional to the weight of the provided study. As indicated by the diamond at the bottom, the pooled mean difference (MD) is present.

are all possible outcomes of T2DM (76). Studies have shown significant associations between increased levels of circulating Cer and insulin resistance as well as T2DM (25, 77–80). Jensen et al. discovered a positive link between elevated blood levels of Cer and C16-lactosyl-Cer and increased glucose levels. However, no significant associations were detected between SM and glucose levels (80). An intriguing study also shows that diabetic patients had higher circulating Cer levels than nondiabetic individuals (77). In contrast to blood Cer, which is found in conjunction with LDL and very-low-density lipoprotein (VLDL), plasma S1P is bound to both albumin and apolipoprotein M, which associates preferentially with HDL (75). In addition to elevated levels of LDL-Cer, obesity and diabetes are correlated with reduced HDL-S1P levels. It has been demonstrated that LDL-Cer inhibits insulin signaling in muscle, while HDL-S1P improves insulin signaling and enhances the function and survival of pancreatic cells (77, 81, 82). HDL-S1P and LDL-Cer exhibit contrasting effects on the progression of T2DM (76). Hence, the process of interconversion among these sphingolipid species is subject to strict regulation. Even a minor disruption in anabolism, catabolism, or substrate accessibility can result in the atypical accumulation of one or more sphingolipid species, thereby causing an imbalanced supply of fatty acids within the metabolic system (83). As a result, changes in the profiles and concentrations of sphingolipids have emerged as a crucial area of

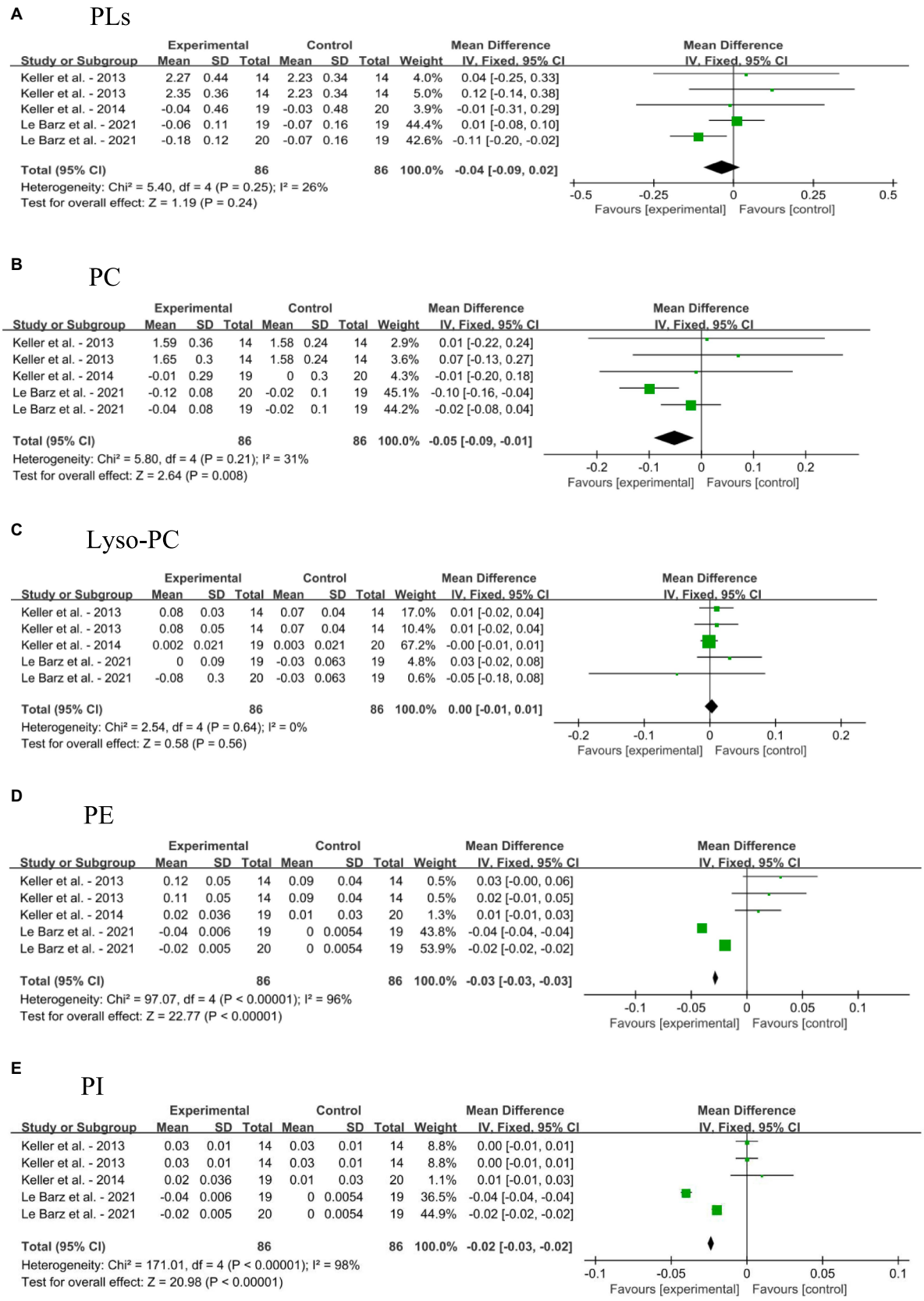


FIGURE 6
Forest plots for the effects of sphingomyelin (SM) on blood phospholipid levels in adults without metabolic syndrome (MetS). **(A)** PLs, total phospholipids; **(B)** PC, phosphatidyl cholines; **(C)** Lyso-PC, Lyso-phosphatidyl cholines; **(D)** PE, phosphatidyl-ethanolamine; **(E)** PI, phosphatidyl-inositol. The horizontal bar represents the 95% confidence interval (CI). The magnitude of the rectangle at the center of the horizontal bar is proportional to the weight of the provided study. As indicated by the diamond at the bottom, the pooled mean difference (MD) is present.

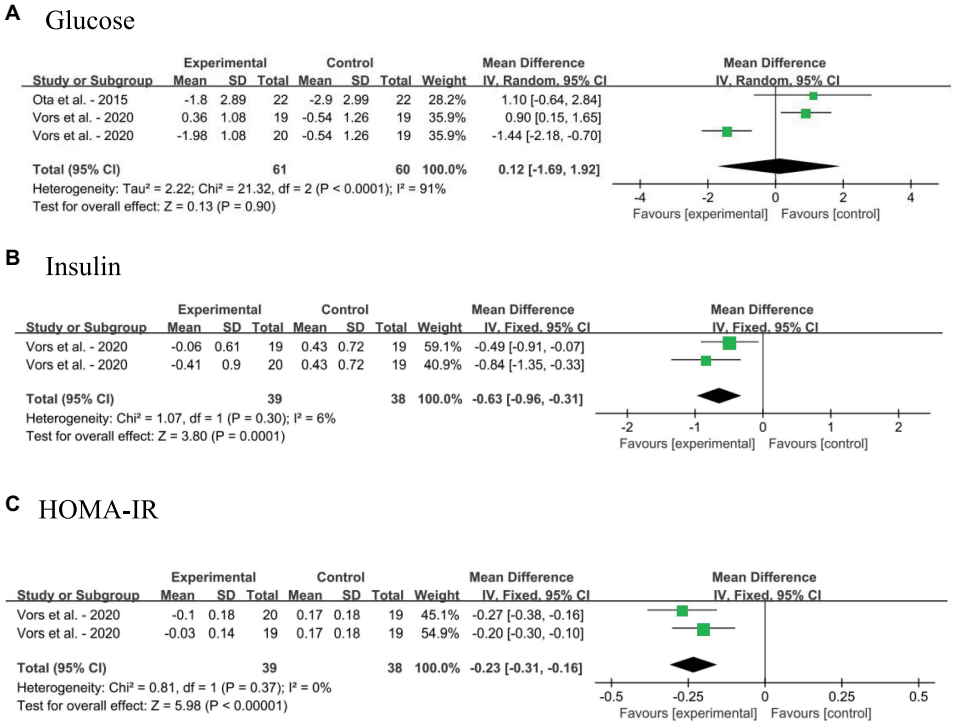


FIGURE 7
Forest plots for the effects of sphingomyelin (SM) on blood glycemia aspect in adults without metabolic syndrome (MetS). **(A)** Glucose, **(B)** insulin, **(C)** HOMA-IR, homeostasis model assessment of insulin resistance. The horizontal bar represents the 95% confidence interval (CI). The magnitude of the rectangle at the center of the horizontal bar is proportional to the weight of the provided study. As indicated by the diamond at the bottom, the pooled mean difference (MD) is present.

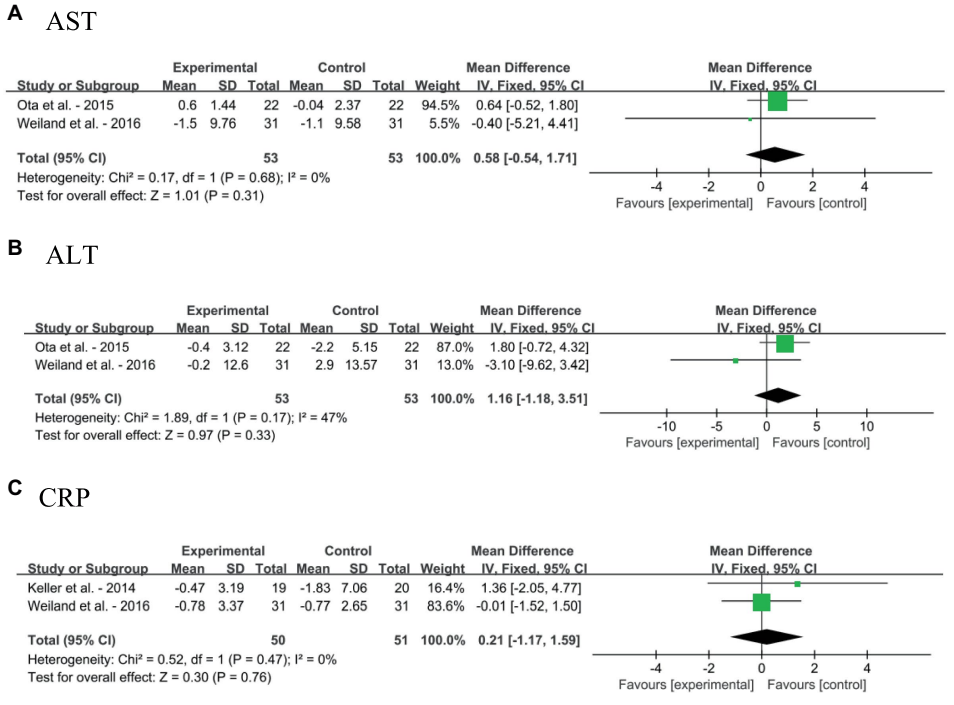


FIGURE 8
Forest plots for the effects of sphingomyelin (SM) on liver function biomarkers in adults without metabolic syndrome (MetS). **(A)** AST, aspartate transaminase; **(B)** ALT, alanine transaminase; **(C)** CRP, C-reactive protein. The horizontal bar represents the 95% confidence interval (CI). The magnitude of the rectangle at the center of the horizontal bar is proportional to the weight of the provided study. As indicated by the diamond at the bottom, the pooled mean difference (MD) is present.

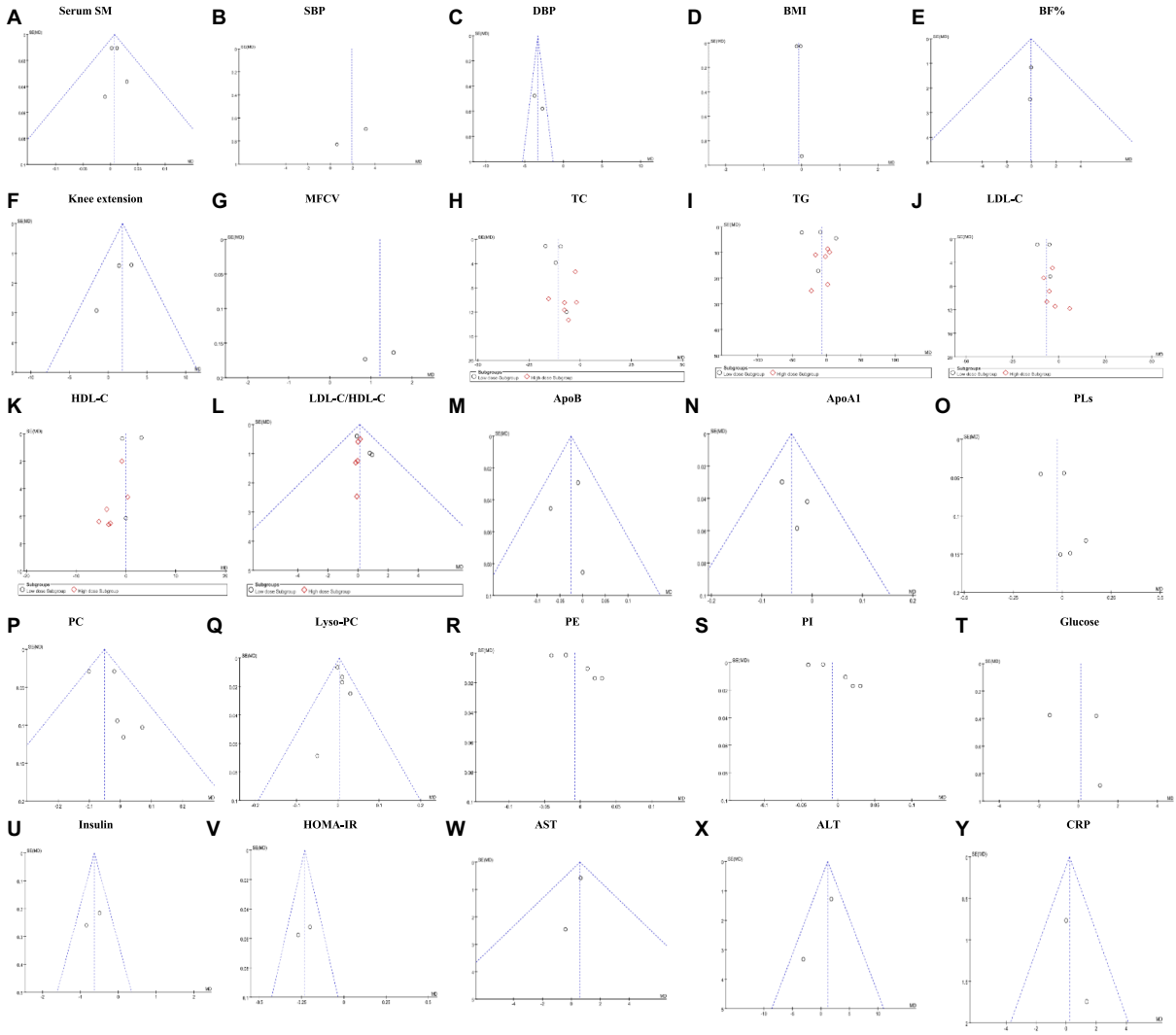


FIGURE 9
Sensitivity analysis plot. **(A)** Serum SM, Serum sphingomyelin; **(B)** SBP, systolic blood pressure; **(C)** DBP, diastolic blood pressure; **(D)** BMI, body mass index, **(E)** BF%, body fat percentage; **(F)** Knee extension; **(G)** MFCV, muscle fiber conduction velocity; **(H)** TC, total cholesterol; **(I)** TG, triglyceride; **(J)** LDL-C, low-density lipoprotein cholesterol; **(K)** HDL-C, high-density lipoprotein cholesterol; **(L)** LDL-C/HDL-C, **(M)** ApoA, apolipoprotein A; **(N)** ApoB, apolipoprotein B; **(O)** PLs, phospholipids; **(P)** Lyso-PC, Lyso-phosphatidyl choline; **(Q)** PC, phosphatidyl choline; **(R)** PE, Phosphatidyl-ethanolamine; **(S)** PI, Phosphatidyl-inositol; **(T)** glucose; **(U)** insulin; **(V)** HOMA-IR, homeostasis model assessment of insulin resistance; **(W)** AST, aspartate transaminase; **(X)** ALT, alanine transaminease; **(Y)** CRP, C-reactive protein. Fixed effects model: **(A,C,E,F,L–N,P,Q,U–Y)**. Random effects model: **(B,D,G–K,O,R–T)**.

study in MetS research. It is very interesting to find that dietary SM supplementation can reduce blood insulin level. Whether this can be attributed to the abundant long-chain fatty acyl groups in Cer derived from milk SM remains to be analyzed. In addition, whether this reduced insulin level is due to the reduction of insulin secretion or an increase in insulin clearance is also deserved to be investigated.

Nevertheless, the current meta-analysis has a few limitations. Due to the limited number of articles included, the number of participants may not have been sufficient to create a relatively large sample size, thereby increasing the type-2 statistical error. The absence of waist circumference data for anthropometric parameters is the second issue of this study. Furthermore, in some of the included RCTs, the intervention group was given a diet or beverage that various doses of SM supplementation were based on normal intakes. A comprehensive stratified analysis of the blood lipid profile was conducted. However, the dose–response relationship between supplementation and biomarkers including anthropometric parameters, glycemia aspect, and inflammatory response factors could not be determined. Therefore, further comprehensive prospective investigations are required to examine the impact of dietary SM supplementation on the sphingolipidome and Cer-S1P in the gastrointestinal tract and bloodstream. Finally, sample size, intervention period, or other factors in this meta-analysis may also be source of heterogeneity, and stratified analyzes of these factors are also necessary.

5 Conclusion

In summary, dietary SM supplementation did not have a detrimental effect on metabolic indexes in adults without MetS, but had a protective effect on blood lipid profiles and insulin

level. From the analysis results of the forest map, dietary SM supplementation can improve DBP, levels of TC, LDL-C, HDL-C, and insulin, and HOMA-IR. However, it does not affect BMI, SBP, and levels of TG, LDL-C/HDL-C and glucose. Therefore, dietary SM may serve as a protective factor for MetS, which needs to be confirmed via further clinical trials and basic research.

Data availability statement

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

Author contributions

C-ZL: Conceptualization, Data curation, Investigation, Software, Validation, Writing – original draft. L-MW: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Writing – original draft. C-XZ: Data curation, Investigation, Methodology, Software, Writing – original draft. H-YD: Conceptualization, Data curation, Investigation, Software, Writing – original draft. G-XC: Data curation, Project administration, Supervision, Validation, Writing – review & editing. FY: Conceptualization, Data curation, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1363077/full#supplementary-material

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Association of multiple serum minerals and vitamins with metabolic dysfunction-associated fatty liver disease in US adults: National Health and Nutrition Examination Survey 2017–2018

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Background: Despite the rapid increase in the global prevalence of Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD), there are no approved therapeutic drugs for MAFLD yet. Nutrient supplementation might mitigate the risk of MAFLD. It is more typical for individuals to consume multiple nutrients simultaneously. However, the studies exploring the combined effects of multiple nutrients on MAFLD are limited. This study aimed to investigate the relationship between both individual nutrients and their combined influence on the risk of MAFLD.

Methods: Data were obtained from National Health and Nutrition Examination Survey (NHANES), and 18 types of nutrients were considered in this study. Logistic regression analysis was performed to evaluate the correlation between single nutrients and the risk of MAFLD. The Least Absolute Shrinkage and Selection Operator (LASSO) regression analysis was performed to pinpoint the most relevant nutrient associated with the risk of MAFLD. Subsequently, both Weighted Quantile Sum (WQS) regression and Quantile g-computation (Qgcomp) were used to assess the combined effects of multiple nutrients on the risk of MAFLD.

Results: A total of 3,069 participants were included in this study. LASSO regression analysis showed that Se, α-tocopherol, and γ-tocopherol exhibited a positive association with the risk of MAFLD. In contrast, the serum levels of Co, P, α-cryptoxanthin, LZ, and trans-β-carotene were inversely associated with the prevalence of MAFLD. When Se and two types of vitamin E were excluded, the WQS index showed a significant inverse relationship between the remaining 15 nutrients and the risk of MAFLD; α-cryptoxanthin showed the most substantial contribution. Similarly, Qgcomp suggested that the combined effects of these 15 nutrients were associated with a lower risk of MAFLD, with α-cryptoxanthin possessing the most significant negative weights.

Conclusion: This study suggested that the complex nutrients with either a low proportion of Se, α-tocopherol, and γ-tocopherol or without them should be recommended for patients with MAFLD to reduce its risk.

KEYWORDS

vitamins, minerals, joint effect, MAFLD, NHANES

1 Introduction

In 2020, an international panel of experts proposed the concept of Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) (1). In contrast to Non-Alcoholic Fatty Liver Disease (NAFLD), MAFLD is defined using different diagnostic criteria (1) and is often depicted as a hepatic insulin-resistant disease, which is predominantly instigated by the dysfunction of lipid metabolism (2). The global prevalence of MAFLD is estimated to be 50.7% in overweight or obese individuals (3) and 33.87% in overweight or obese children and adolescents (4). Studies suggest that MAFLD is significantly correlated with adverse health outcomes, such as cardiovascular disease, hepatic events, extrahepatic malignancies, and renal disease (5). A distinguishing characteristic of MAFLD is excessive hepatic fat accumulation, which can be accurately and non-invasively assessed using FiberScan with Vibration-Controlled Instantaneous Elastography (VCTE) (6).

Hepatic steatosis is typically induced by unhealthy diets rich in fructose, saturated fats, and cholesterol. As there are no approved pharmacological treatments for MAFLD, the primary management strategies include physical activity and dietary modifications (2). Previous studies have indicated that a healthy lifestyle can decrease the risk of metabolic syndrome (7). Specific dietary nutrients can mitigate the symptoms of MAFLD and decrease its risk. For instance, vitamins C and D3 can modulate the gut microbiota and bile acid metabolism, thereby alleviating MAFLD symptoms (8). Moreover, a study indicated a positive correlation between the serum vitamin D concentration and a reduced risk of MAFLD (9). Similar findings have been reported regarding the dietary intake of vitamin K (10). On the other hand, a mendelian randomization study showed an elevated risk of MAFLD associated with an increase in liver iron concentration (11). Multiple factors, such as oxidative stress-induced lipotoxicity and cellular senescence, contribute to the pathogenesis and progression of MAFLD (12). Additionally, inflammatory responses accelerate the development of MAFLD (13). A study reported that several components of traditional Chinese herbal medicine could inhibit liver inflammation and alleviate liver damage (13). Therefore, supplementing the antioxidant or anti-inflammatory nutrients might have similar effects.

Numerous studies have focused on the correlations between individual nutrients and MAFLD. However, due to the intricate interactions among various nutrients, evaluating the effect of a single nutrient might lead to skewed results. For instance, the higher intake of calcium (Ca) and phosphorous (P) might affect the absorption of Mg, inducing various metabolic diseases (14). Moreover, a higher molar ratio of zinc (Zn) to copper (Cu) can cause Cu deficiency, leading to the dysregulation of metabolic factors (15). Notably, patients with liver diseases have dysregulated absorption and metabolism of several nutrients (16). Therefore, when the patients with MAFLD are supplemented with nutrients, the ratio among various nutrients is different from that of normal people. Mixed effects are commonly reported in studies assessing the effects of environmental pollution on human health (17–19). Correspondingly, individuals consume a combination of nutrients; however, the studies examining the combined effects of multiple nutrients are limited. Therefore, this study aimed to evaluate the association between individual serum nutrient levels and the combined effects of multiple serum vitamins and minerals on the risk of MAFLD. Meanwhile, the ratio among various nutrients was also determined. The evaluation was performed using Weighted Quantile Sum (WQS) regression and Quantile g-computation (Qgcomp) regression models on the data obtained from the National Health and Nutrition Examination Survey (NHANES) 2017–2018.

2 Materials and methods

2.1 Study population

The data of participants included in this study were obtained from the NHANES 2017–2018 survey. Among the 9,254 participants in the NHANES survey, 5,494 individuals went through the liver elastography examination. The participants below the age of 18 were subsequently excluded ($n = 748$). Among the remaining subjects, the detection of serum cobalt (Co) concentration was performed for 3,133 individuals aged 40 years and above. Moreover, 53 participants who could not be definitively diagnosed with MAFLD, and 11 participants who responded with "refuse" or "do not know" during questionnaire interviews about alcohol use and education level were excluded. Ultimately, this study included a total of 3,069 participants.

2.2 Definition of MAFLD

As per the international panel of experts, MAFLD diagnosis is based on hepatic steatosis coupled with one of the following three conditions: overweight/obesity, type 2 diabetes mellitus, or metabolic dysregulation (20). Metabolic dysregulation is defined as the conditions with at least two of the following indicators (20): (a) waist circumference ≥ 102/88 cm in Caucasian men and women; (b) blood pressure ≥130/85 mmHg or specific drug treatment; (c) plasma triglycerides ≥1.70 mmoL/L or specific drug treatment; (d) plasma high density lipoprotein-cholesterol (HDL-C) <1.0 mmol/L for men and <1.3 mmol/L for women or specific drug treatment; (e) prediabetes, such as fasting glucose level 5.6 to 6.9 mmol/L, or HbA1c 5.7 to 6.4%; (f) homeostasis model assessment (HOMA)-insulin resistance (IR) score ≥2.5; and (g) plasma high-sensitivity C-reactive protein (hs-CRP) level >2 mg/L. According to the existing literature, the condition with a controlled attenuated parameter (CAP) score of ≥248 dB/m is diagnosed as liver steatosis (21); the CAP score is determined using the FibroScan® model 502 V2 Touch.

2.3 Covariates

The covariates considered in this study included age, sex, race, education level, family income, physical activity (PA), and smoking and drinking status. The patients were grouped into ≤60 and >60 based on age and Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and other races based on race. Based on education level, the patients were divided into less than high school, high school, and more than high school groups (22). Family income was assessed using the poverty income ratio (PIR), and the patients were classified into low-income (PIR < 1.30), middle-income (1.3 ≤ PIR < 3.5), and high-income (PIR ≥3.5) groups (23). Based on smoking status, the patients were divided into non-smoker (smoked

less than 100 cigarettes in their lifetime) and smoker (smoked more than 100 cigarettes in their lifetime) groups. Based on drinking status, they were divided into non-drinker (those who had never consumed alcohol or had not consumed alcohol in the past 12 months) and drinker (those who had consumed alcohol in the past 12 months) groups. According to the literature (24), PA was calculated using Eq. 1.

$$\mathrm{PA}\left(\mathrm{MET}-\mathrm{h}/\mathrm{wk}\right)=\text{metabolic equivalent}\left(\mathrm{MET}\right)\times\text{weekly frequency}\times\text{duration of each physical activity} \quad (1)$$

The three variables in Eq. 1 were obtained from the PA questionnaire on the NHANES website. Then, based on PA, the patients were classified into low PA (<1MET-h/week), moderate PA (1–48 MET-h/week), and high PA (>48 MET-h/week) groups.

2.4 Statistical analyses

All the statistical analyses were performed using the R software (4.2.2). A total of 18 nutrients, including vitamin A, vitamin C, vitamin D, α-tocopherol, γ-tocopherol, lutein + zeaxanthin (LZ), α-carotene, trans-β-carotene, α-cryptoxanthin, β-cryptoxanthin, lycopene, iron (Fe), selenium (Se), Ca, Co, sodium (Na), potassium (K), and P were included in the analysis in this study. If the serum concentration of a specific substance was below the limits of detection (LOD), the value was represented as LOD divided by $\sqrt{2}$. For the 3,069 participants, any missing data, including house income, PA, smoking status and 18 nutrients, was imputed using grouping-based (age, gender, and race) median of the available samples for continuous variable, as well as the grouping-based mode for category variable, including drinking status. Variables, such as house income, PA, and smoking status, were then classified into category variables. Furthermore, the serum contents of the 18 nutrients were natural log-transformed. Continuous variables were represented as the means ± SD (standard deviation), and a student's *t*-test was used to compare differences between the two groups. Categorical variables were expressed as frequencies (percentages), and the differences between groups were compared using a chi-square test.

Initially, two logistic regression models, including model 1 and model 2, were used to explore the association between single nutrients and MAFLD. Model 1 was an unadjusted model, while, model 2 was adjusted for age, gender, race, education level, family income, smoking status, drinking status, and PA. The least absolute shrinkage and selection operator (LASSO) regression analysis was used to identify the nutrient most relevant to the risk of MAFLD (25). The data of 18 nutrients were standardized and centralized and then subjected to LASSO regression analysis. The dose-response relationship between MAFLD and various vitamins and minerals was evaluated using a restricted cubic spline (RCS) model. The combined effects of multiple minerals and vitamins were evaluated using WQS and Qgcomp regression models. Notably, the RCS model, WQS regression model and Qgcomp regression model were adjusted for age, gender, race, education level, family income, smoking status, drinking status and PA. For the WQS regression, 40% of the participants were randomly selected as training dataset, and 60% of the participants served as validation datasets. The bootstrap value was set to 1,000 in the parameter estimations. The Qgcomp regression estimated the joint effects of increasing every nutrient simultaneously by one quantile (26). The Qgcomp regression was conducted without bootstrap to estimate the weight of each nutrient and with bootstrap set to 500 to estimate the marginal odds ratio of the joint effect. Finally, a sensitivity analysis was performed to validate the robustness of the results, and the data were re-analyzed after removing all the participants with missing values.

3 Results

3.1 Baseline characteristics of study participants

As per the definition of MAFLD, 1,968 individuals were diagnosed with MAFLD among the 3,069 participants (Table 1). The average age of all the participants was approximately 60 years. The incidence of MAFLD was higher in males than females (52.0% vs. 45.0%). There were no significant statistical discrepancies between the MAFLD and non-MAFLD groups in terms of education level, family income, smoking status, PA and drinking status. Moreover, the patients with MAFLD were more likely to have higher aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP). Strikingly, as compared to non-MAFLD patients, the serum concentrations of γ-tocopherol (1.43 ± 0.52 vs. 1.23 ± 0.54), α-tocopherol (3.38 ± 0.31 vs. 3.35 ± 0.31) and Se (0.89 ± 0.13 vs. 0.87 ± 0.14) were elevated in the MAFLD patients; all the differences were statistically significant ($p < 0.05$).

3.2 Impacts of individual nutrients on MAFLD

Logistic regression analysis was performed to assess the effects of individual nutrients on the risk of developing MAFLD (Supplementary Table S1). For minerals, the serum levels of Co (OR = 0.503, 95% CI: 0.389–0.648) and Fe (OR = 0.704, 95% CI: 0.564–0.877) in only the Q4 group exhibited an inverse correlation with the risk of MAFLD in model 2. In both the Q3 and Q4 groups, the serum Se concentration was associated with the prevalence of MAFLD. Vitamins and carotenoids, including vitamin C, vitamin D, LZ, β-cryptoxanthin, trans-β-carotene, α-cryptoxanthin and α-carotene, were associated with a reduced risk of MAFLD; the serum trans-β-carotene levels in the Q4 group showed the highest efficacy (OR = 0.350, 95% CI: 0.277–0.442). Conversely, the serum levels of γ-tocopherol and α-tocopherol were positively correlated with the risk of MAFLD. LASSO regression analysis revealed the most critical variables for the risk of MAFLD. A 10-fold cross-validation was performed to select the optimal penalty parameter ($\lambda = 0.016$) (Figure 1A). As the parameters λ achieved this value, the Se, α-tocopherol, γ-tocopherol, P, Co, α-cryptoxanthin, trans-β-carotene, and LZ remained in the model (Figure 1B), indicating that the former three nutrients were positively correlated with the risk of MAFLD, while the latter five nutrients showed the opposite trend.

3.3 Dose-response relationship between nutrients and MAFLD

The RCS results revealed that an increase in the serum levels of Co, Fe, lycopene, LZ, trans-β-carotene, vitamin C, vitamin D, α-carotene, α-cryptoxanthin, and β-cryptoxanthin was significantly associated

TABLE 1 Basic characteristics of participants.

Characteristic	MAFLD		*p*
	No, *N* = 1,101	Yes, *N* = 1968	
Age, year	60.38 ± 12.21	60.39 ± 11.17	0.987
Age, %			0.665
≤60	555 (50.4%)	976 (49.6%)	
>60	546 (49.6%)	992 (50.4%)	
Sex, %			<0.001
Female	605 (55.0%)	944 (48.0%)	
Male	496 (45.0%)	1,024 (52.0%)	
Race, %			<0.001
Mexican American	97 (8.8%)	290 (14.7%)	
Other Hispanic	81 (7.4%)	206 (10.5%)	
Non-Hispanic White	386 (35.1%)	692 (35.2%)	
Non-Hispanic Black	310 (28.2%)	420 (21.3%)	
Other Race	227 (20.6%)	360 (18.3%)	
Education level, %			0.386
<High school	222 (20.2%)	432 (22.0%)	
High school	254 (23.1%)	466 (23.7%)	
>High school	625 (56.8%)	1,070 (54.4%)	
Family income, %			0.315
Low	255 (23.2%)	433 (22.0%)	
Middle	514 (46.7%)	975 (49.5%)	
High	332 (30.2%)	560 (28.5%)	
Smoking status, %			0.786
Never smoking	607 (55.1%)	1,075 (54.6%)	
Smoking	494 (44.9%)	893 (45.4%)	
Physical activity, %			0.294
High	305 (27.7%)	530 (26.9%)	
Middle	493 (44.8%)	844 (42.9%)	
Low	303 (27.5%)	594 (30.2%)	
Drinking status, %			0.904
Drinker	707 (64.2%)	1,268 (64.4%)	
Non-drinker	394 (35.8%)	700 (35.6%)	
Ln ALT, U/L	2.83 ± 0.47	3.02 ± 0.50	<0.001
Ln AST, U/L	3.01 ± 0.35	3.02 ± 0.35	0.178
Ln GGT, IU/L	3.06 ± 0.66	3.31 ± 0.67	<0.001
Ln ALP, IU/L	4.32 ± 0.29	4.37 ± 0.28	<0.001
Ln LZ, μmol/L	−1.06 ± 0.59	−1.18 ± 0.57	<0.001
Ln lycopene, μmol/L	−0.50 ± 0.60	−0.51 ± 0.54	0.799
Ln β-cryptoxanthin, μmol/L	−1.98 ± 0.86	−2.08 ± 0.83	0.002
Ln trans-β-carotene, μmol/L	−1.11 ± 0.97	−1.42 ± 0.88	<0.001
Ln α-cryptoxanthin, μmol/L	−3.08 ± 0.60	−3.21 ± 0.56	<0.001
Ln α-carotene, μmol/L	−2.69 ± 1.06	−2.94 ± 0.99	<0.001
Ln γ-tocopherol, μmol/L	1.23 ± 0.54	1.43 ± 0.52	<0.001
Ln vitamin A, μmol/L	0.60 ± 0.30	0.62 ± 0.29	0.103
Ln α-tocopherol, μmol/L	3.35 ± 0.31	3.38 ± 0.31	0.014
Ln vitamin D, nmol/L	4.24 ± 0.45	4.19 ± 0.46	0.001
Ln vitamin C, μmol/L	3.82 ± 0.73	3.71 ± 0.71	<0.001
Ln Se, μmol/L	0.87 ± 0.14	0.89 ± 0.13	<0.001
Ln Fe, μmol/L	2.68 ± 0.42	2.66 ± 0.39	0.180
Ln Co, nmol/L	1.13 ± 0.48	1.03 ± 0.47	<0.001
Ln P, mmol/L	0.13 ± 0.14	0.11 ± 0.14	<0.001
Ln K, mmol/L	1.41 ± 0.09	1.41 ± 0.09	0.556
Ln Na, mmol/L	4.94 ± 0.02	4.95 ± 0.02	0.582
Ln Ca, mmol/L	0.84 ± 0.04	0.84 ± 0.04	0.759

Data are represented as proportions (%) for categorical variables and as mean ± SD for continuous variables. Student's *t*-test and chi-square test were used for continuous and categorical variables to explore the difference between groups. LZ, lutein + zeaxanthin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase.

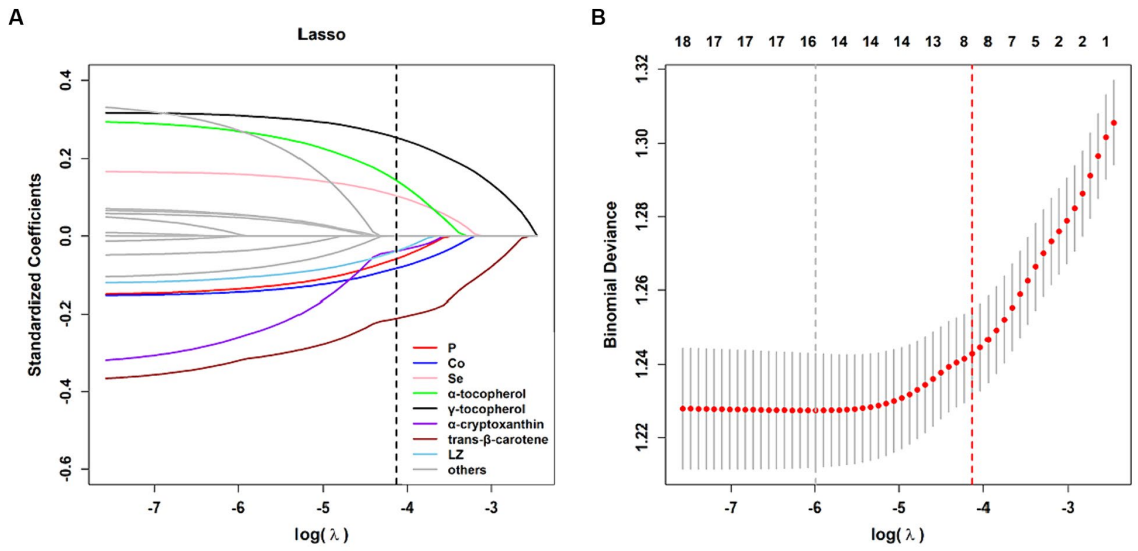

FIGURE 1
Results of LASSO regression model. **(A)** Plot for the coefficients of LASSO regression. **(B)** Ten-fold cross-validation for LASSO regression to select the optimal penalty coefficient.

with the lower risk of MAFLD (Supplementary Figures S1A–J). Conversely, an increase in the serum levels of Se and γ-tocopherol was correlated with a higher risk of MAFLD, particularly the γ-tocopherol level (Supplementary Figures S1K,L). Furthermore, an increase in the serum levels of Ca, Na, Vitamin A, and α-tocopherol could moderately increase the risk of MAFLD (Supplementary Figures S1M–P), while an increase in the serum levels of K and P could marginally mitigate the risk of MAFLD (Supplementary Figures S1Q,R).

3.4 Combined effects of multiple nutrients on MAFLD

The WQS regression showed statistically significant results in both the positive and negative constrains, suggesting that the mixture of nutrients might not affect MAFLD; this result was confirmed by Qgcomp regression analysis (Supplementary Table S2).

Given the significant contribution of five nutrients, including α-tocopherol, γ-tocopherol, Se, Na and Ca, to the positive relationship between the WQS index and MAFLD (Supplementary Figure S2A), WQS regression analysis was performed after consecutively excluding any one, two, or three of the five nutrients. As shown in Supplementary Figures S2B–D, the combined exclusion of Se, α-tocopherol and γ-tocopherol diminished the positive correlation between the WQS index and MAFLD. A 10% increase in the serum levels of the remaining 15 nutrients correlated with a 26.3% reduced risk of MAFLD (OR = 0.736; 95% CI: 0.682–0.794) (Table 2), among which, α-cryptoxanthin, Co, α-carotene, vitamin C, P, LZ, and vitamin D significantly contributed to this combined effect (Figure 2A). Concurrently, the Qgcomp regression analysis also revealed that the combined effect of these 15 nutrients was negatively associated with the risk of MAFLD (Marginal OR = 0.792; 95% CI: 0.732–0.856) (Table 2). The weights of these nutrients in the combined effect calculated using Qgcomp are shown in Figure 2B.

TABLE 2 Combined effect of 15 nutrients.

Methods	OR (95% CI)
WQS positive	1.046 (0.986, 1.109)
WQS negative	0.736 (0.682, 0.794)
Qgcomp	0.792 (0.732, 0.856)

Combined effects were evaluated using WQS regression with positive and negative constrains and Qgcomp. WQS, weighted quantile sum; Qgcomp, quantile-g-computation; OR, odds ratio; 95% CI, 95% confidence interval. Both WQS regression and Qgcomp were adjusted for age, gender, race, education level, smoking status, drinking status, physical activity and poverty income ratio.

3.5 Sensitivity analysis

The data of participants containing at least one missing value was discarded leaving a total of 1,974 subjects eligible for further analysis. Initially, both the WQS and Qgcomp regression analyses were employed to assess the combined effects of the 18 nutrients. The WQS results revealed both positive and negative constraints to be statistically significant (Supplementary Table S3), and the Qgcomp results suggested that the combined effect of the 18 nutrients did not confer any benefits towards the risk of MAFLD (Supplementary Table S3). Interestingly, excluding Se and two types of vitamin E resulted in retaining statistical significance in only the negative WQS index (Supplementary Table S4). Concurrently, the combined effects evaluated using the Qgcomp regression model displayed an inverse correlation with the risk of MAFLD (Supplementary Table S4).

4 Discussion

At present, MAFLD poses a significant threat to global health. Therefore, a cross-sectional study was conducted to investigate the combined effects of multiple nutrients on the risk of MAFLD. Three

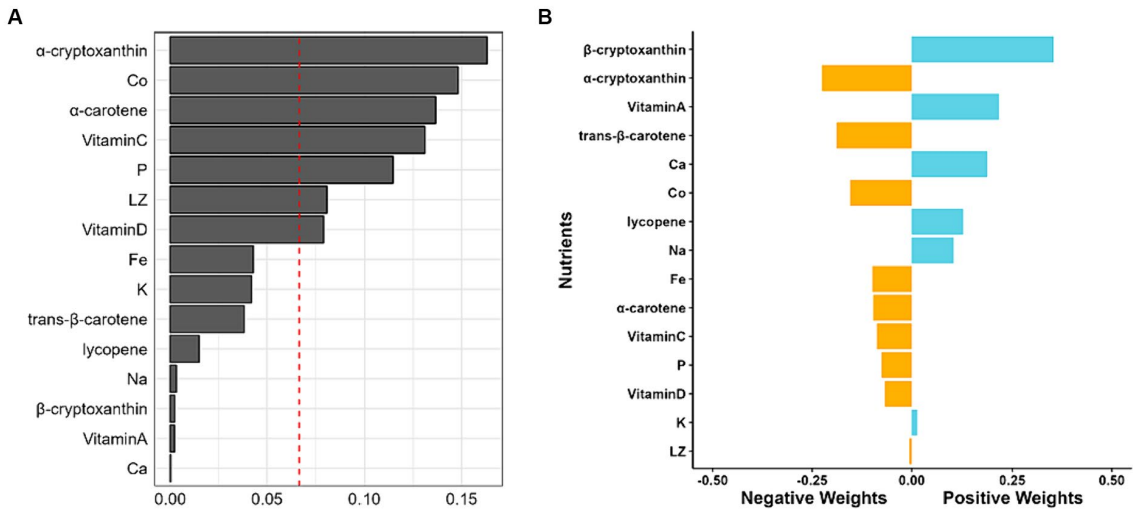

FIGURE 2
Weights of each nutrient in combined effect. **(A)** Weights calculated with WQS regression with negative constrain. **(B)** Weights of each nutrient revealed by Qgcomp, with the negative weights being statistically significant.

different statistical models, including LASSO regression, WQS regression, and Qgcomp models, consistently highlighted that the serum levels of Se, α-tocopherol, and γ-tocopherol were positively correlated with the risk of MAFLD. Conversely, all three methods demonstrated that the serum levels of Co, P, α-cryptoxanthin, LZ, and trans-β-carotene were inversely related to the risk of MAFLD. Subsequently, the result showed that the combined effects of 15 nutrients in serum, excluding Se, α-tocopherol, and γ-tocopherol, were advantageous in reducing the incidence of MAFLD. These findings suggested that a complex nutrient supplement, which can lower the serum levels of Se, α-tocopherol and γ-tocopherol, should be advised for patients with MAFLD to mitigate its risk.

Typically, people are simultaneously exposed to countless minerals and vitamins in day-to-day life, resulting in potential interactions among various nutrients. The over-supplementation of one nutrient can disrupt the absorption of others. Hence, examining the health impacts of a single nutrient on human wellness might yield skewed results. Previous studies have illustrated that co-exposure to a mixture of nutrients, including β-carotene, vitamin A, vitamin D, vitamin C, α-tocopherol, folate, vitamin B6, and vitamin B12, could diminish the risk of all-cause mortality in diabetic patients (27). Furthermore, the combined effects of vitamin C, vitamin B9, and vitamin B12 could limit the risk of metabolic disorders (28). Researchers demonstrated that adherence to a mineral-based nutrient pattern, which refers to the inclusion of multiple vitamins and minerals in an individual's daily diet, was associated with healthier metabolic factors (29). They also suggested that increasing the plant-based nutrients, such as vitamins D, B6, B3, C, B1, E, etc., were associated with a lower risk of metabolic syndrome (30). These results demonstrated the beneficial effects of combined exposure to multiple nutrients, which were consistent with the findings in the current study. Moreover, a cocktail of 11 antioxidant nutrients, including Se and α-tocopherol, could decrease the risk of specific cardiovascular diseases (CVDs), with Se making the most substantial contribution (31). However, the findings in the current study indicated that a complex nutrient solution, including Se and two types of vitamin E, might not present beneficial effects on mitigating the risk of MAFLD. Interestingly, a low serum α-tocopherol level was correlated with a lower likelihood of overweight/obesity. Consequently, it was speculated that this phenomenon might be related to the age of participants, all of whom were over 40 years of age. Additionally, some unhealthy lifestyle choices or other unseen factors might diminish or even reverse the beneficial effects of certain nutrients. For instance, β-carotene supplementation in smokers might amplify the incidence of cardiovascular disease and mortality, as well as the risk of lung cancer (32, 33).

Vitamin E exerts its health benefits primarily through its antioxidant and anti-inflammatory properties (34, 35). Both the α-tocopherol and γ-tocopherol can modulate mitochondrial oxidative metabolism to ameliorate Alzheimer's disease (36). Furthermore, γ-tocopherol could inhibit the inflammatory response and oxidative stress to enhance wound healing in diabetic rats (37), and α-tocopherol could also suppress inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-18, IL-12, and IL-6 (38). Although the onset and progression of metabolic disorders and fatty liver diseases are partially attributed to and concurrent with oxidative stress and inflammatory factors (39–41), Sabina reported that an increase in circulating α-and γ-tocopherol levels were positively associated with the risk of metabolic syndrome (42). This finding aligns with the current study results. This phenomenon might be partially explained by metabolic syndrome inhibiting the metabolism of α-tocopherol (43). Furthermore, the health effects of vitamin E and its metabolites are largely dependent upon the individual's lifestyles, such as smoking status and alcohol consumption (44). Gut dysbiosis induced by MAFLD might also affect the serum levels of vitamin E and its metabolites (45). In conclusion, further research is needed to elucidate the specific mechanisms of this.

Although Se has been recognized as an indispensable trace element, its effects remain a subject of debate. Several studies have suggested that Se possesses numerous health-enhancing effects, such as reducing fasting insulin (46), fasting plasma glucose (47) and serum CRP levels (48). In obese individuals, Se can decrease body fat mass and augment lean body and muscle mass (49). However, researchers

have discerned a positive correlation between plasma Se levels and fasting plasma glucose levels in men (50). Furthermore, high dietary Se intake is positively correlated with HOMA-IR in obese/overweight adults (51). Shao et al. (52) reported that an elevated Se status could increase the risk of diabetes in individuals aged 40 years. Therefore, combined with the current study results, it was suggested that middle-aged or elderly individuals might have elevated Se levels, increasing the risk of metabolic-dysfunction-associated diseases.

Both WQS and Qgcomp models revealed that α-cryptoxanthin played the most significant role in a negative correlation between the complex nutrients and the risk of MAFLD. To the best of our knowledge, though α-cryptoxanthin could induce mammalian phase 2 proteins to shield cells from damage by oxidants and electrophiles (53), few studies have reported beneficial effects of α-cryptoxanthin. Notably, numerous studies have focused on another cryptoxanthin known as β-cryptoxanthin, which is an antioxidant and a retinoid precursor that can mitigate the risk of NAFLD and other lifestyle-related diseases (54); however, it has deleterious effects on smokers and drinkers (55). In the current study, after excluding Se and two types of vitamin E, the Qgcomp demonstrated that β-cryptoxanthin had the most substantial positive weights. Moreover, WQS results indicated that β-cryptoxanthin slightly contributed to the negative correlation between the WQS index and MAFLD. These findings conflicted with the results of single nutrient analysis in RCS results. Similarly, Qgcomp results indicated that excluding Se and two types of vitamin E changed serum K from the largest positive weight to a slightly negative weight. These phenomena might be attributed to potential interactions among various nutrients. Further studies should investigate the contrasting effects of α-and β-cryptoxanthin on MAFLD.

The current study also suggested that trans-β-carotene played a pivotal role in decreasing the risk of MAFLD by ameliorating the indicators related to metabolic disorders. It may function as an antioxidant to decrease serum CRP levels and individual inflammatory burden (56). Researchers showed that a mixture of carotenoids was inversely associated with blood pressure, among which, trans-β-carotene had the most substantial contribution (57). Moreover, trans-β-carotene, as a provitamin A, could decrease children's body mass index (BMI), truncal fat mass, and total body fat mass, while vitamin A exhibited the opposite effects (58). Studies have indicated that increasing serum retinol level is positively associated with a higher prevalence of obesity and other metabolic indicators, including lower HDL-C and higher fasting blood glucose levels (59, 60). Moreover, in this study, the Qgcomp and RCS models showed that vitamin A was positively associated with the risk of MAFLD. This might be because vitamin A could exacerbate high-fat diet-induced hepatic steatosis, which dominates MAFLD patients (61). In addition, vitamin A is mainly dependent on its metabolite retinoic acid, which acts as a transcription factor, activating retinoic acid and retinoid X receptors to improve metabolic disorders (62). Thus, it was hypothesized that various dietary patterns might affect vitamin A, and vitamin A metabolism may be disturbed in patients with MAFLD.

Currently, numerous studies have mainly focused on the specific molecular mechanism of a single vitamin and mineral on NAFLD. For instance, VD3 could increase the levels of the mitochondrial contact site and cristae organizing system (MICOS) 60 by regulating vitamin D receptor (VDR) to ameliorate age-associated NAFLD (63). Vitamin E could activate the AMPK signaling pathway to reduce fatty acid synthesis and decrease oxidative stress (64). Ascorbic acid could activate the FGF21/FGFR2/adiponectin pathway to alleviate hepatocyte stress as well as peroxisome proliferator-activated receptor α (PPARα) and improve the visceral obesity and NAFLD (65). Moreover, studies have demonstrated that vitamin B_{12} and folate could facilitate the β-oxidation of fatty acids and regulate autophagy and inflammation by modifying multiple hepatic proteins to improve non-alcoholic steatohepatitis (66). In summary, a single vitamin and mineral can improve NAFLD by decreasing the level of oxidative stress and inflammation, inhibit the synthesis of fatty acids, and promote the utilization of fatty acids, providing a way forward in the MAFLD research. Moreover, the combined effects of multiple vitamins and minerals on NAFLD or MAFLD are not clear yet. Therefore, further studies are needed to explore it.

The current study has several strengths. First, this study probed into the combined effects of multiple nutrients using various statistical models to circumvent biased results. Second, the weight of individual nutrients on the combined effect was assessed, which helped in determining the relative contents of diverse nutrients in supplementation or dietary intake. Lastly, the data was obtained from NHANES, which strengthens the reliability of the results. However, there were also certain limitations to this study. A cross-sectional study design inherently poses challenges in establishing a causal relationship, necessitating further corroboration through longitudinal cohort studies or clinical trials. Additionally, although adjustments were made for several factors, some potential confounders might have been overlooked. Moreover, a singular measurement might not accurately reflect the long-term nutrient status of participants. Furthermore, this study did not include some crucial vitamins and trace elements, such as vitamin B groups and Zn. Future studies should incorporate a longitudinal follow-up of comprehensive nutrient concentrations and various metabolic indicators. Lastly, while VCTE is extensively used to diagnose liver steatosis and fibrosis due to its high efficiency and non-invasive nature, the cut-off value for diagnosis is still debatable, and liver biopsy retains its position as the gold standard.

5 Conclusion

This study suggested that the combination of 15 nutrients, excluding Se, α-tocopherol and γ-tocopherol, was inversely associated with the risk of MAFLD. Simultaneously, this study offered an appropriate compositional ratio. Consequently, it was suggested that the supplementation of multiple vitamins and minerals, either with reduced ratios of Se and two types of vitamin E or entirely without them, might reduce MAFLD prevalence. Nevertheless, additional research is imperative to corroborate these findings.

Data availability statement

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

Ethics statement

The studies involving humans were approved by National Center for Health Statistics Ethics Review Board. The studies were conducted

in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

PG: Formal analysis, Visualization, Writing – original draft. JY: Methodology, Supervision, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1335831/full#supplementary-material

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Metabolic dysfunction-associated fatty liver disease: current therapeutic strategies

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The definition of "Metabolic Associated Fatty Liver Disease – MAFLD" has replaced the previous definition of Nonalcoholic Fatty Liver Disease (NAFLD), because cardiometabolic criteria have been added for the prevention of cardiological risk in these patients. This definition leads to an in-depth study of the bidirectional relationships between hepatic steatosis, Type 2 Diabetes Mellitus (T2DM), Cardiovascular Disease (CVD) and/or their complications. Lifestyle modification, which includes correct nutrition combined with regular physical activity, represents the therapeutic cornerstone of MAFLD. When therapy is required, there is not clear accord on how to proceed in an optimal way with nutraceutical or pharmacological therapy. Numerous studies have attempted to identify nutraceuticals with a significant benefit on metabolic alterations and which contribute to the improvement of hepatic steatosis. Several evidences are supporting the use of silymarin, berberine, curcumin, *Nigella sativa*, *Ascophyllum nodosum*, and *Fucus vesiculosus*, vitamin E, coenzyme Q10 and Omega-3. However, more evidence regarding the long-term efficacy and safety of these compounds are required. There is numerous evidence that highlights the use of therapies such as incretins or the use of Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) inhibitors or other similar therapies which, by assisting existing therapies for pathologies such as diabetes, hypertension, insulin resistance, have given a breakthrough in prevention and the reduction of cardiometabolic risk. This review gave an overview of the current therapeutic strategies that are expected to aid in the treatment and prevention of MAFLD.

KEYWORDS

Metabolic Associated Fatty Liver Disease (MAFLD), Nonalcoholic Fatty Liver Disease (NAFLD), Cardiovascular Disease (CVD), nutraceuticals, innovative therapies

1 Introduction

In recent years, disapprovals took place on the traditional naming of Nonalcoholic Fatty Liver Disease (NAFLD) which excludes excessive alcohol assumption and the absence of many chronic liver diseases. Therefore, the knowledge on the metabolic alterations of the disease and its high risk of cardio-metabolic complications have raised the necessity to find a new denomination that introduces valid criteria that describe the underlying pathophysiological mechanisms. The new definition "Metabolic Associated Fatty Liver Disease – MAFLD" identifies a condition of hepatic steatosis associated with metabolic alterations that are defined by means of clear and easy-to-apply diagnostic criteria (1) and which underlines the importance of obesity, diabetes, and Metabolic Syndrome (MetS) in the pathogenesis of fat liver (Figure 1) (2). Numerous evidences suggest a strong association between an increase in cardiovascular risk and NAFLD. The Adolescent Brain Cognitive Development (ABCD) study shows that patients affected by fatty liver disease, matched to those who are free of the disease and after managing obesity, have higher Atherosclerotic Cardiovascular Disease (ASCVD) score values and express a higher 10-year risk of atherosclerotic cardiovascular disease events (3). Other clinical trials underline that fat liver is an independent risk factor both for enhancement carotid intima-media thickness (4) and for the presence of echocardiographic signs of diastolic cardiac dysfunction (5).

The association between NAFLD and cardiovascular risk is increasingly documented by studies that highlight ultrasound evaluations relating to the presence of carotid plaques after at least 2 years and the presence of fatty liver at the time of baseline evaluation as a factor of independent risk for the appearance of carotid atherosclerotic plaques (6).

MAFLD tends to include more than NAFLD patients with metabolic alterations, and therefore with greater cardiovascular risk, older patients, subjects with advanced fibrosis, and patients with other associated liver diseases (the diagnosis is no longer one of exclusion). The internist and the diabetologist become a key figure in the diagnosis and initial care of subject affected by metabolic hepatic pathology. The criteria for the MAFLD diagnosis are important for the appropriate use of innovative therapies and nutraceutical supplements.

Therefore, tailored therapies are needed for the corollary cardiovascular risk lower factors in subject affected by MAFLD and to improve liver function.

1.1 Aim of the review

There is no consensus on the best therapeutic approach to manage patients with MAFLD. The aim of this review is to provide an overview of the current therapeutic strategies, whether pharmacological or otherwise, for the treatment and prevention of MAFLD.

2 Metabolic dysfunction-associated fatty liver disease (MAFLD)

Currently, NAFLD is the most frequent hepatic alteration; NAFLD is considered as the presence of fat in liver echography and/or biopsy in the absence of one more hepatic injury (alcohol, hepatotoxic drugs, toxins, viral infections, genetic liver disease) (7).

However, the term NAFLD refers only to liver disease unrelated to alcohol and not to alterations on metabolism and correlated cardiometabolic risk, for that reason, specialists in scientific societies

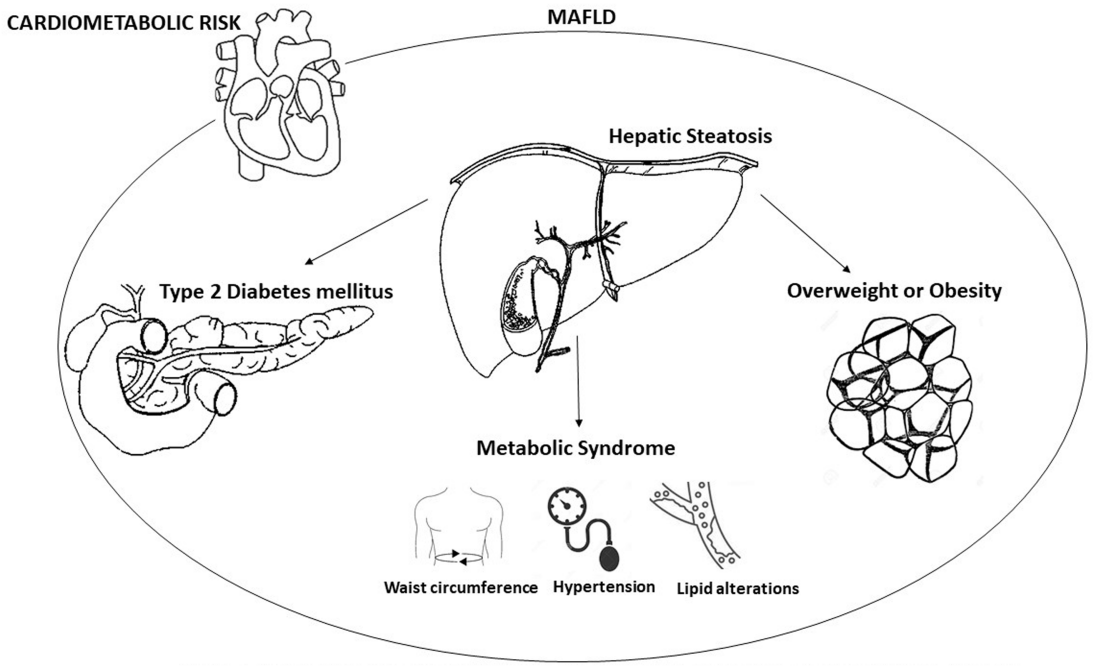

FIGURE 1
Metabolic factors involved in metabolic dysfunction-associated fatty liver disease (MAFLD).

have taken action to modify and improve this definition. The term MAFLD was proposed in 2020 and is diagnosed on the presence of fatty liver disease (verified by liver echography and/or biopsy) and the presence of one of the following comorbidities: Type 2 Diabetes Mellitus (T2DM), obesity and metabolic dysfunction (1).

The prevalence of this systemic disease is growing and in the future it could be the main cause of chronic hepatic condition (8).

The prevalence of obesity is implicated in the genesis of MAFLD, and the liver is often involved in obesity because the major metabolic processes linked to glucose and lipids take place in this organ. However, the metabolic dysfunctions do not only occur in the liver but also in other body area; in fact, Chronic Kidney Disease (CKD), osteoporosis, Obstructive Sleep Apnea Syndrome (OSAS), endocrine disorders, depression, cognitive impairment and cardiovascular consequences happen in these patients (9).

There is not accord on how the degree of fatty liver influence atherosclerosis. The association between the severity of fatty liver disease and atherosclerosis may be explained in part by severe lipotoxicity, inflammation, and marked hepatic insulin resistance (10). However, NAFLD/MAFLD still needs to receive enough interest in the community of cardiologists (11). The relationship between MAFLD and Cardiovascular Diseases (CVD)/Subclinical Carotid Atherosclerosis (SCA) is generally assigned to presence of shared risk factors (lipid abnormalities and obesity) (12). MAFLD focuses in the systemic metabolic conditions accompanying steatotic hepatic pathology.

Thus, the relationship between the development of MAFLD and SCA needs to be further explored.

2.1 Metabolic profile of MAFLD

MAFLD is defined as the presence of fatty liver disease associated with at least two metabolic alterations among (13):

- Waist circumference ≥ 102/88 cm in Caucasian men/women or ≥ 90/80 cm in Asian men/women;
- Blood pressure ≥ 130/85 mmHg or antihypertensive drugs;
- Plasma Triglycerides (TG) ≥ 150 mg/dL or TG lowering drugs;
- Plasma high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL for men and < 50 mg/dL for women or lipid-lowering drugs;
- Fasting plasma glucose levels between 100 and 125 mg/dL or 2 h post-load;
- Glucose levels between 140–199 mg/dL or glycosylated hemoglobin (HbA1c) between 5.7–6.4%;
- Homeostasis model assessment (HOMA) with insulin resistance score ≥ 2.5;
- High-sensitivity C-reactive protein levels >2 mg/L.

Hepatic steatosis is diagnosed by ultrasound and the hepatic parenchyma appears brightness (14). The presence of steatosis is not always an indication of MAFLD. The possible alternative causes of steatosis (drugs, celiac disease, prolonged fasting, severe weight loss, lipid metabolism disorders) must always be sought in the absence of the clinical criteria of MAFLD.

The pathophysiology of MAFLD is complicated and heterogeneous. The "two-hit hypothesis" proposed by some colleagues predicts that the first hit causes fat accumulation in hepatocytes and the second hit causes oxidative stress, increasing inflammation and leading to fibrosis (15). The second hypothesis instead proposed that MAFLD develops when the synthesis of triglycerides in the liver exceeds the catabolism of non-esterified fats and depends on the oxidation in the mitochondria and the export of the same triglycerides in Very Low-Density Lipoproteins (VLDL) ("multiple hit hypothesis") (16). In patients with MAFLD, the expression of Acetyl-Coenzyme-A Carboxylase 1 (ACC1), an enzyme in *de novo* lipogenesis, was decreased and acetyl-coenzyme-A was shown to be converted to malonyl-CoA. Accumulation of malonyl-CoA inhibits Carnitine Palmitoyl Transferase (CPT)-1, which transports fatty acids into the mitochondria and decreases β-oxidation. Fatty acid synthase (FAS) induces the conversion of malonyl-CoA to palmitic acid, and its expression is reduced in NASH patients (17), but the mechanism leading to the decrease in triglyceride synthesis appears to be the accumulation of harmful free fats.

MAFLD has been associated with MetS and it has been considered the liver expression of the MetS. MAFLD may facilitate the development of components of the metabolic syndrome, which may in turn predispose to MAFLD (14).

Patients with diabetes have a prevalence of MAFLD two to three times higher than patients without. The improper deposit of fat in the liver causes alterations in energy metabolism and the inflammatory state, generating insulin resistance. Due to this chronic hyperinsulinemia, patients with diabetes tend to accumulate more fat in the liver. In these patients the severity, morbidity, progression, and liver-related mortality associated with MAFLD are much higher (16, 17).

A distinction is no longer made between steatosis and steatohepatitis (therefore, the diagnostic concept of Metabolic dysfunction-Associated Steatohepatitis (MASH) does not exist), but the disease activity as well as the fibrosis are evaluated as a continuum based on the data of non-invasive methods (Table 1) or liver biopsy (NAFLD Activity Score and degree of fibrosis).

Laboratory tests support the MAFLD diagnosis and allow us to evaluate the conditions associated with hepatic steatosis evolution. Generally, liver fibrosis biomarkers reflect matrix turnover but not the extent of extracellular matrix deposition. None of the biomarkers available today are specific to fibrosis in the liver, and inflammatory

TABLE 1 Clinical-laboratory indexes for monitoring the steatosis activity and the fibrosis.

Clinical-laboratory indexes
AST/platelet ratio index (APRI)
BARD score
Enhanced Liver Fibrosis (ELF) score
Fatty Liver Index (FLI)
Fibrosis-4 (FIB-4) index
Fibrotest
Hepatic Steatosis Index (HSI)
HepaScore
Nonalcoholic Fatty Liver Disease (NAFLD) Fibrometer
Nonalcoholic Fatty Liver Disease (NAFLD) Fibrosis score
Nonalcoholic Fatty Liver Disease (NAFLD) Liver Fat Score
SteatoTest

and oxidative states in other sites can contribute to increasing circulating levels.

The indirect markers, which reflect alterations in liver function such as ASpartate aminoTransferase (AST), ALanine aminoTransferase (ALT), platelet count, Gamma-Glutamil Transferas (GGT), total bilirubin, alpha 2-macroglobulin or alpha 2-globulin (mainly haptoglobin), individually provide rather limited clinical information regarding the presence or absence of fibrosis. Hence, scores that consider multiple biomarkers combined in various ways which increase diagnostic accuracy (18) have been proposed (Table 1).

Direct markers which instead reflect liver fibrosis contain biomarkers of collagen synthesis or degradation, extracellular matrix glycoproteins, proteoglycans, and glycosaminoglycans (PIIINP: amino-terminal Propeptide of type III Procollagen; TIMP-1: Tissue Inhibitor of Metalloproteinase; TNF: Tumor Necrosis Factor; MMP: Matrix MetalloProteinase) must be consider. Furthermore, in the pathophysiological mechanisms involved in MAFLD, pro-inflammatory molecules such as Transforming Growth Factor beta-1 (TGF-β1), Insulin-Like Growth Factor (IGF-1) and endothelin-1 and inflammatory mediators such as C-Reactive Protein (CRP), Interleukin (IL)-6, and pro-coagulant factors such as fibrinogen, factor VIII and plasminogen activator inhibitor-1 (19), which also determine insulin resistance (20) must also be considered.

There are different pathophysiological mechanisms underlying the link between MAFLD and atherosclerosis (21). In conditions of insulin resistance, the action of lipases leads to an abnormal flow of fatty acids and the production of chylomicrons (CM) at the intestinal level and VLDL to the liver. Hyperinsulinemia leads to increase fatty acid esterification and inhibit beta-oxidation which determining the triglycerides formation in liver. Dysmetabolic subjects have systematically more active processes of oxidative stress, increase levels of glucose in circulating and increase in blood lipoproteins, leading to foam cell formation and atherosclerotic disease (22).

2.2 Cardiovascular risk in MAFLD

The arterial stiffness and endothelial dysfunction present in MAFLD determine the increase in mortality for cerebro-cardiovascular events. The cerebral hemodynamics modifications are detected by transcranial Doppler. This instrumental investigation allows to measure alterations such as the blood flow velocity of the middle cerebral artery, the Pulsatility Index (PI) and the Resistance Index (RI), markers of cerebrovascular vasoconstriction and the Index of Respiratory Retention (RRI).

Subclinical atherosclerosis is significantly correlated with an elevated risk of cardiovascular disease. Carotid Intima-Media Thickness (CIMT) and carotid plaque are surrogate markers for Acute Coronary Syndrome (ACS) risk (23). Through B-mode ultrasound, it is possible to examine the carotid artery, observing the bilateral parts of the internal, external, common, and bifurcations sites (24). High CIMT is defined as CIMT >1.1 mm and identified as carotid plaque CIMT >1.5 mm. ACS is diagnosed with carotid artery plaque or increased CIMT (24).

Subclinical atherosclerosis is closely related to NAFLD as demonstrated in previously conducted studies (25). This close association also applies to MAFLD as reflected in CIMT alterations in these patients (26). The impact of carotid atherosclerosis in MAFLD are most evident in younger subjects or those with severe fatty liver disease. NAFLD has insulin resistance as its pathophysiological mechanism and is regarded a manifestation of cardiometabolic alteration (27). Unfortunately, however, NAFLD is underestimated as an independent risk factor for ASCVD (28) and better attention should be paid to the diagnosis, monitoring, and management of these patients.

Several studies and meta-analyses have established the correlation between NAFLD and the onset of ACS. Surrogate markers of cardiovascular disease such as the presence of carotid plaque, carotid intima-media thickness, brachial artery vasodilatory responsiveness and coronary artery calcification score have been associated to NAFLD (29). MAFLD has been shown to identify high-risk subjects for fibrosis, metabolic dysfunction, and chronic kidney disease (30).

3 Current treatment of MAFLD

To date, there is no consensus on the best therapeutic approach, whether pharmacological or otherwise, for patients with MAFLD.

The therapeutic goals in patients with MAFLD are to reduce steatosis, chronic inflammation, and fibrosis and control the main cardio-metabolic risk factors. In this way, the reduction of hepatic and extrahepatic complications and cardiovascular mortality is hypothesized. According to clinical practice guidelines, optimal therapeutic strategies to halt the progression and development of MAFLD and CVD include lifestyle modification, smoking cessation, weight reduction, dietary intervention, and exercise (31). Smoking cessation is essential for the prevention of the primary causes of MAFLD and CVD (31). In overweight and obese patients with MAFLD, a weight loss of 7–10% is desirable to attain a reduction in hepatic steatosis and vascular and metabolic complications (31). The recommendations for both the treatment of MAFLD and the prevention of cardiovascular diseases envisage a low-carbohydrate, ketogenic, low-fat, high-protein, Mediterranean diet, which causes a reduction in dyslipidemia, fatty liver disease and of its associated comorbidities (31). High-intensity interval exercise, which has been shown to improve plasma lipid levels and insulin resistance as well as CVD risk factors, such as plasma levels of triglyceride-rich VLDL1 particles and LDL cholesterol, should be recommended in combination with proper diet (32).

In addition to modifying lifestyle, which must gradually leads to weight loss and therefore reduction of visceral fat, in recent years, attention has focused on the use of nutraceuticals and/or innovative therapies with proven improving effects on liver enzymes, hepatic steatosis, the reduction of insulin resistance, the lipid profile and that can in a more specific and tailor reduce the possibility of the onset of a cardiovascular event in patients affected by this condition. There is no pharmacological therapy with special indication for MAFLD. All therapies are off-label.

3.1 Nutraceutical supplements

The use of nutraceuticals for the management of NAFLD is a proposition that should not be underestimated. Nutraceuticals alone or in combination with diet and lifestyle modifications promoting weight loss and reducing insulin resistance (33). The use of

nutraceuticals in terms of therapeutic intervention in the presence of NAFLD is evidence-based. However, partly due to the relative limitations of studies in this area compared to marketing studies on new drugs and partly due relatively small sample size with a short interval of follow-up, studies are basing their conclusions on surrogate endpoints, rather than purely clinical outcomes (34). The nutraceuticals considered are those bring a significant advantage on cardio metabolic risk, that contribute to the improvement of hepatic steatosis and act by improving metabolic factors. Some of these have rather low bioavailability, which could compromise their effectiveness. Individual genetic composition influences how nutraceuticals are assimilated, stored, and excreted and represents an individualized approach to disease.

Nutraceuticals have effects on health through different actions (inflammation, glycemia and insulinemia, LDL-C, hypertension). In addition, they have complementary actions and can have effects on different biomolecular targets (35).

Silymarin is an antioxidant composed of seven flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, iso-silychristin, and silydianin) and one flavonoid (taxifolin). It represents one of the highly used natural compounds in the management of liver disorders for its anti-inflammatory, antioxidant, antifibrotic and insulin-sensitive properties. In preclinical studies performed in mice models, it has been shown that a nutraceutical containing silymarin together with chlorogenic acid, guggul, curcumin and inulin was able to prevent NAFLD and atherosclerosis (36). Also, in patients with NAFLD, the combination of silybin, phosphatidylcholine and vitamin E, administered for 12 months, reduced transaminases, GGT and the degree of steatosis (37). A meta-analysis of eight RCTs showed a significant decrease in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Several other studies confirmed a reduction in HOMA-IR, glycemia and insulinemia in patients affected by NAFLD (38).

Berberine is a quaternary ammonium salt of the isoquinoline alkaloid group known for its lipid-lowering and insulin-sensitizing activity (39). Treatment with 0.5 g of berberine resulted on a significant improvement in the lipid profile and a significant reduction in body weight, HOMA-IR, and hepatic steatosis (40). Furthermore, the coadministration of berberine and silymarin has been shown to be associated with several significant ameliorations in both the lipid and glucose profiles, suggesting that cardiometabolic and health level can be promoted with the potential use of this nutraceutical combination (41).

Curcumin, extracted from *Curcuma longa*, is an insulin-sensitizing nutraceutical that significantly reduces hepatic steatosis (42). Supplementation with curcumin for 8 weeks resulted in a significant improvement in insulinemia, HOMA-IR, waist circumference, blood pressure, TG, HDL-C, hepatic transaminases, GGT and hepatic steatosis in subjects with prediabetes (43).

Nigella sativa belongs to the Ranunculaceae family, whose antioxidant and anti-inflammatory outcomes are attributable to thymoquinone and is used for the treatment of liver diseases (44). Supplementation with *Nigella sativa* significantly improves transaminases, fasting blood sugar, lipid profile, high-sensitivity C-reactive protein and the degree of hepatic steatosis (45).

The combination of *Ascophyllum nodosum* and *Fucus vesiculosus* which slows the intestinal absorption of cholesterol by increasing intestinal viscosity and reduces the absorption of sugars by inhibiting the enzymes α-amylase and α-glucosidase, significantly reduces insulinemia, HOMA-IR, blood glucose, and also waist circumference. In addition, it significantly increases plasma HDL-C levels after 6 months of therapy (46).

Coenzyme Q10 has anti-inflammatory properties taken into consideration in managing hepatic steatosis and metabolic alterations (47). Coenzyme Q10 regulates adipokine levels and decreases oxidative stress in patients with metabolic syndrome. The intake of 100 mg/day of coenzyme Q10 for 3 weeks led to a reduction in transaminases and GGT, an improvement in the adiponectin/leptin ratio and better glucose control (48). Coenzyme Q10 has a high safety profile without relevant pharmacological interactions. However, its relatively low bioavailability raised limitation concerns for its use.

Polyunsaturated fatty acids of the omega-3 series could play a role in the treatment of metabolic and hepatic disorders characterizing MAFLD (49). Long-term supplementation with omega-3 is associated with a significant improvement in AST, ALT, and the degree of hepatic steatosis (50), TG, HOMA-IR and glycemia (51).

The synergistic nutraceutical combination therapy prescribed for MAFLD could benefit a series of diseases perhaps unknown to the patient and indirectly treated can help in the prevention of cardiovascular risk factors. Silymarin, polyunsaturated fatty acids of the ω-3 series, coenzyme Q10, berberine and curcumin possess hepatoprotective activity and exert a favorable action on the CV system. Traditional Chinese herbal combinations such as Artemisia capillaris (Thunb), *Gardenia jasminoides* (Ellis), and *Rheum palmatum* (L) exert anti-NAFLD effects (33).

The consumption of chlorogenic acid and its derivatives and luteolin and its derivatives by individuals with MetS with a follow-up period of 6 months shown a significant improvement in body weight ($p < 0.001$), waist circumference ($p = 0.003$), HbA1c ($p < 0.001$), plasma lipids ($p < 0.001$ for Tchol. LDL and TG), Fatty Liver Index (FLI), a surrogate marker of fatty liver disease ($p < 0.001$), liver transaminases, flow-mediated dilatation ($p < 0.001$), and carotid intima-media thickness ($p < 0.001$), regardless of the degree of fatty liver disease (52). The effect of a supplement containing *Curcuma longa*, silymarin, guggul, chlorogenic acid, and inulin was evaluated in patients with MetS for 4 months. There were significant reductions in body weight ($p < 0.0001$), Body Mass Index (BMI) ($p = 0.001$), waist circumference ($p = 0.0004$), fasting glucose ($p = 0.014$), and total cholesterol ($p = 0.03$) (53).

3.2 Pharmacological treatment

Drug therapies for MAFLD management tend to decrease fat accumulation in the liver, activate metabolic pathways, and improve liver damage.

Acetylsalicylic acid (aspirin) is recommended in an established atherosclerotic disease, reduces liver fibrosis, and prevents cardiovascular events (31). Statins are key elements in the pharmacological modification of cardiovascular risk and are recommended for patients with MAFLD and NASH (54). In subjects with elevated transaminases, atorvastatin reduced cardiovascular risk due to both cardiological and hepatological benefits (31). The use of ezetimibe is tolerable and effective for the prevention of CVD in MAFLD. It reduces hepatic lipid synthesis and improves liver histology (55).

Bempedoic acid, an inhibitor of ATP citrate lyase (an enzyme involved in the synthesis of cholesterol upstream of HMGCoA reductase), reduces LDL cholesterol by 28% in monotherapy and in combination with statins and ezetimibe (56).

Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) influences muscle and hepatic lipid accumulation and contribute to the pathogenesis and progression of MAFLD. No liver-related negative signals were reported in the Fourier (Evolocumab) and Odyssey (Alirocumab) studies, and it appears to be safe in the patients with liver disease (31). A beneficial activity is exerted by Pemafibrate, which is much more selective for PPAR alpha than PPAR gamma or PPAR delta (56). Pemafibrate improved macrophage accumulation, and ballooning degeneration of hepatocytes without a noticeable change in TG accumulation in the liver (57). It also improved markers of liver inflammation, function and fibrosis (58).

Obeticholic acid (OCA), a semisynthetic variant of the naturally occurring bile acid chenodeoxycholic acid, increased insulin sensitivity and decreased markers of liver fibrosis in NAFLD patients with T2DM (59).

The presence of MAFLD in T2DM raise the risk of microvascular complications (especially diabetic nephropathy), increases cardiovascular risk, and has a three times greater risk of presenting itself in the form of advanced fibrosis or cirrhosis or hepatocellular carcinoma. The hypoglycemic drugs used for the treatment of diabetes, a disease often associated with the onset of MAFLD, have been used for a long time in the treatment of MAFLD. Metformin used in MAFLD patients with T2DM had a greater benefit on improving aminotransferase levels than vitamin E treatment (60). In MAFLD, metformin directly reduced fat deposition and inhibited inflammation in the liver by enhancing phosphorylation of hepatic 5′ adenosine monophosphate-activated protein kinase (AMPK) and ACC and reduced lipogenic enzymes and proinflammatory cytokines (61). Sodium-glucose cotransporter-2 (SGLT2) inhibitors improve liver enzymes AST and ALT and liver fat accumulation (62).

SGLT2 inhibitors reduce body fluids and body weight and may be recommended in obese subjects with MAFLD (63). Treatment with canagliflozin for 20 weeks delayed the onset of NASH and caused reduction in liver enzymes [ALT, AST, Alkaline Phosphatase (ALP), and GGT] and body weight, and increased bilirubin (64). Empagliflozin reduces liver fat content and improves hepatic steatosis and fibrosis, decreases AST and ALT in MAFLD patients with or without T2DM (64). Furthermore, it decreased the expression of hepatic inflammatory genes such as TNF-α, interleukin-6 and Monocyte Chemoattractant Protein-1 (MCP-1) in NASH and in combination with linagliptin, a DPP-4 inhibitor, it reduces mRNA expression for genes associated with fatty acid synthesis, collagen deposition and expression of Alpha Smooth Muscle Actin (αSMA), which is an indicator of fibrosis (64). Empagliflozin reduces insulin resistance and attenuates inflammasome and triglyceride NLR family pyrin domain containing 3 (NLRP-3) activation in the liver (64). Empagliflozin has shown a beneficial effect on steatosis, ballooning, and fibrosis (65). Ipragliflozin reduced thiobarbituric acid reactive substances and carbonyl and inflammatory protein markers in NASH (66).

Another class of drugs that benefit patients with MAFLD are the Glucagon-Like Peptide-1 (GLP-1) Receptor agonists (GLP-1RAs). GLP-1RAs delay the progression of MAFLD by inhibiting inflammation, insulin resistance, oxidative stress, enzymes participated in hepatic lipogenesis, and stimulating the autophagy/mitophagy pathway, as well as enzymes responsible for β-oxidation (67–71). There is a downregulation of GLP-1 receptors in MAFLD (72): GLP-1 analogs inhibit MAFLD by inhibiting the NLRP3 inflammasome through potentiation of autophagic/mitophagic pathways, increasing antioxidant defense in the liver and inhibiting macrophage recruitment and activation (73). They improve insulin sensitivity by reducing JNK phosphorylation and enhancing PPARγ expression and activity (74). These drugs decrease liver enzymes, body weight, and liver content. Exenatide treatment reduced fatty liver disease associated with decreased body weight, visceral fat, and fasting glucose levels (75). In the Liraglutide Efficacy and Action in NASH (LEAN) study, 39% of patients treated with liraglutide showed resolution of steatohepatitis on liver biopsy with improvements in blood glucose, HbA1c, GGT, and HDL, along with a weight loss of approximately 5 kg (76). Use of Liraglutide in patients with T2DM and NAFLD reduced CIMT, a surrogate marker of atherosclerosis, independently of glucometabolic changes (77). The use of semaglutide in patients with diabetes reduces hepatic fibrosis and steatosis parameters and the anthropometric, hepatic, and glycemic indices, as well as plasma lipids independent to the variations in CIMT and HbA1c (78).

Dipeptidyl Peptidase-4 (DPP-4) inhibitors work by impeding the activities of (DPP-4) to increase incretin levels and decrease glucagon release by increasing exocytosis of the insulin and fatty acid oxidation in the liver, decreasing gastric emptying, and lowering hepatic glucose production (79). Sitagliptin, a glyptin-based drug, has been found to cause reduction in liver enzymes, body weight, and hepatocyte swelling in patients with diabetes and NASH (80), but randomized clinical trials of this class of DPP-4 inhibitors in patients with NASH are needed.

4 Conclusion

MAFLD has a very important impact on the healthcare system. MAFLD is a fairly complex pathology, a condition for which strict metabolic control a tailored therapy that can also be efficient in the prevention of cardiovascular complications as well as in the treatment of the pathologies present in its corollary as T2DM is required (Figure 2). The replacement of the term "non-alcoholic" with the term "metabolic" of fatty liver disease brings with it series of psychological mechanisms that favor greater awareness and attention to the disease on the part of patients, doctors, and the pharmaceutical industry.

There are no specific therapies for MAFLD. It is clear that drugs used for the treatment of diabetes, obesity, insulin resistance, hypercholesterolemia and hypertension could be proposed for the treatment of MAFLD and studies on the mechanisms that regulate lipid accumulation in the liver may lead to promising future therapies. Incretin therapies have an anti-inflammatory, lipid-lowering action, and act directly on the atheromatous plaque, blocking its progression. In addition, they act on body weight directly on the metabolism of adipocytes and have a direct effect on hepatic steatosis. The PCSK9 inhibitor acts instead by modulating both the internalization of the LDL receptor at the liver level, but also by improving hepatic steatosis. All this leads to a reduction in serum and endothelial LDL with a reduction in the risk of plaque formation and progression. Innovative therapies have proven to be effective in managing patients with

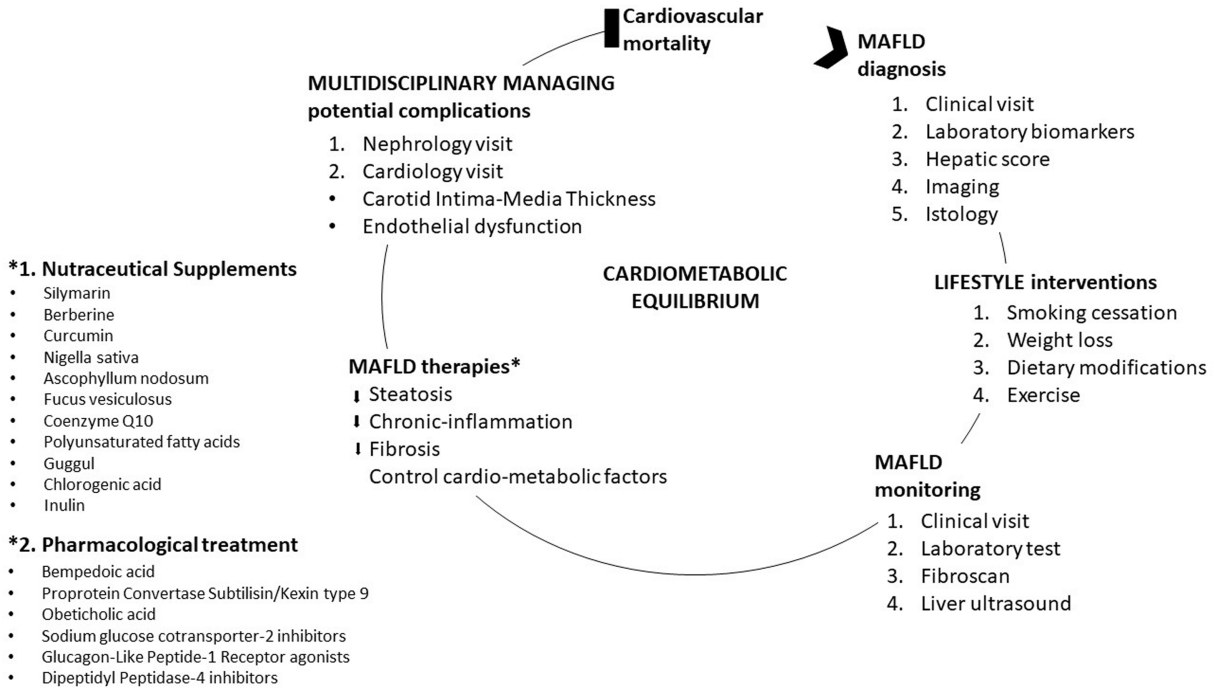

FIGURE 2
Management of metabolic dysfunction-associated fatty liver disease (MAFLD).

diabetes, obesity, insulin resistance and hypercholesterolemia, but also in patients with MAFLD (56–81).

The evidence currently available regarding the use of nutraceuticals suggests hepatoprotective effects and positive outcomes on the metabolic front (33–53, 82, 83). Further studies are required to confirm observations and optimize nutraceutical treatment for MAFLD and to ensure their long-term efficacy and safety. In addition, it is wise to emphasize that dietary modification represents a very important lifestyle changes that over the years has been associated with lower cardiometabolic risk. The burden of cardiometabolic diseases is lowered by appropriate diet rich in proteins of animal and vegetable origin and poor in meat (84). The Mediterranean diet is still proposed by international guidelines as the treatment of choice (85, 86). To successfully alleviate MAFLD and associated comorbidities, new molecular markers should be identified and use as specific targets for the treatment of this pathology with high cardio-metabolic risk (87, 88).

Author contributions

KAH: Supervision, Writing – original draft. RVG: Conceptualization, Supervision, Writing – original draft. APS: Supervision, Writing – review & editing. AMP: Conceptualization, Supervision, Writing – original draft. KAW: Writing – review & editing. KAR: Writing – review & editing. MC: Conceptualization, Supervision, Writing – review & editing. MR: Conceptualization, Supervision, Writing – review & editing.

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Mechanisms of body fat distribution and gluteal-femoral fat protection against metabolic disorders

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Obesity is a major health problem that affects millions of individuals, and it is associated with metabolic diseases including insulin resistance (IR), type 2 diabetes (T2D), and cardiovascular diseases (CVDs). However, Body fat distribution (BFD) rather than crude obesity is now considered as a more accurate factor associated with these diseases. The factors affecting BFD vary, from genetic background, epigenetic factors, ethnicity, aging, hormonal changes, to lifestyle and medication consumptions. The main goal of controlling BFD comes from the fact that fat accumulation in different depots has a different effect on the overall health and metabolic health of individuals. It is well established that fat storage in the abdominal visceral depot is associated with metabolic disorder occurrence, while gluteal-femoral subcutaneous fat depot seems to be protective against these diseases. In this paper, we will summarize the factors affecting fat distribution. Then, we will present evidence connecting gluteal-femoral fat depot with protection against metabolic disorders including IR, T2D, and CVDs. Finally, we will list the suggested mechanisms that lead to this protective effect. The abstract is visualized in Graphical Abstract.

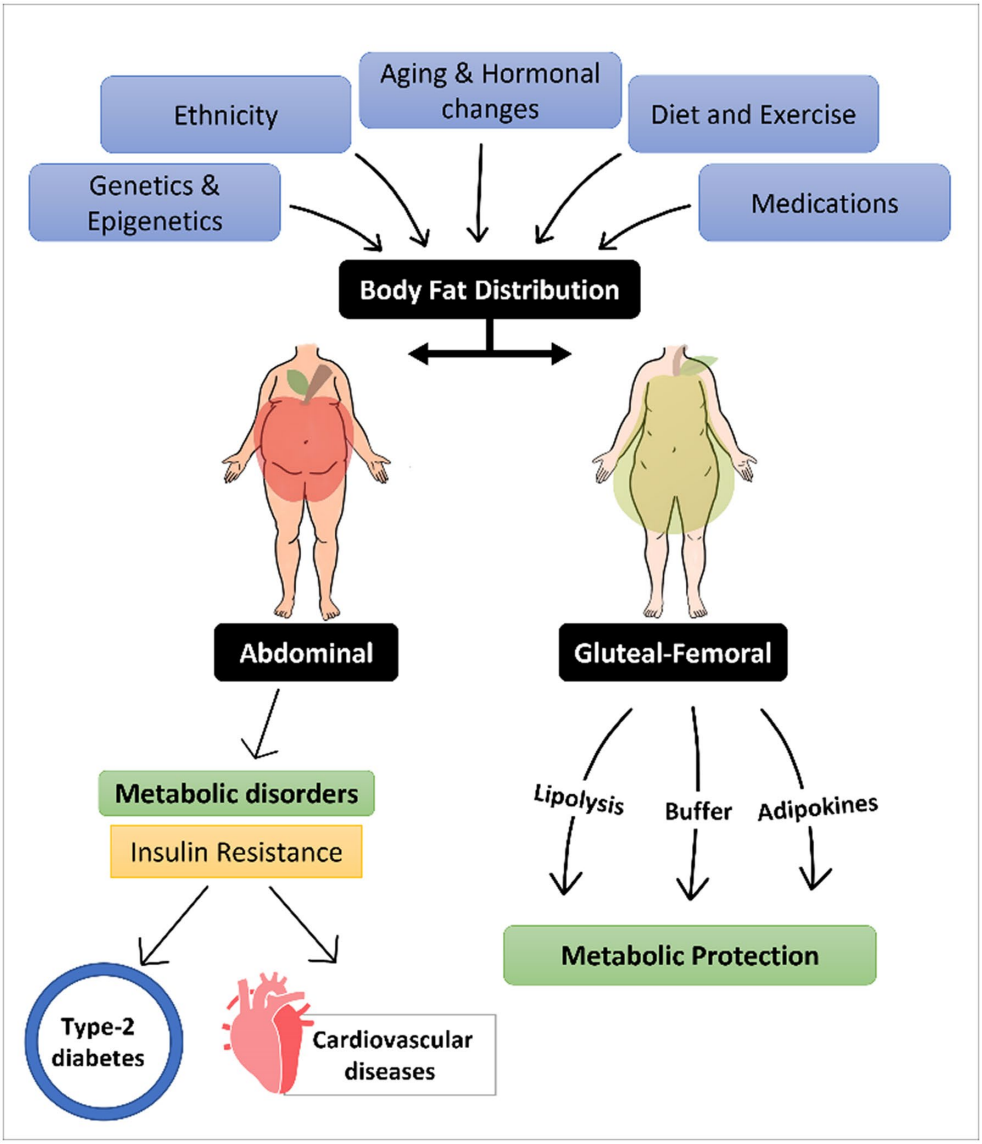
KEYWORDS

metabolic protection, prediabetes, type 2 diabetes, cardiovascular diseases, body fat distribution, thigh fat

1 Introduction

Obesity is a major public health problem, affecting millions of individuals worldwide. According to World Obesity Atlas (1), the proportion of the obese population (BMI $\geq$ 30) will reach 17% in 2025, and 20% in 2030 affecting more than 1.5 billion people. It is noteworthy to mention that COVID-19 pandemic has worsened the obesity epidemic, and the restrictions from 2020 to 2022 have increased the risk of weight gain by increasing sedentary and unhealthy dietary behaviors, and significantly reduced access to health care (2).

Obesity is a complex multifactorial disease, meaning it is caused by multiple interacting factors, including genetics, environmental, and behavioral factors (3). The long-term serious effect of obesity lies in the increasing likelihood of many diseases, including insulin resistance (IR) and type 2 diabetes (T2D) (4), chronic kidney disease (CKD) (5), non-alcoholic fatty liver disease (NAFLD), and some cancers (6). Additionally, both obesity and T2D increase the prevalence of cardiovascular disorders (CVDs) (7). Although obesity and BMI represent a good indicator of the overall metabolic health profile, studies have shown that the distribution of body fat into fat depots plays a more important role in the co-morbidity of the metabolic



GRAPHICAL ABSTRACT
Graphical abstract showing a summary of the factors affecting body fat distribution, the difference between abdominal and gluteal-femoral obesity on metabolic disorders progression, and the protective mechanisms of gluteal-femoral fat.

diseases (8). This indicates that BFD measurements along BMI need to be considered to assess metabolic health. These measurements include simple waist to hip ratio, where the waist circumference (reflecting abdominal adiposity) is divided by the hip circumference [reflecting gluteal femoral (GF) fat adiposity]. Other more accurate measurements include body imaging to measure actual fat depot sizes.

Adipose tissue functions not only as a fat storage organ, but also as a dynamic organ (9); therefore, fat storage in different depots have diverse impact on human health. In fact, each fat depot contributes differently as metabolic and endocrine organ, leading to different levels of metabolic disorders (10). It is well established that over-accumulating fat in the abdominal fat depot is associated with metabolic disorders; Increases of abdominal adiposity as opposed to overall adiposity, is associated with increases in the likelihood of getting T2D and CVDs 4 (11). Some studies showed hip circumference and thigh fat adiposity alone as an independent indicator of obesity-related metabolic risks (12). More recent evidence suggests that GF fat depot shows a protective role against these metabolic disorders; fat storage in the GF depot is preferred over storage in the abdominal depot to gain a metabolically healthy state (13–16). These opposing relationships reflect the unique intrinsic characteristics of different fat depots.

Explaining the factors that lead to distinct BFD and resolving the mechanisms behind the protective role of some depots, may help in finding novel therapeutic venues for preventing or treating obesity-related diseases. In this review, we summarized the important factors affecting BFD, whether uncontrollable (genetics, epigenetic factors, age, and hormonal profile) or controllable (diet, exercise, and consumption of certain medications). Then, we compiled detailed evidence supporting the protective role of the GF fat depot against metabolic disorders, including IR, T2D, and CVDs. Finally, we summarized the reported possible mechanisms explaining how this protection is accomplished.

2 Fat depots and gluteal-femoral fat biology

Adipose fat depots are the specific location where the fat tissue is built and located in the body. The human body stores fat in 2 anatomically and physiologically distinct fat depots; subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT). SCAT is located between the skin and superficial musculature, and it can be further classified into superficial SCAT and deep SCAT (17). The main depots where SCAT is stored are the inguinal region (GF regions), and the anterior and the back of the abdominal wall as shown in Figure 1. On the other hand, VAT depot, also known as intra-abdominal fat, is the fat tissue stored within/between the visceral organs, such as liver and intestines (8). Physiologically, VAT is more vascular, innervated, and contains less preadipocyte differentiating capacity, greater percentage of large adipocytes and larger number of inflammatory and immune cells as compared to SCAT (18). Individuals with enhanced abdominal obesity, more specifically with high VAT depot content are referred to apple shaped, as compared to pear shaped individuals that express higher GF content (19), as illustrated in Graphical Abstract.

The GF fat as a combined depot is referred to as gluteal-femoral fat (GF fat/GF adipose tissue) as shown in Figure 1. In literature, GF fat is referred to as thigh, hip, or lower body fat, and could be further classified into different depots. These depots differ in their biology, histology, and physiological role. GF subcutaneous adipose tissue (GF-SCAT) is the fat tissue stored under the skin of the lower body part. Another thigh fat depot is the thigh intermuscular adipose tissue (thigh IMAT) illustrated in Figure 1, which is considered as an ectopic fat depot; fat stored in tissues other than specialized adipose tissue, skeletal muscle tissue in this case (8). In the following section, we discussed the factors affecting BFD, favoring specific depots over the others.

3 Factors affecting body fat distribution

Body fat distribution (BFD) is affected and mediated by multiple factors. Genetic background plays a significant role in determining BFD. Studies have shown that certain genes are associated with increased abdominal fat, while others are associated with increased fat in the hips and thighs (20–25). Epigenetic differences, which impact obesity, may also have an influence on body fat distribution (26–28). Ethnicity, aging, and hormonal changes also play a role in BFD (7, 29, 30). On the other hand, certain factors can be induced to manipulate

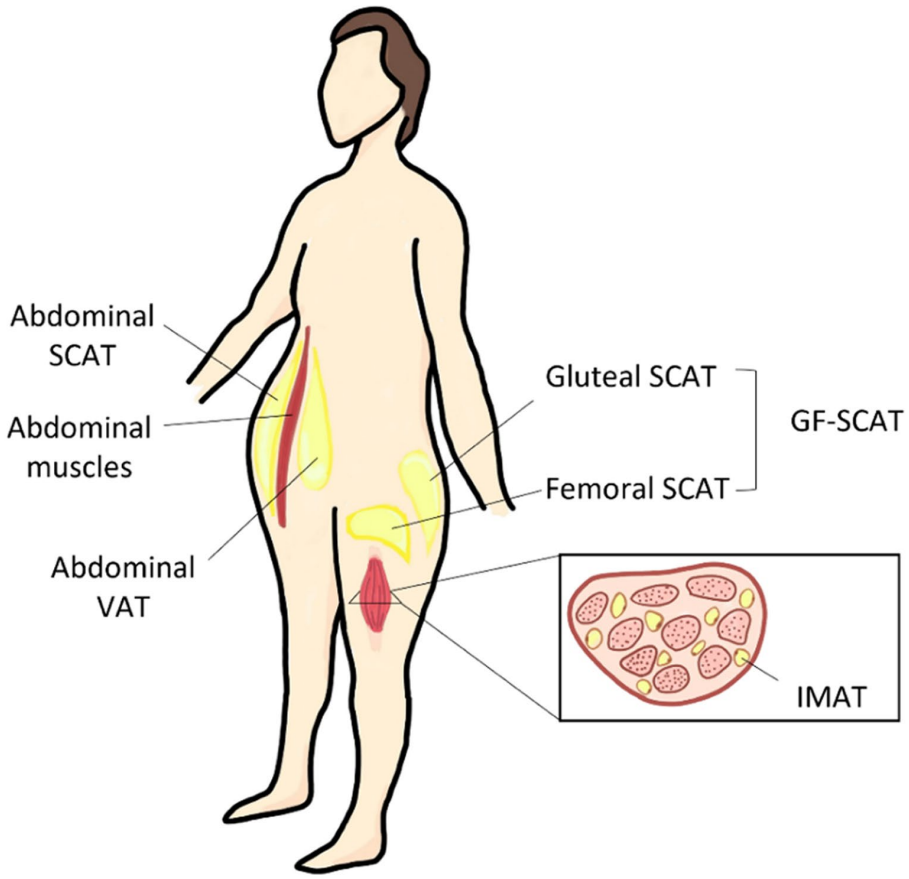


FIGURE 1
Different body fat depots and their anatomical location. SCAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; GF, gluteal-femoral adipose tissue; and IMAT, intermuscular adipose tissue.

BFD. Diet restriction and exercise can lead to fat reduction in the abdominal region (31). Certain medications, such as those used to treat diabetes or high blood pressure, can also lead to a redistribution in body fat (19). The factors that affect BFD are summarized in Table 1.

4 Gluteal-femoral fat protection against insulin resistance

Insulin resistance (IR) is a defined as a state where the body cells become irresponsive to the physiological insulin levels secreted in the blood (46). IR is a risk factor for many chronic metabolic disorders. The major consequence of IR is the progression of type 2 diabetes, where individuals diagnosed with IR are expected to develop T2D in 5 ~ 10 years (46). T2D is highly diverse disease. To personalize the treatment of T2D, a study attempted to classify the disease into clusters, depending on six variables: including glutamate decarboxylase antibodies, age, BMI, HbA1c, and homoeostatic model assessment 2 estimates of β-cell function and insulin resistance. The significance of this classification is to generate a way to estimate the risk of diabetic complications, which they successfully showed in their study. According to the researchers, one cluster (highest insulin resistance) showed more association to kidney disease, another cluster (insulin deficient) showed the highest risk of retinopathy, while a third cluster associated with higher fat liver, indicating a relevance of VAT to IR (47).

Previously, obesity was believed to be a major leading cause of IR. However, recent investigation showed that abdominal obesity is positively associated with IR, while considerable debate remains concerning the potential of a positive effect of thigh fat in metabolic protection against IR progression (19). This theory explains the metabolically healthy obese individuals and how their bodies escape the negative metabolic consequences of obesity (48). Thigh fat is protective against IR; a study demonstrated that lower thigh SCAT and higher VAT both contribute to insulin resistance profile. The study was conducted on a cohort from the general population, it demonstrated that high thigh SCAT alone or low VAT alone were positively associated with IS state while Individuals with combination of high SCAT and low IMAT had the highest IS state among all groups (49). A more recent study investigated both thigh fat depots (thigh SCAT and thigh IMAT) in a female cohort. They showed that higher thigh-SCAT and lower thigh-IMAT are associated with insulin sensitivity (14). The same findings were supported with studies done on children obese cohort (age of 7–17 years old), where a study suggested a protective effect of thigh fat, and its association to a healthy metabolic profile [more favorable HOMA-IR score, triglyceride serum level, systolic blood pressure (SBP), C-reactive protein (CRP), resistin, high-density lipoprotein (HDL), cholesterol, adiponectin, and blood lipid profile] (15). Whereas another study conducted on postmenopausal women suggested that thigh fat was associated with a favorable metabolic profile, including serum triglycerides, HDL-cholesterol, and various IR markers (50). Due to the reported association between thigh fat and protection against IR,

TABLE 1 Summary of the factors affecting body fat distribution.

Factor	Description	References
Genetic	Many loci associated with BFD, that are greatly distinct from those of BMI and obesity risk. *S70C* variant in Coiled-Coil Domain Containing 92 gene is associated with decreased visceral fat (VAT) and increased leg fat (GF-SCAT). Dynein axonemal heavy chain 10 (*DNAH10*), Plexin-D1 (*PLXND1*), R-spondin 3 (*RSPO3*), and lysophospholipase-like 1 (*LYPLAL1*) genes are high determinants of BFD. HOXA5 expression is upregulated in abdominal subcutaneous adipose tissue compared to gluteal subcutaneous adipose tissue.	(20–22, 24, 26, 29)
Epigenetic	DNA promoter methylation of IRS1 in human adipose tissue is related to fat distribution.	(32)
Ethnicity	Asian ethnic group has more visceral fat as compared to Europeans. Asian women have less GF-SCAT, and greater abdominal VAT compared to Caucasians and African Americans. Mexican Americans have more visceral obesity as compared to non-Hispanic white Americans.	(13, 29, 33)
Aging and hormonal changes	Aging leads to changes in BFD, with a shift from subcutaneous to visceral fat, especially in men. Estrogen accumulates fat in GF-SCAT depot, and not in abdominal fat depot. Menopausal transition is associated with fat accumulation in the central body depot (abdominal) as compared to thigh depot.	(7, 30, 34)
Diet and exercise	Aerobic exercises and dietary restrictions have been found to be independent factors contributing to a substantial decrease in visceral adipose tissue (VAT). Combined effect of exercise and diet may have more effect on BFD. The Mediterranean diet leads to mobilizing ectopic fat to other healthy depots, while exercise only leads to ectopic fat loss.	(31, 35–37)
Medication	Glucocorticoid VAT mass through lipolysis stimulation by activating the hormone-sensitive lipase and increasing catecholamine sensitivity. Metformin significantly decreases abdominal obesity, including SAT and VAT reduction. Thiazolidinediones were associated with a reduction of VAT/SAT ratio in a dose-dependent manner. SGLT2 inhibitors were associated with a significant reduction of liver and pancreatic fat. Glucagon-like Peptide Receptor Agonists (GLP-1) lead to a significant reduction in VAT. New GLP-1/GIP/glucagon triple agonist therapy normalizes body weight. DPP-4 inhibitors, in combination with metformin are associated more reduction in visceral fat compared to metformin alone.	(38–45)

a study suggested that regional adiposity, including thigh fat size could be used as a predictive way to predict the risk of developing IR. They reported that each increase in thigh fat led to a 59% decrease in the odds of becoming insulin resistant, independently from confounders (age, BMI, visceral adiposity, and gender) (51).

It has been reported that a larger gluteal femoral fat is associates with lower prevalence of IR, prediabetes, and dyslipidemia (52). However, as explained in the previous section, not all thigh fat seems to be protective. A study assessed critical biomarkers and how their levels are different among individuals with different BFDs. The findings suggested a significantly lower insulin resistance and triglycerides levels in individuals with higher GF fat (13), but according to Snijder et al. (52), only thigh-SCAT was found to be associated with favorable metabolic profiles. Higher thigh-SCAT was shown to be associated with lower transformed triglycerides (in both genders), higher HDL-cholesterol, and lower blood glucose level (in males only).

The revised studies showed how GF-SCAT is beneficial as a protective fat depot against metabolic disease. In contrast, IMAT is an ectopic depot for fat storage. In contrary with SCAT, fat deposition in IMAT is highly associated with increase IR and T2D (53). One suggested mechanism of how IMAT promotes IR progression is because fat accumulation within the thigh muscular tissue leads to reduced blood circulation, and decreased insulin uptake efficiency by the muscle cells (54). These studies confirm the association between GF SCAT levels, not GF IMAT, and developing IR leading to multiple metabolic disorders, including type 2 diabetes and cardiovascular disease as explained in the following section.

5 Gluteal-femoral fat cardio protection

Cardiovascular diseases (CVDs) are a general term that describes a range of pathologies affecting the health of the heart, the blood vessels, or the pericardium. It is critical to study the risk factors affecting and leading to CVDs as these diseases can be fatal. Previous studies have shown a clear association between BFD and the risk of developing CVDs (19, 50, 55). While increased fat storage in the abdominal depot; mainly ectopic visceral fat is associated with high CVDs progression risk, high GF fat storage was shown to be cardioprotective (56).

The cardioprotective role of GF fat has been confirmed in many studies involving diverse human subjects with different ages, BMIs, and diseases. As early as 1991, thigh fat has a negative association with cardiovascular diseases was first hypothesized. High thigh fat was shown to contribute to lipoprotein plasma profile; an indicator of lower risk of cardiovascular diseases (57). Following that finding, more research groups started showing the same correlation among different models. Accumulating evidence shows an association between increased lower body adiposity and GF fat and healthy lipid profile. Many studies showed evidence that elevated GF fat is associated with better serum lipid levels, including lower low-density lipoprotein-cholesterol profile, and higher high-density-cholesterol levels, which are indicators of improved cardiovascular health[8]. Van Pelt et al. (50) showed an enhanced triglycerides level in individuals with higher GT fat as compared to others with the same BMI.

In addition to improved serum lipid profile, increased GF mass has been shown to be directly associated with enhanced vascular health, including lower aortic calcification, decreased progression of present aortic calcification, as well as decreased arterial stiffness in a women cohort (58). Furthermore, studies showed a direct effect between GF fat and cardiac diseases. Yusuf et al. (59), conducted a study that involved 27,000 participants, they showed a negative association between hip circumference and the risk of myocardial infarction. Another study confirmed that a larger hip circumference is associated with lower risk of developing coronary heart disease in a population involving both genders (60). Shay et al. (16) reported that women characterized with higher GF fat content and suffer from type-1-diabetes have shown less risk of developing coronary artery disease, which was not shown in men.

The protective effect of GF fat against CVD risk persists even with aging. A study was conducted on healthy elderly women, they confirmed a strong association between GF-fat and protection against vascular damage (55). These studies confirm the tight association between GF levels and the risk of CVDs. More studies were well revised by Manolopoulos et al. (61). In the following section, we will revise the reported mechanisms that explain this association, as well as the possible mechanisms that need more investigation to be proven.

6 Mechanisms of gluteal-femoral fat metabolic protection

As mentioned previously, thigh fat shows protective properties against IR and CVDs. While the mechanism behind this association remains not fully understood, research suggests three major mechanisms by which this protection is achieved. These mechanisms ultimately enhance metabolic health and lower the risk of developing IR and CVDs.

6.1 Catecholamine-mediated lipolysis

Lipids are mobilized by adipocyte lipolysis, a fundamental process of hydrolyzing triacylglycerol to fatty acids for internal or systemic energy use. The rate of lipolysis is low in the subcutaneous femoral-gluteal region, intermediate in the subcutaneous abdominal region and high in the visceral region (62). Indeed, abdominal adipocytes showed β-adrenergic lipolytic sensitivity 10–20-fold greater than gluteal adipocytes; this is due to an increase in the total number of β-adrenoceptors in this depot (63).

A study showed that the steady-state mRNA levels of beta-adrenergic receptors BAR 1 and BAR 2 were about twice as high in abdominal as in gluteal adipocytes of men and women ($p < 0.01$) explained by an increased expression of the genes that encode for *BAR 1* and *BAR 2* (64). In women, variation in the affinity properties of the α-2 adrenoceptor is an additional factor. Abdominal adipocytes showed a 40 times lower α-2 adrenergic antilipolytic sensitivity than did gluteal adipocytes (63).

The decreased action of β-adrenergic receptors and increased activity of α_2-adrenergic adrenoceptors in combination with defects in hormone sensitive lipase function inhibits the lipolytic effect of catecholamines in subcutaneous fat cells, whereas increased activity of β-adrenergic receptors and decreased activity

of α_2-adrenoceptors increases the lipolytic response in visceral fat cells. These abnormalities in catecholamine function promote release of free fatty acids from the visceral fat cells to the liver through the portal system and might cause several of the metabolic complications to upper-body obesity (62).

6.2 Gluteal-femoral adipose depot acts as a buffer to control excess lipids

The protective properties of GF fat against insulin resistance and cardiovascular diseases relies mainly on the fact that it is physiologically different from other fat depots, especially abdominal fat depot. This mechanism suggests that the protection is due to the nature of thigh depot. Most of the thigh stored fat is stored in the subcutaneous fat depot (GF SCAT) rather than the ectopic thigh depot (GF IMAT). Fat storage in the GF SCAT depot keeps the excess fat away from the visceral region, which is linked to high risk of metabolic disorder progression. Additionally, accumulating evidence from *in vivo* and *in vitro* studies showed a differential regulation of GF SCAT fat mechanisms of fatty acid uptake and release at the adipocyte level. In case of increased dietary lipid intake, GF SCAT depot becomes a long-term store of free fatty acids, preventing them from accumulating in ectopic fat depots, especially in the viscera and within vital organs (65). This is likely since GF fat depot has a relatively high lipoprotein lipase activity as compared to other depots, making it a better reservoir of excess circulating fatty acids. This storage prevents lipid overflow within abdominal visceral depot, causing reduced lipotoxicity, as well as decreased risk of IR and CVDs on the long run (13).

This theory simply shows that the protective association is due to the absence of the risk, rather than an actual protection mechanism (66). However, more recent research has shown other mechanisms that contribute to this protection. According to Tran et al. (67), the protective effect of GF SCAT is not due to its depot, but rather to intrinsic differences between this depot and visceral fat depot. This research group assessed how transplanting GF SCAT from healthy mice to the visceral depot of metabolically unhealthy mice, which lead to enhanced metabolic profile in the recipient mice, including decreased total body weight, fat mass, blood glucose, and insulin levels. These findings recommend that the difference is not as simple as GF SCAT working as a buffer to control excess fat from getting stored in the visceral region. GF SCAT certainly plays a more crucial role at the physiological level to gain this protection and improve insulin sensitivity. Recent studies suggest that GF SCAT is intrinsically different from other depots as is it plays a critical role as an endocrine organ that is associated with a protective adipokine profile. The protective role of GF SCAT as an endocrine organ is discussed in detail in the next section.

6.3 Gluteal-femoral adipose depot contributes to lipoprotein profile and secretes protective adipokines

Lipoproteins are molecular structures that consist of proteins associated with fat with the main function to transport lipids (triglycerides and cholesterols) throughout the body (68) and serve an important role in metabolic health. The two major types of lipoproteins are the high-density lipoprotein (HDL) and low-density lipoprotein (LDL), which are often referred to as "good" and "bad" cholesterol in the metabolic disorder context. Terry et al. (57) showed a positive correlation between thigh fat and HDL levels, and where the first to suggest that thigh fat contributes to plasma lipoprotein profiles and might predict lower risk of metabolic disorders, mainly CVDs.

Conversely, Adipokines are special cytokines secreted by adipose tissue components, including adipocytes and other cells as macrophages. Adipokines (adiponectin and Leptin) are mainly produced by the adipocyte component of the adipose tissue, while interleukins (such as IL-6) are produced by residential macrophages (61). It is known that a healthy adipokine profile is associated with reduced risk developing CVDs. Recent studies suggested that GF depot has a different secretome profile than other depots. It was shown to be associated with elevated levels of beneficial adipokines, and less pro-inflammatory adipokines as opposed to abdominal fat depot.

6.3.1 Leptin

Leptin is a critical adipokine in terms of energy metabolism by controlling energy intake and storage as a fat mass (69). Leptin is predominantly expressed by isolated subcutaneous adipocytes as opposed to omental adipocytes, particularly in women (70). Leptin secretion levels are higher in SCAT as compared to VAT. In terms of mRNA expression of Leptin, no previous studies have compared leptin expression level among different SCATs. However, some studies indirectly suggested that the basal expression level of leptin does not differ among SCAT, and it is the same among abdominal SCAT and thigh SCAT (71). As a secreted adipokine, leptin levels negatively correlate with waist to hip ratio (72). A study conducted by Picó et al. (73) reported that the hormone leptin plays a key role in regulating the size and function of fat depots. Based on that, the authors suggest that targeting the leptin pathway may be a potential therapeutic strategy for managing metabolic disorders (73). All previous studies recommend a differential level of leptin and its association with fat distribution. However, the direct association and definition of leptin level as a mechanism of thigh fat protection is an area that needs more investigation.

6.3.2 Adiponectin

Adiponectin is a hormone, an adipokine associated with insulin sensitivity and inflammation. Low levels of adiponectin are associated with increased risk of several metabolic diseases, including CVDs. Recently, adiponectin levels were shown to be significantly lower in individuals with high abdominal fat depot, while adiponectin levels were higher with high GF fat depot (74). This opposing effect of different depots on differential serum adiponectin levels lead a more recent study to assess that as a suggested mechanism of GF fat cardioprotection (75). Gradidge et al. (75) conducted a cross sectional study on a female cohort. They first confirmed the positive association of GF fat with adiponectin serum levels. Then, they proved a negative correlation between GF fat with triglyceride level and insulin resistance, a critical risk factors of developing CVDs (75). Although the study was not conclusive, it gives insight of a tight association between GF fat, adiponectin serum levels, and CVD risk factors (75). Another study showed that higher thigh fat results in higher adiponectin level, leading to enhanced insulin sensitivity (76). In conclusion, a higher GF fat is associated with higher adiponectin secretion and enhanced metabolic health.

6.3.3 IL-6

Interleukin-6 (IL-6) is an inflammatory cytokine produced by multiple tissues including adipose tissue, mainly by macrophages residing in the tissue (77). The secreted level of IL-6 correlates with obesity, the higher the BMI the higher the IL-6 levels in the plasma (78). In terms of different depots, IL-6 increases with visceral fat mass increase, and is negatively correlated with thigh fat mass (79). A study compared the IL-6 profile among obese individuals expressing different BFD profiles with and without metabolic syndrome. They first showed that BFD explains the difference between the obese participants in metabolic health, which they linked to higher levels of IL-6 blood levels, concluding that individuals with a more favorable BFD (lower VAT and higher thigh IMAT) express a more favorable inflammatory profile and metabolic health as compared to their unhealthy counterparts (same BMI, gender, and age group) (48). There are not enough studies to show a causality link between IL-6 profile and thigh fat protective role, and more investigation is needed in this area.

6.3.4 DPP4

DPP4 plays a major role in glucose metabolism. It is responsible for the degradation of incretins such as GLP-1. A comprehensive proteomic profiling of the human adipocyte secretome identified dipeptidyl peptidase 4 (DPP4) as a novel adipokine that may impair insulin sensitivity in an autocrine and paracrine fashion, the protein levels of DPP4 are fivefold higher in VAT compared with SAT in a cohort of obese patients (80).

7 Conclusion

The BFD is a highly accurate indicator of metabolic health due to its association with IR, T2D, and CVDs. Th factors that contribute to fat distribution include genetic background, age, hormonal changes, lifestyle (diet and exercise), and medication consumption. It is necessary to understand BFD, as fat storage in certain depots shows a protective effect against metabolic disorders. The gluteal femoral fat depot is one of these depots. Many reports showed its protection against IR, and its metabolic complications (T2D and CVDs). The mechanisms of this protection include three major mechanisms: first, GF depot demonstrates a lower rate of lipolysis. Second, GF depot acts as a metabolic reservoir of excess lipids, preventing them from being stored in the visceral fat depot. And finally, GF depot has a unique physiological nature of GF in terms of lipoproteins, adipokines, and cytokines secretion, it produces protective cytokines (leptin and adiponectin) in higher levels as compared to other depots, while secreting lower levels of proinflammatory adipokines and cytokines (DPP4 ND IL-6). The protective effect of GF fat is interesting, and the mechanisms behind it is still an active area of research that merits further studying to be fully understood.

Author contributions

MA: Investigation, Visualization, Writing – original draft. KN: Supervision, Writing – review & editing. ME: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

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

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Impact of low-carbohydrate diet on health status: an umbrella review

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Introduction: The prevalence of diet-related non-communicable diseases has increased. A low-carbohydrate diet (LCDs) is one of the most popular interventions. Several systematic reviews and meta-analyses of randomised clinical trials (RCTs) and non-RCTs have linked LCDs to the management of obesity, diabetes, cardiovascular disease, epilepsy, and cancer. However, there has been limited appraisal of the strength and quality of this evidence.

Objective: To systematically appraise existing meta-analyses and systematic reviews of RCTs and non-RCTs on the effects of LCDs on different health conditions. To understand their potential efficacy, we summarised the studies' findings and assessed the strength of the evidence.

Methods: A search was conducted using the PubMed database from inception to October 2021 for systematic reviews and meta-analyses of RCTs and non-RCTs investigating the association between LCDs and multiple health outcomes in humans. The Academy of Nutrition and Dietetics Quality Criteria was used for the quality assessment. In addition, the evolution of heterogeneity, strength of the included studies, and effect sizes were extracted from each systematic review and meta-analysis.

Results: Ten systematic reviews and meta-analyses were included. Of the included reviews, 70% were of positive quality, 30% were neutral, and none were negative. The majority of the studies included strength in each systematic review, and the meta-analyses were of low to medium strength. The existing literature indicates that LCDs may help promote weight reduction in adults who are obese or overweight. This conclusion is supported by the findings of studies included in the analysis, which were of low to moderate strength. Furthermore, compelling data indicates a significant association between low-carbohydrate diets (LCDs) and a reduction in haemoglobin A1c levels among those diagnosed with type 2 diabetes mellitus. In contrast, there was a lack of evidence of this correlation in type 1 diabetes mellitus patients or those with cardiovascular diseases. Additionally, there was limited evidence regarding the effectiveness of LCDs in epilepsy and adult cancer patients.

Conclusion: This review thoroughly examines the current body of information on how LCDs affect various health outcomes. Studies have presented evidence to support the idea that incorporating LCDs can positively influence weight management and HbA1c levels. However, there is a lack of information regarding the association between LCDs and individuals with Type 1 diabetes mellitus and cardiovascular diseases. Additionally, there is limited empirical evidence to substantiate the effectiveness of LCDs in the treatment of epilepsy and adult

cancer patients. The long-term effects of LCDs on mortality and other chronic diseases that account for different carbohydrate subtypes is unclear. Further longitudinal cohort studies are required to reach definitive conclusions.

KEYWORDS

low-carbohydrate diet, ketogenic diet, nutrition, health, umbrella review

1 Introduction

Dietary interventions may affect Non-Communicable Diseases (NCDs), which are considered a global public health challenge (1). NCDs are the leading cause of death worldwide, killing over 41 million people annually and accounting for 74 % of all deaths (2, 3). Although the aetiology of NCDs remains unclear, unhealthy diet is emerging as a major modifiable risk factor (4, 5). Thus, there has been an increased interest in the roles of diets and different dietary macronutrient distribution patterns in preventing and managing the severity of NCDs.

Dietary carbohydrates (CHO) are essential macronutrients that provide the body with energy and support its physiological functions (6). Consequently, the recommended intake differs according to age, body weight, physical activity and health conditions. However, the Acceptable Macronutrient Distribution Range for healthy adults is 45%−65% of the total calorie requirement (7). The various low carbohydrate dietary approaches all restrict the total consumption of carbohydrates to some degree, yet there is a lack of definitive consensus regarding the exact parameters that define low-carbohydrate diets (LCDs). Nonetheless, most LCDs involve decreasing carbohydrate intake to <45% of an individual's total caloric intake.

The different terms used for LCDs often imply differences in the distribution of macronutrients. For example, the Atkins, South Beach, and Zone diets are characterised by <40% CHO, ∼30% protein, and 30%−55% fat (8, 9). Additionally, the ketogenic diet (KD) was the most restrictive diet among LCDs, which includes 5%−10% CHO, about 10% protein, and replaces the remaining with dietary fat (9).

Several epidemiological studies have shown that high CHO intake is potentially associated with an increased risk of many diseases such as cancer, heart disease, diabetes, and metabolic syndrome (10–14). However, numerous systematic and meta-analysis reviews have shown that subsequent LCDs may lead to some potential improvements in metabolic risk factors, such as haemoglobin A1C (HbA1c) and lipid profile and may help in weight loss (8, 15–22). Nonetheless, studies on the strength and quality of this evidence are limited (8, 15–22). To date, two recent umbrella reviews have been published 2021-2023 have assessed the quality of evidence (23, 24). The first review included 17 meta-analyses of randomised control trials (RCTs) that assessed the association between KD and health outcomes. They found that the KD reduced triglyceride (TG) by MD, −18.36 mg/dl after 3 months and by MD, −24.10 mg/dl after 12 months compared with regular diet and increased low-density lipoprotein (LDL) by MD, 6.35 mg/dl for 12 months compared with regular diet in adults (23). In contrast, the KD decreased seizure frequency in children and adolescents with epilepsy by RR, 5.11 for 3–16 months when compared with regular diet (23).

The second review included 43 meta-analyses of observational studies addressing the association between CHO and 23 health outcomes categorised into five primary domains: (1) 11 types of cancer, (2) all-cause and cause-specific mortality, (3) metabolic diseases including T2DM and metabolic syndrome, (4) digestive system conditions including ulcerative colitis, Crohn's disease, and inflammatory bowel disorders, and (5) other outcomes comprising coronary heart disease, stroke, Parkinson's disease, and bone fracture (24). Based on a rigorous assessment of the quality of evidence, the study found high-quality evidence associating higher CHO intake with an increased incidence of metabolic syndrome, and potential link of high mortality, as well as a decreased likelihood of developing oesophageal cancer. However, the evidence regarding the relationship between carbohydrate intake and other health outcomes appears to be inconclusive or lacking.

Previous efforts of published umbrella reviews have focused on assessing either one type of LCDs (KD) or the included meta-analyses of observational studies. Hence, the present umbrella review aimed to systematically appraise existing meta-analyses and systematic reviews of RCTs and non RCTs on the effects of LCDs on multiple health conditions to understand their potential efficacy, summaries the studies' findings, and assess the strength of evidence to provide a comprehensive vision.

2 Materials and methods

2.1 Design

This umbrella review was conducted to synthesis and investigate the quality of all existing published systematic reviews and meta-analyses of RCTs and non-RCTs to assess the association between LCDs and diverse health conditions. This review was performed according to the 2020 PRISMA Statement and all items of the PRISMA checklist were completed (25).

Abbreviations: BW, body weight; CHO, carbohydrates; CVDs, cardiovascular diseases; FBG, fasting blood glucose; HbA1C, haemoglobin A1c; HDL, high-density lipoprotein; KD, ketogenic diet; LCD, low carbohydrate Diet; LCKD, low carbohydrate ketogenic diet; LDL, low-density lipoprotein; LFD, low fat diet; N\A, Not available; NCDs, non-communicable diseases; PSA, prostate-specific antigens; RCTs, randomised control trials; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TEI, total energy intake; TG, triglycerides; WC, waist circumference.

2.2 Search strategy

Two independent investigators conducted a search using the PubMed database from its inception to October 2021 to identify systematic reviews and meta-analyses of RCTs and non-RCTs that assessed evidence regarding the effects of LCDs on health. The keywords used in this search strategy were: "carbohydrates," "carbohydrate," "carb," "fat," "proteins," "proteinous," "protein," "ketogenic," "keto," and "atkins." Search philtres were used to identify systematic reviews, meta- analyses, and full- text articles. All titles and abstracts were screened to remove duplicates and select potentially eligible articles. Discrepancies were resolved through discussion with a third investigator.

2.3 Study eligibility criteria

Articles were eligible for inclusion if they were systematic reviews and meta-analyses of RCTs and non RCTs including human studies across all age groups, and articles that only aimed to investigate the associations between LCDs and health outcomes, with a restriction that the amount of CHO did not exceed 45% of the total daily caloric intake, were included. Articles meeting the following criteria were excluded: systematic reviews and meta-analyses based on animal studies, experiments with designs other than RCTs or non RCTs, studies that included diets with more than 45% CHO, and articles published in language other than English.

2.4 Data extraction

The research team developed a data extraction form. The data extracted from each systematic review and meta-analysis included the first author's name, publication year, study design, number of studies included in each systematic review and meta-analysis, study population, intervention, comparison group, duration, parameters of interest, evolution of heterogeneity (I^2%), and quality of the studies included in each systematic review and meta-analysis. Data were independently extracted by two investigators. Disagreements were resolved through discussion with a third investigator.

2.5 Assessment of the quality of systematic reviews and meta-analyses

The quality of systematic reviews and meta-analyses was evaluated using the Quality Criteria Checklist created specifically for primary research articles by the Academy of Nutrition and Dietetics (26) and graded into three categories: "(1) Positive: indicates that the review has clearly addressed issues of inclusion/exclusion, bias, generalizability, and data collection and analysis. (2) Negative: indicates that these issues have not been adequately addressed. (3) Neutral: indicates that the review is neither exceptionally strong nor weak." Quality assessment was independently performed by four investigators. The decision was made when three of the four investigators agreed, and all discrepancies were resolved through a discussion with a fifth investigator. To ensure the accuracy of the evaluation, we shared the quality assessment with five Ph.D. nutrition experts. The evaluation was subsequently revised based on the expert feedback and comments.

2.6 Data analysis

The calculation of the effect size was measured in current review by Cohen's *d*. The effect size, denoted as *d*, is calculated manually using the following equation (27): $d = (M1 - M2)/spooled$. Here, M1 represents the mean of group 1, M2 represents the mean of group 2, and spooled refers to the pooled standard deviations for the two groups. It is crucial to note that the interpretation of the effect size depends on its value. If d falls within the range of $\geq 0.2 \leq 0.499$, it is considered a "small" effect. A value of $\geq 0.5 \leq 0.799$ indicates a "medium" effect, while a value of ≥ 0.8 suggests a "large" effect (28). We assessed heterogeneity using the I^2 statistics, considering $I^2\% \geq 50\%$ as indicative of high heterogeneity (29).

3 Results

3.1 Search results

A total of 274 articles were initially identified, and 255 were excluded after title and abstract screening. Nine of the 19 articles were excluded after applying the inclusion criteria. Finally, 10 eligible articles were included, as shown in Figure 1. A list of the excluded articles is presented in Supplementary material.

3.2 Characteristic of the included systematic reviews and meta-analyses

Among eligible systematic reviews and meta-analyses, different outcomes associated with five health conditions were examined: obesity, cardiovascular disease, diabetes, cancer, and epilepsy.

The characteristics of the included reviews are summarised in Table 1. The average number of studies included in each systematic review and meta-analysis was 31 (range: 6–121), and the average number of participants was 4,067 (range: 222–21,942). The participants in all the included reviews were adults, except for one review that included both children and adults (30). Most participants in the included studies were obese or overweight (80%) (8, 17–22, 31), followed by those with T2DM (40%) (17, 19, 22, 31). The most frequently measured health indicators in the included studies were weight (body weight (BW), waist circumference (WC), and body mass index), blood sugar levels [fasting blood glucose (FBG) and haemoglobin A1C (HbA1c)], and lipid profiles.

3.3 Quality and strength of evidence

Quality of evidence indicates the extent to which the article has clearly addressed the issues of inclusion/exclusion, bias, generalizability, and data collection and analysis. The quality of

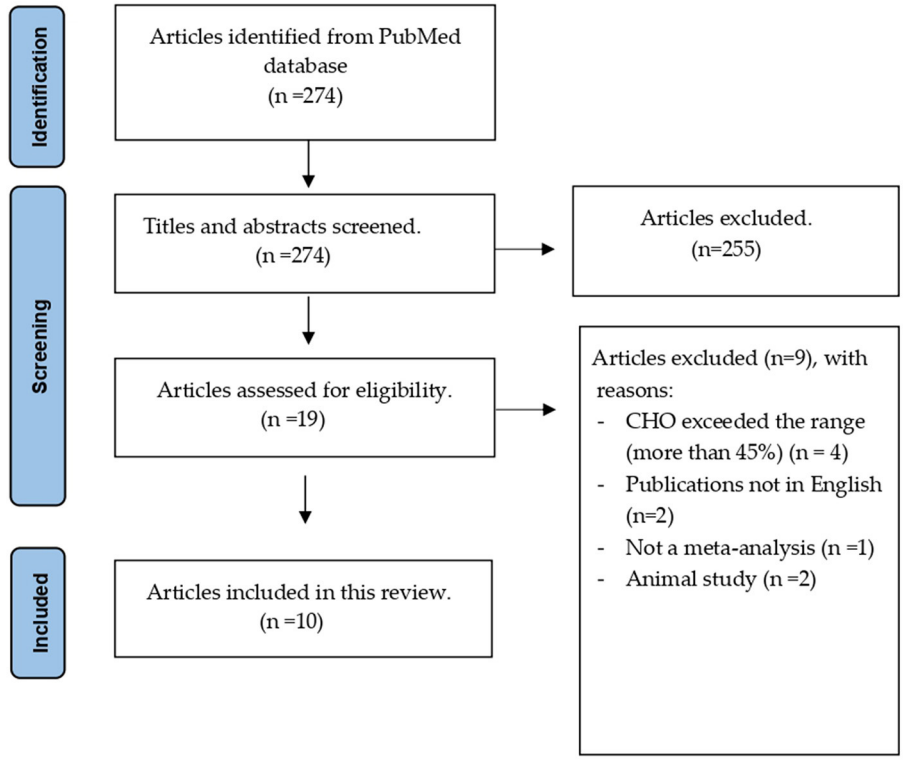

FIGURE 1
Articles selection flowchart.

the included systematic reviews and meta-analyses was assessed using the Academy of Nutrition and Dietetics Quality Criteria Checklist, which categorised the quality of evidence into three categories (positive, negative, and neutral) (26). Seven of the 10 eligible reviews (70%) were positive (8, 17, 19–21, 30, 32), three of the 10 eligible reviews were neutral (30%) (18, 22, 31), and none of the included reviews were negative, as shown in Table 2. Based on the findings, the strengths of the included studies as reported by the included systematic reviews and meta-analyses were low to moderate in four of 10 eligible reviews (40%) (8, 17, 19, 21); low to very low in two reviews (20%) (20, 30); and moderate to high strength evidence in one review. Grading information was unavailable for three of 10 included reviews (30%) (18, 22, 31) as shown in Table 2.

3.4 Summary effect size

The results showed that TG, HbA1c, high-density lipoprotein (HDL), and BW were significantly associated with LCD in most of the included systematic reviews and meta-analyses. Figure 2 summarises the effect sizes of all included reviews with similar health indicators for comparison. The findings revealed that most of the reviews reported statistical significance associations between HDL and LCD; however, the effect size was small. In terms of blood glucose indicators, HbA1c was the most frequently reported statistically significant indicator with a medium effect size. Among weight measurement indicators, the association between BW and LCD was considered to have a large effect size.

3.5 Heterogeneity between studies

There was high heterogeneity in most health indicators in the included studies (52%) and low heterogeneity in six health indicators (26%). In addition, 22% were not available. Systematic reviews and meta-analyses with high heterogeneity included health indicators such as TC, HbA1c, and FBG, as shown in Table 2.

3.6 Weight reduction outcomes

Five of the included reviews measured the indicators of weight gain (BW and WC) in obesity or overweight participants (8, 18, 20) and in participants with T2DM (17, 31). Among the included reviews, BW was the most frequently measured weight indicator. LCDs were linked to a BW decrease in individuals who were obese or overweight (8, 18), as well as those who had T2DM in adults (17, 31). However, only two of the included reviews had a strength rating of low to moderate, and their quality was positive, demonstrating that LCDs were associated with BW reduction with the mean difference of BW (−1 to 2, −3.81, and −7.78 kg in diabetes patients) (8, 17). Moreover, the effect size were medium to large Conversely, the reduction of WC by −0.74 cm was observed in one review with large effect size and $P = 0.01$; however, the strength

TABLE 1 General characteristics of included systematic reviews and meta-analyses.

Study number	References	Study design of studies included in each systematic review and meta-analyses	Number of studies included in each systematic review and meta-analyses	Population	Intervention	Comparison group	Duration	Parameters of interest
1	Yuan et al. (31)	7 RCTs and 6 non-RCTs	13	Obese or overweight adults with T2DM	KD	Pre-KD	1–56 weeks	TC TG HDL LDL BW HbA1C FBG
2	Choi et al. (17)	RCTs	14	Obese or overweight adults with or without T2DM	KD	LFDs	2 h to 12 months	HDL TG BW HbA1C
3	Sackner-Bernstein et al. (18)	RCTs	17	Obese or overweight adults	LCDs	LFDs	2–24 months	BW
4	Ge et al. (8)	RCTs	121	Obese or overweight adults	LCDs	Moderate macronutrient dietary patterns (CHO: >40% of TEI)	6 months	BW
5	Schwingshackl et al. (19)	RCTs	56	Obese or overweight adults	LCKDs + exercise	Usual diet + exercise	4–24 weeks	HbA1C
6	Lee and Lee (20)	RCTs	7	Obese or overweight adults	LCKDs + exercise	Usual diet + exercise	4–24 weeks	WCTG
7	Chawla et al. (21)	RCTs	38	Obese adults	LCDs	LFDs	1–24 months	HDL TG
8	Yang et al. (32)	RCTs	6	Adults with cancer	KD	Non-KD	4-24 weeks	PSA—Tumour marker
9	Martin-McGill et al. (30)	RCTs	13	Children and adults with epilepsy	KD	Usual care	2–16 months	- Seizure freedom - 50% or greater reduction in seizure frequency
10	Hu et al. (22)	RCTs	23	Adults with metabolic risk	LCDs	LFDs	6-24months	HDL TG

T2DM, Type 2 diabetes mellitus; KD, ketogenic diet; CHO, carbohydrate; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BW, body Weight; HBA1C, Haemoglobin A1C; FBG, fasting blood glucose; LFDs, low-fat diets; LCDs, low carbohydrate diet; LCKDs, low-carbohydrate ketogenic diets; WC, Waist Circumference; PSA, Prostate-Specific Antigen; TEI, Total Energy intake; RCTs, randomised clinical trials.

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TABLE 2 Heterogeneity, strength of the included studies, quality of the included reviews, and effect size.

References	Parameters of interest	Evolution of heterogeneity (I^2%)	The strength of the studies included in each systematic review and meta-analyses	The quality of the included systematic reviews and meta-analyses	Effect size
Yuan et al. (31)	TC	75%	N\A	Neutral	Medium effect
	TG	67%			Large effect
	HDL	78%			Small effect
	LDL	71%			Small effect
	BW	92%			Large effect
	HbA1C	68%			Medium effect
	FBG	71%			Large effect
Choi et al. (17)	HDL	10%	Low to moderate	Positive	Medium effect
	TG	59%			Medium effect
	BW	78%			Medium effect
	HbA1C	23%			Medium effect
Sackner-Bernstein et al. (18)	BW	N\A	N\A	Neutral	Large effect
Ge et al. (8)	BW	N/A	Low to moderate	Positive	Large effect
Schwingshackl et al. (19)	HbA1C	N\A	Low to moderate	Positive	Small effect
Lee and Lee (20)	WC	0%	Low	Positive	Large effect
	TG	0%			Medium effect
Chawla et al. (21)	HDL	N/A	Low to moderate	Positive	Small effect
	TG	N\A			Small Effect
Yang et al. (32)	PSA—Tumour marker	78.3%	Moderate to high	Positive	Large Effect
Martin-McGill et al. (30)	Seizure freedom	0%	Low to very low	Positive	The association not statistically significant
	50% or greater reduction in seizure frequency	0%			The association not statistically significant
Hu et al. (22)	HDL	78.6%	N\A	Neutral	Large effect
	TG	55.6%			Large effect

TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BW, body Weight; HBA1C, haemoglobin A1C; FBG, fasting blood glucose; WC, waist circumference; PSA, prostate-specific antigen; NA, not available.

of evidence was low (20). Furthermore, the findings suggest that weight reduction is achievable if proper calories are estimated for weight loss regardless of the carbohydrate composition of the diet.

3.7 Cardiovascular diseases outcomes

The types of LCDs had different effects on risk factors associated with CVDs (total cholesterol, TG, HDL, and LDL) in different populations (17, 20–22, 31). However, there is a lack of studies that directly measure the incidence of CVDs or that were conducted on CVD patients. LCDs have been associated with decreased TG and increased HDL levels which are considered to reduce the risk of CVD. Different effect sizes between studies for TG and HDL as shown in Figure 2. These results were obtained in obese and overweight participants, with or without T2DM, and in participants with metabolic syndrome (17, 20–22, 31). Nevertheless, these results are supported by a low-to-moderate level of evidence. A limited review of Neutral qualities found that LCD was associated with decreased LDL with small effect size and TC levels with medium effect size (31).

3.8 Diabetes outcomes

Three of the included reviews compared the efficacy of LCDs and other diets on HbA1C percentage. The results from these reviews showed that LCDs resulted in a significant reduction in the

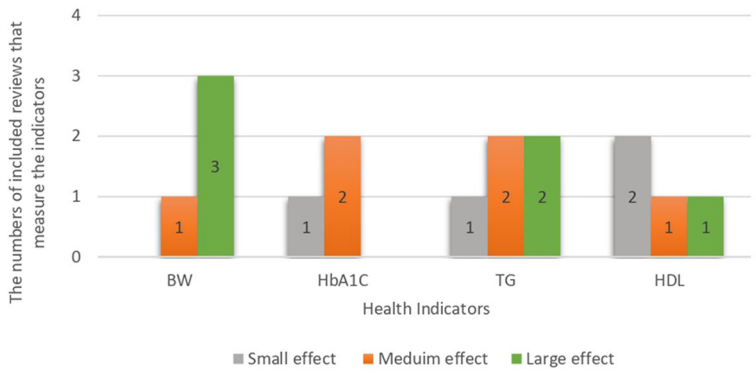

FIGURE 2
Effect size of most common health indicators in the studies included regardless of the disease.

percentage of HbA1c compared to other diets (−0.82 to −0.47%, −0.5% to −0.42%, and 1.07%) in T2DM patients with medium effect size (17, 31), and small effect size (19). Furthermore, data from one review with neutral quality showed that a who consumed the control diet (−1.29 mmol/L) with large effect size (31).

3.9 Cancer outcomes

Overall, there is limited evidence regarding the safety and effectiveness of LCDs in cancer patients. One included review with positive quality, moderate-to-high strength of included studies, and a large effect size found a statistically significant association between LCDs and one of the crucial tumour markers for prostate cancer (PSA); $P = 0.03$ in prostate cancer (32).

3.10 Epilepsy outcomes

A Cochrane review suggested that high levels of ketones in the blood caused by KD may reduce the frequency of epileptic seizures in children with drug-resistant epilepsy (30). However, it should be noted that some children experience frequent adverse effects of LCDs, such as constipation, vomiting, nausea, and diarrhoea (30). In adults, the current review showed that there are limited systematic reviews and meta-analyses of RCTs and non-RCTs on the effectiveness of LCDs in epilepsy patients, and the results of the evidence are uncertain (30).

4 Discussion

4.1 Principal findings and possible mechanisms

This umbrella review provides an overview and appraisal of 10 systematic reviews and meta-analyses o RCTs and non RCTs on the effects of LCDs on different outcomes associated with five health conditions obesity, CVDs, T2DM, cancer, and epilepsy. Seventy percent of the included reviews were of positive quality (seven reviews), 30% were neutral (three reviews), and none were negative.

Although most reviews were of positive quality, the strengths of the studies included in each systematic review and meta-analysis ranged from low to medium. The current review includes a systematic and meta-analysis that evaluated prospective cohorts and RCTs studies. These studies included participants from several geographic locations, as well as ethnic and cultural diversity, to minimise variations that could potentially bias the findings. Some of the included meta-analyses contained duplicate articles (primary studies), due to the use of similar keywords during the search phase. In addition, most of the meta-analyses were published between 2020 and 2021. However, this review design focused on summarising the evidence, comparing the effect sizes across all investigated factors, and reporting the heterogeneity across the included studies in each meta-analysis. Pooling the data derived by the same studies, create forest plots, and other summing analyses may brought together to provide one estimate and cause overlapping by over/underestimation of some results, for that reason each meta-analysis review was assessed separately.

Multiple studies have identified various biological mechanisms that indicate significant metabolic and hormonal changes upon achieving high ketone body production or ketosis, in relation to the five health conditions focused in this review (33, 34). Ketone bodies produced by the liver serve as oxidative fuels and markers of carbohydrate deficiency. They may help conserve carbohydrates during periods of low carbohydrate availability by reducing insulin levels and anabolic processes and promoting the conversion of fatty acids from fat storage and diet into ketone bodies for energy (35, 36).

The utilisation of fats for ketone bodies production as energy has led some studies to suggest potential weight loss as a risk factor for the five health conditions on our review. This concept, known as the carbohydrate-insulin model of obesity, suggest that high-glycemic carbohydrates encourage fat storage and hunger, which can be mitigated through lifestyle changes, such as prolonged fasting, extended exercise, or a low-carbohydrate diet to lower insulin levels (37, 38). According to this model, low-carbohydrate diets may reduce the risk of obesity, CVDs, T2DM, and cancer by decreasing body weight and increasing fat utilisation by limiting

carbohydrate intake. However, for epilepsy, there was no direct mechanism that suggests how weight loss in the carbohydrate-insulin model of obesity might help improve the outcomes of this condition.

According to the epidemiological study, a reduction of 5 kg/m^2 of height can lead to an average of 27% decrease in the risk of CVDs and a 17% decrease in the risk of T2DM. Additionally, this reduction in body mass may decrease the risk of pancreatic cancer by 14%, colon cancer by 3%, breast cancer by 3%, uterine cancer by 52%, kidney cancer by 31%, bladder cancer by 23%, and liver cancer by 59% (39, 40). Consequently, the effectiveness and efficiency of weight loss resulting from LCD are crucial factors in achieving results that are comparable to those achieved from these epidemiological studies.

Although the expected physiology and mechanisms appeared promising, the evidence reviewed in this paper showed varying degrees of effectiveness when such diet was tested. There was evidence suggesting the effectiveness of LCDs on weight reduction in obesity or overweight adults, supported by the low to moderate strength of the included studies. Moreover, evidence strongly suggested a correlation between LCDs and HbA1c reduction in T2DM patients. In contrast, systematic reviews and meta-analyses of RCTs and non-RCTs that included T1DM patients or those with CVDs are lacking. In addition, the current review showed that there is limited evidence regarding the effectiveness of LCDs in epilepsy and adult cancer patients.

Epidemiological studies suggests that the harmful impact of such diets may be attributed to the significant concern of increased overall fat intake (41). The results indicate that an increase in calories derived from fats can lead to a rise in total serum cholesterol levels by ~0.02 mm/L. Additionally, a change of 1 mm/L in total cholesterol could potentially increase the risk of mortality from CVDs by 20%, as indicated by research (42). This might explain the lack of significant findings regarding CVDs on our review. However, it is important to note that the majority of the studies included in this review have not measured the change in cholesterol levels resulting from high fat intake and their subsequent association with CVDs risk outcomes. Instead, the primary focus has been on the direct dietary intake of LCDs in relation to CVDs. Therefore, further research in this area may be necessary to better understand the dose-response relationship, particularly with regards to this specific focus.

4.2 Comparison with other reviews and possible explanations

According to the European Food Safety Authority, individuals are recommended to consume carbohydrates within 45%−60% of their total energy intake (43). This dietary guideline effectively improves metabolic risk factors associated with chronic diseases when combined with reduced total fat and saturated fat consumption (44). Nevertheless, a precise consensus about the definition of LCDs has yet to be reached. Most LCDs typically involve reducing CHO intake to below 45% of the total caloric intake.

Two umbrella reviews have examined the impact of LCDs on various health outcomes. An umbrella summarises and evaluates 43 meta-analyses of observational studies investigating the association between CHO intake and health outcomes, including cancer, mortality, metabolic diseases, digestive system outcomes, and other outcomes (coronary heart disease, stroke, Parkinson's disease, and bone fracture). The findings of this review provided evidence in favour of the correlation between carbohydrate intake and metabolic syndrome. In addition, the researchers proposed that the evidence presented on the negative impact of CHO consumption on coronary heart disease and T2DM was lacking in strength. This finding is consistent with the results of our present review, which indicate that LCDs may enhance risk factors associated with cardiac illnesses and T2DM. Nevertheless, the findings presented in this study are supporting by a low to moderate degree quality of evidence. Additionally, a statistically significant correlation was seen between the use of LCDs and the proportion of HbA1c levels in individuals with T2DM (24).

A recent umbrella review was conducted to systematically identify and summarise relevant meta-analyses of RCTs on KD (23). The analysis included data from 17 meta-analyses, which consisted of a total of 68 RCTs (23). The primary objectives of the review were to identify the effects of KD on health outcomes and to assess the strength of the evidence supporting these effects (23). The study demonstrated positive correlations between KD and various cardiometabolic markers. Furthermore, it was observed that KD had inconsistent effects on TC and LDL levels, resulting in unfavourable outcomes. However, the authors considered 76% of the RCTs to be critically low quality (23). On the contrary, our findings demonstrated an inverse relationship between LCDs and CVD, as evidenced by reduced TG levels and elevated HDL levels. However, it should be noted that the findings presented in this study are supported by a level of evidence that ranges from low to moderate. Additionally, the review demonstrated positive results linked to the KD, including changes in BW, TG, HDL-C, and HbA1c levels. The discovery above aligns with the outcomes obtained from our research. Furthermore, high-quality evidence supports the notion that a KD can effectively reduce the frequency of seizures by 50% or more in children and adolescents (23). This finding is consistent with a large retrospective cohort study that compared KD with usual care, which aim to assess the safety, effectiveness, and retention rate of KD for paediatric with drug-resistant epilepsy found that after KD, the retention rate significantly increased over time by 82.0% at 3 months, 60.6% at 6 months, and 34.1% at 12 months. Additionally, the response rate improved dramatically over 3 months by 55.5%, 6 months by 43.2%, and 12 months by 31.5% (45).

When looking at the effect of other diets such as high-protein diet, the Mediterranean diet, and high fibre diet. We found that there were evidence supporting the improvement of health marks by following these diets. However, there were limited meta-analysis that have been compared the LCDs with different diets types other than control diets. Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes was published in 2013, aimed to assess the effect of various diets including (low-carbohydrate, vegetarian, vegan, high-fibre, low glycemic index, Mediterranean, and high-protein diets) on glycemic control, lipids, and weight loss. The results showed that the LCDs, low glycemic, Mediterranean, and high-protein diets were effective in improving different indicators of cardiovascular risk in diabetic people (46).

4.3 Strength and limitations

To the best of our current understanding, this umbrella review represents a pioneering effort in offering a methodical and all-encompassing evaluation of published systematic reviews and meta-analyses of RCTs and non-RCTs that examine the impact of LCDs on various human health-related outcomes. This review can assist dietitians, nutritionists, and researchers in evaluating the comparative efficacy of LCDs on many health outcomes. Furthermore, we assessed the methodological rigour. We examined the magnitude of the effect size and heterogeneity among the included reviews to guide future research endeavours.

One potential constraint of this review is the need for a comparative analysis of various LCD kinds, which exhibit variations in the extent of carbohydrate, fat, and protein limits. This omission may have influenced the outcomes of the study. A further factor worthy of consideration pertains to the predominant focus of existing studies on quantifying immediate results, hence neglecting the possibility of secondary effects associated with LCDs that could be discerned using alternative research methodologies. Furthermore, similar to other literature reviews, umbrella reviews are susceptible to biases, particularly appraisal and selection biases.

In summary, this umbrella review comprehensively examines the available information on the effects of LCDs on various health-related outcomes. Several studies have provided data supporting the notion that the utilisation of LCDs can have a beneficial effect on weight management and HbA1c levels. Nevertheless, there is a scarcity of information about the correlation between this connexion in patients with T1DM and CVDs. Furthermore, there is limited empirical support for the effectiveness of LCDs in treating epilepsy and adult cancer patients. Moreover, it is recommended that future research endeavours prioritise investigating the enduring impacts of adhering to LCDs on mortality rates, the prevention and treatment of other chronic diseases, and the examination of how various sources of CHO influence this process.

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

SAlk: Supervision, Writing – original draft, Writing – review & editing, Conceptualization, Investigation, Methodology. RA: Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Investigation. OA: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. BF: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. AA: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. TA: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. SAla: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. YA: Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1321198/full#supplementary-material

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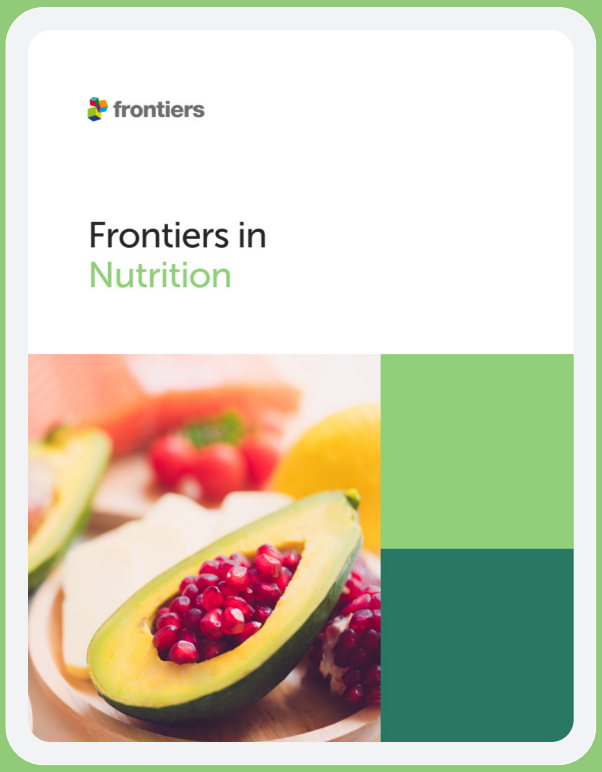

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