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Nutritional approaches in chronic liver diseases

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Nutritional approaches in chronic liver diseases

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Editorial: Nutritional approaches in chronic liver diseases

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liver disease, nutritional approach, therapeutic, cirrhosis, metabolic dysfunction-associated steatotic liver disease (MASLD)

Editorial on the Research Topic
Nutritional approaches in chronic liver diseases

We are currently at a pivotal moment regarding the awareness, diagnosis, treatment, and management of liver diseases worldwide. The burden of these conditions extends beyond mere epidemiological data—over two million deaths annually are attributed to liver diseases (4% of all global deaths). It is also assessed through composite health indicators that combine two variables: quality and quantity of life. Liver disease has the greatest impact on individuals aged 25 to 49 years, where it ranks as the twelfth leading cause of disability-adjusted life-years (DALYs) (1).

Another approach to evaluating the burden of these conditions is through the investment allocated for research. According to data from the National Institutes of Health, the U.S. government is projected to allocate $954 million in 2024 for the study of liver diseases (2).

Despite regional differences, the global significance of liver diseases is evident (1).

Despite efforts to ensure timely diagnosis and advances in treatment (3), delays and inadequacies in routine clinical care hinder the recognition of macro- and micronutrient deficiencies, resulting from metabolic disorders—an inherent characteristic of chronic liver diseases—thereby preventing adequate nutritional support for these patients (4, 5).

While there are recommendations for using nutrition as an adjunctive treatment in the multidisciplinary management of these patients (6, 7), nutritional interventions are not frequently implemented.

The following sections outline key aspects for managing certain liver diseases. In cirrhosis of any etiology, malnutrition and sarcopenia have been associated with higher mortality and reduced survival. A meta-analysis by Cui et al., indicates a prevalence of 41%, and suggests incorporating sarcopenia screening into the initial evaluation of every cirrhotic patient.

On the other end of the spectrum, metabolic dysfunction-associated steatotic liver disease (MASLD), the most common chronic liver disease and a risk factor for cardiovascular disease and mortality worldwide, has been studied by Liu et al., who propose that timely and appropriate supplementation with vitamins and trace elements may aid in disease recovery or delay its progression, thereby improving patient outcomes.

Similarly, studies by Li et al., and Chen et al., demonstrate that dietary intake of folic acid, choline, vitamin B1, and vitamin B2 may be associated with hepatic steatosis, providing insights into potential dietary strategies for postmenopausal women. Additionally, magnesium supplementation may prevent and treat hepatic steatosis, with variations depending on gender and ethnicity.

In summary, nutritional therapy plays a crucial role in the management of liver disease, and its omission could negatively impact clinical outcomes and the quality of life of affected individuals.

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Low geriatric nutritional risk index predicts poor prognosis in patients with cirrhosis: a retrospective study

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Aim: Malnutrition, which increases the risk of liver disease-related events and mortality, is a serious complication in cirrhosis. This study aimed to investigate whether the geriatric nutritional risk index (GNRI) could predict the long-term prognosis in patients with cirrhosis.

Methods: We retrospectively evaluated 266 patients with cirrhosis and classified them into two groups based on baseline GNRI scores: risk (≤98, $n = 104$) and no-risk groups (>98, $n = 162$). The cumulative survival rates were compared between the two groups in patients with compensated and decompensated cirrhosis, respectively. Cox proportional hazards regression analysis was used to identify significant and independent factors associated with mortality.

Results: The median observation period was 54.9 (33.6–61.7) months and 65 (24.4%) liver disease-related deaths occurred during the follow-up period. The GNRI scores significantly and inversely correlated with Child-Pugh score ($r = -0.579$), model for end-stage liver disease score ($r = -0.286$), and Mac-2 binding protein glycosylation isomer ($r = -0.494$). Multivariate analysis identified low GNRI as a significant and independent factor associated with mortality [overall cohort: hazard ratio (HR), 0.926; $p < 0.001$; compensated cirrhosis: HR, 0.947; $p = 0.003$; decompensated cirrhosis: HR, 0.923; $p < 0.001$]. The risk group demonstrated significantly lower cumulative survival rates than the no-risk group in overall cohort, and patients with compensated and decompensated cirrhosis ($p < 0.001$, <0.001, and = 0.013, respectively).

Conclusion: Low GNRI was associated with poor long-term prognosis in both patients with compensated and decompensated cirrhosis. Therefore, the GNRI is a simple and useful tool for predicting prognosis and modifying the nutritional status in patients with cirrhosis.

KEYWORDS

cirrhosis, malnutrition, nutritional assessment tool, geriatric nutritional risk index, prognosis

1. Introduction

The liver is an essential organ in the metabolism of nutrients such as carbohydrates, lipids, proteins, vitamins, and minerals (1). Patients with cirrhosis have reduced liver functional reserve frequently complicated by malnutrition due to altered metabolism of these nutrients, as well as hypermetabolic state, malabsorption, decreased nutrients intake, hormonal imbalance, and systemic inflammation (1–4). Malnutrition is associated with adverse clinical outcomes, including portal hypertension, encephalopathy, variceal bleeding, hepatorenal syndrome, hepatic spontaneous bacterial peritonitis, sarcopenia, reduced quality of life, and mortality (5–8). Patients with cirrhosis may achieve improved survival rates through appropriate multidisciplinary nutritional intervention (9). Therefore, simple and practical methods for assessing nutritional status and predicting clinical outcomes are required to promptly initiate treatment and improve patient prognosis in a clinical setting.

The geriatric nutritional risk index (GNRI), which is calculated based on actual/ideal body weight [or body mass index (BMI)] and serum albumin levels, was proposed to estimate the risk of malnutrition-related complications in older adults (10). This scoring system categorizes individuals into the four nutrition-related risk groups, and lower GNRI scores indicate higher morbidity and mortality risks. The GNRI demonstrated superior 1-year prognostic predictability over the global leadership initiative on malnutrition (GLIM) criteria and mini nutritional assessment-short form (MNA-SF) in hospitalized Japanese older adults (11). Several studies have revealed the usefulness of GNRI in predicting the prognosis of malignancies, including esophageal, gastric, hepatic, pancreatic, and colorectal cancers (12–16). Reportedly, low GNRI is associated with poor prognosis in non-malignant diseases, including heart failure, stroke, and chronic kidney disease (17–19). Our recent study of patients with cirrhosis discovered low GNRI as an independent risk factor for sarcopenia, which has been reported to be associated with malnutrition and poor prognosis (20, 21). Therefore, the GNRI may be a simple and useful indicator of nutritional status and prognosis in both malignant and non-malignant diseases. However, no study has reported the association between GNRI and prognosis in patients with cirrhosis.

This study aimed to determine the usefulness of the GNRI in predicting the long-term prognosis of patients with cirrhosis.

2. Materials and methods

2.1. Study participants

This retrospective study enrolled 266 consecutive patients with cirrhosis who presented to the Jikei University School of Medicine (Tokyo, Japan) and Fuji City General Hospital (Shizuoka, Japan) between 2017 and 2020. The study cohort included 182 patients analyzed in our previous report (20). The inclusion criteria were (i) patient age ≥ 20 years and (ii) presence of cirrhosis diagnosed based on noninvasive alternatives to liver biopsy, such as laboratory tests and imaging/endoscopic findings, as described elsewhere (20). The exclusion criteria were (i) pre-existing malignancies other than hepatocellular carcinoma (HCC); (ii) massive and uncontrollable ascites; (iii) HCC beyond the Milan criteria (22); (iv) acute liver failure; (v) liver transplantation history; and (vi) undergoing hemodialysis. Serum total bilirubin, albumin, creatinine, sodium, Mac-2 binding protein glycosylation isomer (M2BPGi, which is a hepatic fibrosis marker), and prothrombin time (PT) were measured using standard methods. The liver functional reserve was assessed with the Child-Pugh (CP) classification and model for end-stage liver disease (MELD) (23, 24). Alcohol-related cirrhosis was diagnosed based on past and/or current history of excessive alcohol consumption (> 60 g/day) and exclusion of other etiologies (25). Metabolic dysfunction-associated steatotic liver disease-related cirrhosis was diagnosed based on the multi-society Delphi consensus statement on new fatty liver disease nomenclature (26). Decompensated cirrhosis was defined as cirrhosis complicated by encephalopathy, variceal bleeding, ascites, and/or jaundice (27). The endpoint in this study was liver disease-related death. Patients who underwent liver transplantation during the study period were considered as death and censored cases. This study protocol was approved by the ethics committees of the Jikei University School of Medicine (approval number: 34-021) and Fuji City General Hospital (approval number: 279) and complied with the 2013 Declaration of Helsinki.

2.2. Patient classification based on GNRI score

The GNRI was calculated based on actual/ideal body weight and serum albumin levels using the following formula: GNRI = [14.89 × albumin (g/dL)] + [41.7 × (actual body weight/ideal body weight)] (10). This scoring system categorizes individuals into four risk groups: no-risk (>98); low-risk (92 to ≤98); moderate-risk (82 to <92); and major-risk (<82) groups. The present study classified the participants into two groups, referring to the original GNRI classification (10) and previous reports (14, 28): risk group (with nutrition-related risk; GNRI ≤98.0) and no-risk group (without nutrition-related risk; GNRI >98.0).

2.3. Statistical analysis

Continuous variables were presented as medians (interquartile ranges), and between-group differences were compared using the Mann–Whitney U test or the Kruskal–Wallis test, as appropriate. Categorical variables were presented as numbers (percentages), and between-group differences were compared using the chi-squared test. The trend of proportions or continuous variable levels among multiple groups was analyzed using the Cochran-Armitage trend test or the Jonckheere-Terpstra trend test, respectively. Correlations between GNRI scores and continuous variables were analyzed using Spearman's rank correlation test. The cumulative survival rates were estimated using the Kaplan–Meier method, and between-group differences were compared using the log-rank test, followed by the Bonferroni multiple-comparison method, or the log-rank trend test, as appropriate. Univariate analysis was initially performed to identify mortality-related variables that achieved $p < 0.10$. Multicollinearity among these variables was evaluated using variance inflation factor (VIF). Variables with VIF > 5, which was considered as high multicollinearity, were excluded from the analysis. Subsequently, multivariate Cox proportional hazards regression analysis with forward stepwise selection was performed to identify significant and independent factors associated with mortality.

Nomograms were constructed to visualize the prognostic strengths of the significant and independent factors in predicting survival. All statistical analyses were performed using SPSS Statistics version 27 (IBM Japan, Tokyo, Japan) and R software (version 4.3.1). Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Patient characteristics

A flow diagram of patient selection in this study is presented in Supplementary Figure S1. A total of 318 patients with cirrhosis were initially screened for eligibility. Of them, 52 met the exclusion criteria, and the remaining 266 patients were enrolled. The baseline patient characteristics are summarized in Table 1. The median age was 68 (58.0–76.0) years, and 176 (66.2%) patients were men. The median CP and MELD scores were 6 (5–7) and 8 (7–11), respectively. The prevalence of decompensated cirrhosis and HCC were 35.7% (95/266) and 16.9% (45/266), respectively. The median GNRI score was 102.2 (94.0–110.2). Patients with decompensated cirrhosis demonstrated significantly lower GNRI scores than those with compensated cirrhosis (median, 94.0 vs. 107.3; $p < 0.001$; Supplementary Figure S2).

3.2. Clinical characteristics of the risk and no-risk groups

The proportions of the risk and no-risk groups were 39.1% (104/266) and 60.9% (162/266), respectively (Table 1). Hepatitis B virus and metabolic dysfunction-associated steatotic liver disease were significantly more frequent in the no-risk group, whereas the others were significantly more frequent in the risk group ($p = 0.002$). The risk group demonstrated significantly higher decompensated cirrhosis prevalence, CP and MELD scores, and M2BPGi levels ($p < 0.001$ for all), and lower sodium and PT levels ($p = 0.002$ and < 0.001, respectively) than the no-risk group.

3.3. Correlations between GNRI and liver functional reserve and fibrosis marker

The correlations between GNRI and CP scores, MELD scores, and M2BPGi were investigated using Spearman's rank correlation test (Figure 1). The GNRI significantly and inversely correlated with CP score ($r = -0.579$), MELD score ($r = -0.286$), and M2BPGi ($r = -0.494$) ($p < 0.001$ for all).

3.4. Comparison of cumulative survival rates between the risk and no-risk groups

The median follow-up period was 54.9 (33.6–61.7) months. During the observation period, 65 (24.4%) liver disease-related deaths occurred: liver failure ($n = 42$), HCC ($n = 10$), rupture of esophageal varices ($n = 8$), and liver transplantation ($n = 5$) (Supplementary Figure S1). The 1-, 3-, and 5-year cumulative survival rates were 94.2%, 65.0%, and 49.2% in the risk group and 98.7%, 94.1%, and 88.1% in the no-risk group, respectively (Figure 2). The risk group demonstrated significantly lower cumulative survival rates than the no-risk group ($p < 0.001$).

TABLE 1 Comparison of clinical characteristics between the risk and no-risk groups.

Variable	All patients	Risk group	No-risk group	*p-value*
Patients, *n* (%)	266	104 (39.1)	162 (60.9)	
Man, *n* (%)	176 (66.2)	63 (60.6)	113 (69.8)	0.123
Age (years)	68.0 (58.0–76.0)	70.0 (60.3–76.0)	66.0 (57.0–75.0)	0.106
BMI (kg/m^2)	23.8 (21.6–26.4)	21.7 (19.2–23.5)	25.5 (23.5–28.6)	< 0.001
Etiology				
HBV/HCV/alcohol/MASLD/others, *n*	21/76/94/36/39	4/27/41/8/24	17/49/53/28/15	0.002
Decompensated cirrhosis, *n* (%)	95 (35.7)	65 (62.5)	30 (18.5)	< 0.001
Child-Pugh score	6 (5–7)	7 (6–8)	5 (5–6)	< 0.001
MELD score	8 (7–11)	9 (7–13)	8 (7–10)	< 0.001
GNRI	102.2 (94.0–110.2)	91.3 (86.7–95.6)	108.2 (104.0–114.2)	< 0.001
Total bilirubin (mg/dL)	0.9 (0.7–1.3)	1.0 (0.6–1.7)	0.9 (0.7–1.2)	0.264
Albumin (g/dL)	3.8 (3.4–4.2)	3.3 (3.0–3.6)	4.1 (3.8–4.4)	< 0.001
Creatinine (mg/dL)	0.8 (0.7–1.1)	0.8 (0.7–1.1)	0.8 (0.7–1.1)	0.701
Sodium (mEq/L)	140 (138–142)	140 (137–141)	140 (139–142)	0.002
Prothrombin time (%)	81 (65–94)	68 (50–89)	83 (71–96)	< 0.001
M2BPGi (C.O.I.)	3.09 (1.47–5.89)	5.32 (2.68–8.46)	2.45 (1.28–4.18)	< 0.001
HCC, *n* (%)	45 (16.9)	18 (17.3)	27 (16.7)	0.892

Continuous variables are shown as median (interquartile range). Statistical analysis was performed using the chi-squared test or the Mann–Whitney U test, as appropriate. BMI, body mass index; C.O.I., cut-off index; GNRI, geriatric nutritional risk index; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; M2BPGi, Mac-2 binding protein glycosylation isomer; MELD, model for end-stage liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease.

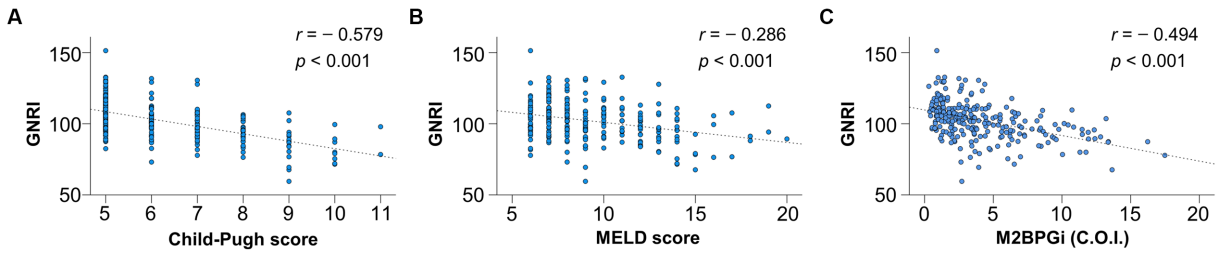

FIGURE 1
Correlation of geriatric nutritional risk index (GNRI) with liver functional reserve-related scores and fibrosis marker. GNRI significantly correlated with **(A)** Child-Pugh score, **(B)** model for end-stage liver disease (MELD) score, and **(C)** Mac-2 binding protein glycosylation isomer (M2BPGi) ($p < 0.001$ for all).

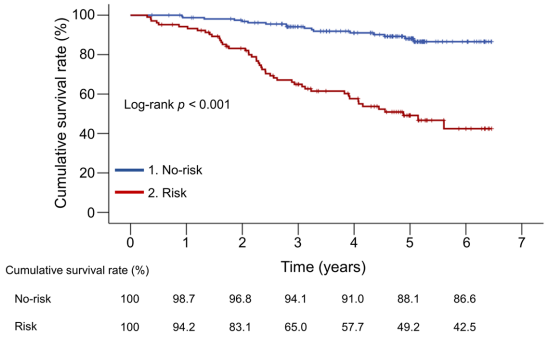

FIGURE 2
Comparison of the cumulative survival rates between the risk and no-risk groups. The cumulative survival rates were significantly lower in the risk group than in the no-risk group ($p < 0.001$).

Furthermore, we investigated whether the cumulative survival rates differed following the original GNRI classification. Age, decompensated cirrhosis prevalence, CP and MELD scores, and M2BPGi levels significantly increased stepwise, whereas BMI, albumin, and PT levels significantly decreased stepwise from the no-risk group to the low-, moderate-, and major-risk groups (Supplementary Table S1). The cumulative survival rates significantly decreased stepwise with increasing GNRI risk ($p < 0.001$; Supplementary Figure S3). The no-risk group demonstrated the highest cumulative survival rates than any other group ($p < 0.001$ for all), whereas the major-risk group had significantly lower survival rates than the no-risk and low-risk groups ($p < 0.001$ for both). Therefore, the combined group had significantly lower cumulative survival rates than the no- and low-risk groups when the major- and moderate-risk groups were combined into one group ($p < 0.001$ and $= 0.012$, respectively; Supplementary Figure S4).

Death from various non-liver-related causes was recorded in 13 patients who were considered censored cases (Supplementary Table S2). Even when they were included in the endpoint cases, similar results were obtained as above: i.e., the no-risk group demonstrated the highest cumulative survival rates than any other group, whereas the major-risk group had significantly lower survival rates than the no- and low-risk groups (Supplementary Figure S5). The cumulative survival rates for the major-risk group were identical to the above-described results, indicating that the major-risk group did not include non-liver-related deaths.

3.5. Comparison of cumulative survival rates between the risk and no-risk groups in subgroup populations

We compared the cumulative survival rates between the risk and no-risk groups in patients with compensated and decompensated cirrhosis, respectively (Figure 3). The 1-, 3-, and 5-year cumulative survival rates were 97.4% vs. 98.5%, 74.9% vs. 96.0%, and 61.6% vs. 90.7% in the risk and no-risk groups, respectively, in patients with compensated cirrhosis, indicating significant differences between the two groups ($p < 0.001$; Figure 3A). These were 92.3% vs. 100%, 59.4% vs. 85.7%, and 42.2% vs. 77.0% in the risk and no-risk groups, respectively, in patients with decompensated cirrhosis, indicating significant differences between the two groups ($p = 0.013$; Figure 3B).

We compared the cumulative survival rates between the risk and no-risk groups in patients with and without HCC, respectively (Supplementary Figure S6). In patients without HCC, the cumulative survival rates in the risk group were significantly lower than those in the no-risk group ($p < 0.001$; Supplementary Figure S6A). In patients with HCC, a marginally significant difference was found in the cumulative survival rates between the risk and no-risk groups ($p = 0.065$; Supplementary Figure S6B).

3.6. Significant factors associated with mortality in patients with cirrhosis

Univariate analysis revealed that the following variables were significantly associated with mortality: decompensated cirrhosis, CP score, MELD score, GNRI, total bilirubin, sodium, PT, and M2BPGi in all patients (Supplementary Table S3); CP score, GNRI, and sodium in patients with compensated cirrhosis (Supplementary Table S4); and age, CP score, MELD score, GNRI, total bilirubin, and M2BPGi in patients with decompensated cirrhosis (Supplementary Table S5). Among them, only CP score of all patients had a VIF > 5 and was excluded from further analysis (Supplementary Table S6). Cox proportional hazards regression analysis identified the following variables as significant and independent prognostic factors: low GNRI [hazard ratio (HR), 0.926; 95% confidence interval (CI), 0.905–0.947; $p < 0.001$] and high total bilirubin levels (HR, 1.391; 95% CI, 1.113–1.739; $p = 0.004$) in all patients (Table 2); low GNRI (HR, 0.947; 95% CI, 0.913–0.981; $p = 0.003$) and low sodium levels (HR, 0.779; 95% CI, 0.666–0.911; $p = 0.002$) in patients with compensated

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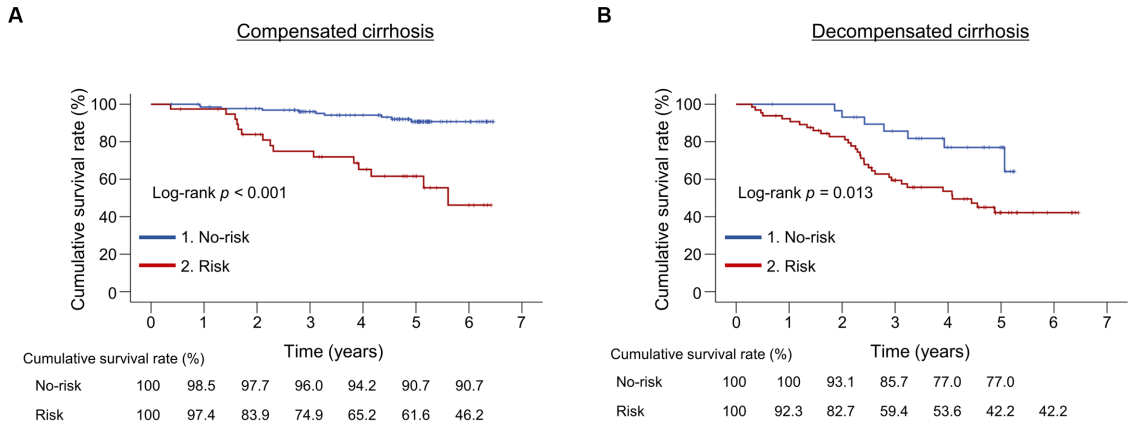

FIGURE 3
Comparison of the cumulative survival rates between the risk and no-risk groups in patients with compensated or decompensated cirrhosis. **(A,B)** The cumulative survival rates were significantly lower in the risk group than in the no-risk group in both patients with compensated and decompensated cirrhosis ($p < 0.001$ and = 0.013, respectively).

TABLE 2 Significant factors associated with mortality in all patients.

Variable	Univariate		Multivariate	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
Decompensated cirrhosis	3.629 (2.196–5.998)	< 0.001		
MELD score	1.200 (1.117–1.290)	< 0.001		
GNRI	0.919 (0.900–0.939)	< 0.001	0.926 (0.905–0.947)	<0.001
Total bilirubin (mg/dL)	1.761 (1.417–2.189)	< 0.001	1.391 (1.113–1.739)	0.004
Sodium (mEq/L)	0.851 (0.775–0.934)	< 0.001		
Prothrombin time (%)	0.970 (0.956–0.984)	< 0.001		
M2BPGi (C.O.I.)	1.167 (0.582–2.135)	< 0.001		

CI, confidence interval; C.O.I., cut-off index; GNRI, geriatric nutritional risk index; HR, hazard ratio; M2BPGi, Mac-2 binding protein glycosylation isomer; MELD, model for end-stage liver disease.

TABLE 3 Significant factors associated with mortality in patients with compensated cirrhosis.

Variable	Univariate		Multivariate	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
Child-Pugh score	1.829 (1.035–3.233)	0.038		
GNRI	0.934 (0.902–0.967)	< 0.001	0.947 (0.913–0.981)	0.003
Sodium (mEq/L)	0.735 (0.633–0.854)	< 0.001	0.779 (0.666–0.911)	0.002

CI, confidence interval; GNRI, geriatric nutritional risk index; HR, hazard ratio.

cirrhosis (Table 3); and low GNRI (HR, 0.923; 95% CI, 0.892–0.954; $p < 0.001$) in patients with decompensated cirrhosis (Table 4).

3.7. Prognostic nomograms for survival prediction

Prognostic nomograms of the independent factors identified in multivariate analysis were constructed to visually estimate 1-, 3-, and 5-year survival probabilities in all patients and those with compensated and decompensated cirrhosis (Figure 4; Supplementary Figures S7, S8, respectively). Points assigned to GNRI, total bilirubin, and sodium, respectively, were determined by drawing a vertical line from the corresponding value to the point scale (top in each figure). The total point was the sum of the points for these factors (middle in each figure). Finally, each survival probability was a value that matched the total point on the corresponding scale (lower in each figure).

4. Discussion

Malnutrition is a serious complication that negatively affects the prognosis of patients with cirrhosis (7, 8). Significant changes in body composition, such as body water, body fat, and body cell mass, occur

TABLE 4 Significant factors associated with mortality in patients with decompensated cirrhosis.

Variable	Univariate		Multivariate	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
Age (years)	1.031 (1.000–1.063)	0.049		
Child-Pugh score	1.624 (1.292–3.233)	< 0.001		
MELD score	1.130 (1.013–1.261)	< 0.001		
GNRI	0.920 (0.890–0.951)	< 0.001	0.923 (0.892–0.954)	<0.001
Total bilirubin (mg/dL)	1.401 (1.080–1.816)	0.011		
M2BPGi (C.O.I.)	1.093 (1.013–1.178)	0.021		

CI, confidence interval; C.O.I., cut-off index; GNRI, geriatric nutritional risk index; HR, hazard ratio; M2BPGi, Mac-2 binding protein glycosylation isomer; MELD, model for end-stage liver disease.

in patients with cirrhosis: a marked loss of body fat in the early stages of cirrhosis (i.e., CP class A) and an accelerated loss of body cell mass in the advanced stages of cirrhosis (i.e., CP class B or C) (29). Therefore, early nutritional status assessment is crucial in managing cirrhosis from a prognostic standpoint. Nevertheless, appropriate nutritional screening and assessment strategies remain to be determined due to the diversity of malnutrition definitions, lack of validated rapid screening methods, and difficulties in interpreting body composition and laboratory data in real-world clinical settings (30).

The GNRI is a simple and objective nutritional status assessment tool that can predict prognosis in patients with malignant (mainly digestive system cancers) and non-malignant diseases (such as heart failure, stroke, and chronic kidney disease) (12–19). However, no study has reported on the usefulness of GNRI in patients with cirrhosis. Hence, the present study is the first to report the association between GNRI and long-term prognosis in patients with cirrhosis. This study revealed that GNRI scores negatively correlated with CP and MELD scores and were the strongest independent prognostic factor, especially in patients with decompensated cirrhosis. The cumulative survival rates were determinately lower in the risk group and decreased stepwise with increasing risk in the original GNRI classification. One study of patients who underwent hepatic resection for HCC (CP class A/B: 96.8%/3.2%) revealed lower recurrence-free survival and overall survival (OS) rates in the low-GNRI (GNRI ≤98) group than the high-GNRI (GNRI >98) group and low GNRI as an independent prognostic factor for both rates (14). Another study of patients with CP class A and hepatitis B virus-related HCC revealed the major- and moderate-risk GNRI groups as independent risk factors for postoperative severe complications and OS (31). Another study of patients who underwent transarterial chemoembolization for HCC (CP class A/B: 45.9%/54.1%) revealed GNRI <98, tumor number ≥ 2, tumor size ≥5 cm, TACE times <3, and alpha-fetoprotein ≥400 as independent risk factors for poor prognosis (32). Furthermore, our recent study of patients with HCC treated with lenvatinib (CP class A/B: 85.2%/14.8%) revealed that low GNRI was independently associated with treatment discontinuation, and that the low-GNRI (GNRI ≤98) group had worse progression-free survival than the high-GNRI (GNRI >98) group (33). The present study revealed lower cumulative survival rates in the risk group than the no-risk group in both patients with and without HCC. These findings indicate that low GNRI is associated with poor prognosis, irrespective of HCC and liver functional reserve, and that the GNRI may be useful for predicting prognosis in patients with cirrhosis.

Various criteria for assessing malnutrition have been reported as useful for predicting prognosis in patients with cirrhosis. The European Society for Clinical Nutrition and Metabolism guidelines recommend the Royal Free Hospital-Nutritional Prioritizing Tool (RFH-NPT) to screen patients with liver disease for malnutrition risk (34). This screening tool comprises three major steps. First, the presence of acute alcoholic hepatitis or tube feeding is evaluated. Second, fluid overload (i.e., edema or ascites) and its impacts on food intake and weight loss are assessed. BMI, unplanned weight loss, and daily nutritional intake are assessed if patients do not have fluid overload. Third, the scores are calculated and patients are classified into the corresponding risk group (35, 36). Reportedly, the RFH-NPT was associated with CP and MELD scores and clinical complications (ascites, hepatorenal syndrome, and encephalopathy), and it was an independent predictor of MELD score deterioration and transplant-free survival (35). In addition, RFH-NPT amelioration caused an improvement in survival time. Furthermore, the RFH-NPT had superior prognostic predictability compared to the Nutritional Risk Screening 2002, including nutritional score (BMI, weight loss, and dietary intake), disease severity score, and age score (36). Meanwhile, the GLIM criteria, which includes unplanned weight loss, low BMI, reduced muscle mass, reduced dietary intake, and the presence of inflammation or disease burden, could predict mortality in patients with chronic liver disease, independent of liver functional reserve and HCC (8). However, a recent study of hospitalized Japanese older adults revealed that the GNRI was superior to the MNA-SF, which includes dietary intake, weight loss, mobility, the presence of psychological stress or acute disease, neuropsychological problem, and BMI, and GLIM criteria in predicting 1-year mortality (11). Muscle mass assessment included in the GLIM phenotypic criteria requires specialized equipment (such as dual-energy X-ray absorptiometry, computed tomography, and bioelectrical impedance analysis) and is not easy to perform in daily medical practice. In addition, the cutoff values for reduced muscle mass are not clearly indicated in the GLIM criteria and vary among races or different diagnostic criteria. Furthermore, the GLIM etiologic criteria components, such as reduced food intake, digestion or absorption, and chronic disease-related inflammation, are difficult to assess objectively. Meanwhile, the GNRI can be easily calculated from body weight and height and serum albumin levels, both of which are objective test items evaluated routinely in outpatient clinics. Thus, the GNRI is a simpler, more objective and convenient, and universal

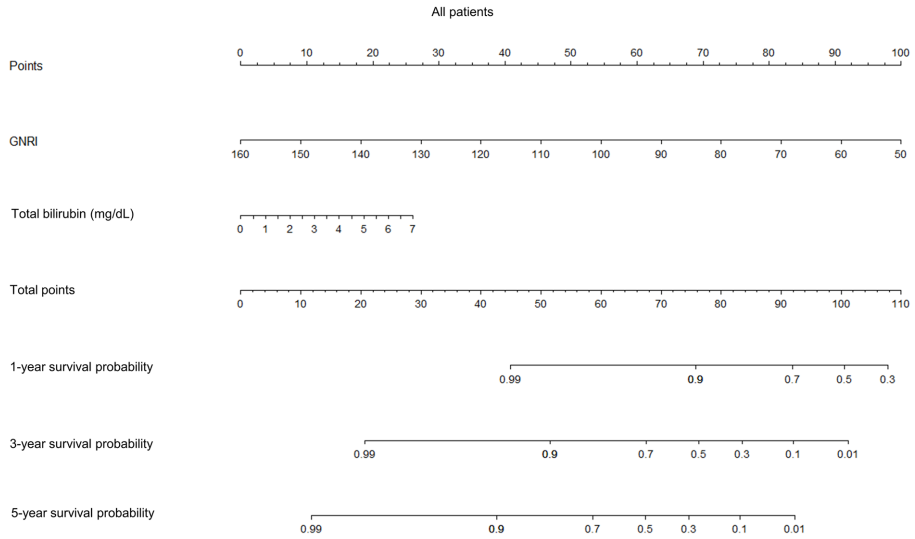

FIGURE 4
Prognostic nomograms for 1-, 3-, and 5-year survival probabilities of all patients. GNRI, geriatric nutritional risk index.

assessment tool for predicting prognosis, compared with the GLIM criteria.

The GNRI may reflect the chronic inflammatory condition. One study of chronic kidney disease revealed higher systemic inflammatory marker levels, such as C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor-α, in patients with low GNRI scores than those with high GNRI scores (37). Plasma IL-6 levels were independently and negatively associated with GNRI scores. Another study of older inpatients revealed the association of a higher GNRI risk with increased CRP levels and low lymphocyte counts (38). Another study of patients with cirrhosis revealed serum IL-6 and CRP levels as independent predictors of mortality and liver transplantation (39). The GNRI is a simple and easy-to-use malnutrition screening tool for predicting prognosis, although the most suitable scoring system for evaluating malnutrition in patients with cirrhosis remains to be determined. In the future, the GNRI must be compared with other assessment tools to determine the method appropriate for which patient, the anthropometric assessment to be included, and the more detailed or simplified assessment methods.

This study has some limitations. First, the presence of ascites may overestimate BMI and GNRI scores. However, this influence was reduced by excluding patients with massive ascites. Nutritional assessment tools, including BMI as an evaluation item, may optimistically interpret the nutrition status in patients with hepatic edema and ascites. Second, the inflammatory markers, which may influence the GNRI scores, were not assessed. Finally, this was a retrospective, two-center study; therefore, prospective, multicenter studies are needed to validate the aforementioned findings.

5. Conclusion

In conclusion, the GNRI score was the strongest factor associated with long-term prognosis in patients with cirrhosis. The GNRI is a simple, objective, and useful tool for predicting prognosis in patients with cirrhosis.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the ethics committees of the Jikei University School of Medicine (approval number: 34-021) and Fuji City General Hospital (approval number: 279). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because The requirement for written informed consent from participants was waived by the ethics committee of the Jikei University School of Medicine and Fuji City General Hospital due to the retrospective nature of the study.

Author contributions

HK: Data curation, Writing – original draft. CS: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. AK: Conceptualization, Supervision, Writing – review & editing. CN: Data curation, Writing – review & editing. TK: Data curation, Writing – review & editing. KU: Data curation, Writing – review & editing. MN: Data curation, Writing – review & editing. TO: Data curation, Writing – review & editing. YT: Data curation, Writing – review & editing. MS:

Supervision, Writing – review & editing. AT: Formal analysis, Supervision, 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

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Association between dietary intakes of B vitamins and nonalcoholic fatty liver disease in postmenopausal women: a cross-sectional study

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Background: Non-alcoholic fatty liver disease (NAFLD) is increasingly common globally, particularly among postmenopausal women. Diet plays a fundamental role in the treatment of NAFLD. However, clinical research on the dietary intakes of B vitamins, specifically in postmenopausal women, is scant. Hence, it is imperative to study the impact of B vitamin dietary intake in postmenopausal women.

Methods: This study utilized National Health and Nutrition Examination Survey (NHANES) data for 668 postmenopausal women. Logistic regression analysis was conducted to investigate the association of the intakes of B vitamins with hepatic steatosis and liver fibrosis prevalence. The analysis accounted for various covariates and employed restricted cubic spline analysis to examine potential nonlinear relationships. Additionally, interactions among age, diabetes, and B-vitamin intakes, as well as the interaction between folate and vitamin B12 intake, were explored.

Results: Higher intakes of folate [0.30 (0.10–0.88)], choline [0.26 (0.07–0.95)], vitamin B1, and vitamin B2 were associated with a reduced risk of hepatic steatosis in postmenopausal women. The associations of niacin (P-nonlinear = 0.0003), vitamin B1 (P-nonlinear = 0.036), and vitamin B2 (P-nonlinear<0.0001) intakes with hepatic steatosis showed a nonlinear pattern. However, no significant associations were observed between the intakes of niacin, vitamin B6 and vitamin B12 and hepatic steatosis. Furthermore, there were no significant associations between B-vitamin intakes and liver fibrosis. No interaction effects were observed.

Conclusion: Dietary intakes of folate, choline, vitamin B1, and vitamin B2 may be associated with liver steatosis in postmenopausal women, these results suggest that optimizing the intake of these specific B vitamins may have a protective effect against liver steatosis in postmenopausal women, offering valuable insights into potential dietary strategies to promote their well-being.

KEYWORDS

B vitamins, postmenopausal women, NAFLD, liver fibrosis, National Health and Nutrition Examination Survey

1. Introduction

In recent years, nonalcoholic fatty liver disease (NAFLD), now referred to as MASLD (Metabolic Dysfunction Associated Steatotic Liver Disease), has surpassed viral hepatitis as the most prevalent liver disease worldwide, with an estimated global prevalence of approximately 25%. It is further estimated that there are approximately 3.6 million new cases annually (1). Despite the renaming of NAFLD to MASLD, there was still controversy over the nomenclature when we conducted this study. Furthermore, the latest research has shown minimal differences between NAFLD and MASLD, with only one out of 414 liver biopsy cases of NAFLD not meeting the criteria for MASLD. Therefore, in the subsequent text, we will continue to use the term "NAFLD "(2).

While NAFLD is more prevalent in males (3), recent reports indicate an increasing trend in NAFLD prevalence among women (4). Notably, postmenopausal women, who lack estrogen, are susceptible to developing NAFLD and advanced NASH fibrosis, as compared to their premenopausal counterparts (5). Furthermore, a study indicates that for women, reaching the age of 50 or older significantly escalates the risk of advanced fibrosis (6). Given these observations, it becomes imperative to delve into the factors that contribute to or shield against NAFLD in postmenopausal women.

There is no specific medication available for NAFLD, and thus lifestyle modification remains the most effective long-term treatment, with dietary interventions being widely considered as the cornerston (7). Considering the significant influence of vitamins on the immune system and their potential to impact NAFLD, it's crucial to investigate the role of dietary vitamin intake. While some studies have explored vitamins D, C, and E in NAFLD treatment (8, 9), there is a lack of studies on vitamin B complex and NAFLD, particularly in the context of postmenopausal women, with choline being the primary focus.

The vitamin B group encompasses eight water-soluble compounds, including thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folate (B9), and cyanocobalamin (B12). However, only a few of these compounds have been studied in relation to NAFLD. Vitamins B1 and B2, along with their active coenzymes thiamine pyrophosphate (TPP) and flavin adenine dinucleotide (FAD), respectively, play a crucial role in the catabolism (breakdown) of carbohydrates and fatty acids. Compared to premenopausal women, postmenopausal women are unable to synthesize choline (vitamin B4) through the endogenous pathway catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) due to the lack of estrogen (10, 11). This leads to significantly more severe fibrosis in postmenopausal women with low choline intake (12). Vitamin B3(niacin), serves as a precursor for the coenzyme nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADPH) and shows potential for the treatment of NAFLD (13). The relationship between vitamin B12, folate, and NAFLD has been a subject of ongoing debate due to the lack of direct clinical evidence for NAFLD treatment (14–17). However, in recent research conducted by Dr. Tripathi and colleagues, they investigated the effects of vitamin B12 and folate supplementation in dietary and Cbs knockdown (Cbs-LKD) mouse models, as well as in 36 patients, and primates. After adding folate and vitamin B12 to the drinking water from weeks 0 to 16, they observed histological improvements in liver cell infiltration of inflammatory cells and fibrosis in mice(except steatosis), suggesting the potential of dietary vitamin B12 and folate supplementation for the prevention or treatment of NASH (18). There is limited research on the other B vitamins and their relationship with NAFLD.

In light of this, our study aims to investigate the association between B vitamin intake (B1, B2, niacin, B6, folate, B12, and choline) and hepatic steatosis in postmenopausal women and assess their clinical correlation with liver fibrosis in postmenopausal women with NAFLD, utilizing data from the National Health and Nutrition Examination Survey (NHANES) 2017–2020 March.

2. Materials and methods

2.1. Study population

NHANES is a cross-sectional, complex, multistage survey conducted with a cycle of 2 years, aiming to evaluate the health and dietary condition of adults and children in the United States. The analysis utilized data from the NHANES cycles of March 2017–2020, as these studies included information related to liver steatosis and fibrosis detected using transient elastography in adult subjects. Due to the COVID-19 pandemic, data for 2020 was not completed and the collected data are not nationally representative. Therefore, the data from March 2019–2020 were combined with the cycles from 2017–2018.

The inclusion criteria for this study consisted of postmenopausal women between the ages of 18 and 80. Exclusion criteria were as follows: (1) participants with missing data on vitamin intake in their diet, red blood cell (RBC) folate levels; (2) presence of hepatitis B or C (defined by the detection of hepatitis C antibodies or the presence of hepatitis B surface antigen) or a history of liver cancer or autoimmune liver disease; (3) missing transient elastography data or failure to meet the criteria; and (4) excessive alcohol consumption (> 10 g/day). After excluding all participants who did not meet the criteria, a total of 668 postmenopausal women remained. Flowchart 1 illustrates the detailed screening process. The protocol received approval from the Institutional Review Board of the National Center for Health Statistics, and all participants provided written informed consent.

2.2. Dietary assessment

The dietary intake of B vitamins and cholesterol was assessed using a 24-h dietary recall method administered by trained interviewers on both weekdays and weekends. Two dietary assessments were conducted for each participant, with a time interval of 3 to 10 days between them. The first assessment was conducted face-to-face, while the second assessment was conducted *via* telephone. The energy, macronutrient, and micronutrient intake from the dietary recalls were analyzed. The intake of B vitamins and cholesterol from dietary supplements was also included in the study. The dietary data were collected using the automated multiple-pass method, and all food and beverage ingredients were adhered to standards set by the US Department of Agriculture Food and Nutrition Database. In this analysis, the dietary intake of B vitamins and cholesterol was evaluated using the single-day values for individuals with single recalls and the two-day average values for others. Due to the inability to establish a

relationship between cholesterol intake exceeding 300 mg/day and cardiovascular diseases, the 2015–2020 Dietary Guidelines for Americans removed the restriction on recommending a maximum intake of 300 mg/day. However, those guidelines still advise minimizing the intake of cholesterol. Therefore, we defined cholesterol intake exceeding 300 mg/day as excessive.

2.3. Laboratory results

The detailed information regarding the measurement of RBC folate and urinary uric acid can be found in the laboratory method files on the NHANES official website (19).

2.4. Transient elastography

The main purpose of NHANES liver ultrasonographic transient elastography is to obtain objective measurements for two important manifestations of liver disease, liver fibrosis and hepatic steatosis, by recording controlled attenuation parameter (CAP), a method used to grade liver fat by measuring the degree of ultrasound attenuation in the liver) and FibroScan readings. The elastography examination is performed by NHANES health technicians who have received training and certification from NHANES staff and equipment manufacturers. Hepatic steatosis is characterized by a controlled attenuation parameter (CAP) score of 248 dB/m or above, while significant fibrosis is defined as a liver stiffness value equal to or exceeding 7.9 kPa (20, 21).

2.5. Other covariates

According to previous studies, we included the following risk factors for NAFLD as covariates: age (divided into an 18–60 years old group and a >60 years old group), race/ethnicity (Mexican–American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Other Races), education (≤ high school, >high school), daily physical activity (categorized based on the intensity of exercise and whether it is sustained for at least 10 min per week), smoking (current smoker, former smoker, never smoker), hypertension, diabetes, "overweight.group" (under/normal weight: <25 kg/m^2, overweight: 25–30 kg/m^2, obesity: ≥30 kg/m^2). Menopausal women were defined as those who had not had a menstrual period in the previous 12 months because of hysterectomy, menopause or change of life. Diabetes was defined as follows: a previous diagnosis of diabetes or the use of diabetes medication for blood glucose control. If there was no previous diagnosis of diabetes, it was defined by a glycated hemoglobin (HbA1c) level of ≥6.5% or a fasting blood glucose level of ≥126 mg/dL. If the subjects were regularly taking antihypertensive medication, if their systolic blood pressure exceeded 130 millimeters of mercury, or if their average diastolic blood pressure exceeded 80 millimeters of mercury (based on the average of three measurements), they were diagnosed with hypertension.

2.6. Statistical analysis

After applying the recommended weighting from NHANES to all variables, we conducted logistic regression analysis to assess the relationships between B vitamin intakes and hepatic steatosis and liver fibrosis. Participants were categorized into four groups based on their B-vitamin intake quartiles, with Quartile 1 serving as the reference group. Odds ratios (ORs) and 95% confidence intervals were calculated to compare Quartile 2, Quartile 3, and Quartile 4 with the reference group regarding the presence of hepatic steatosis (fatty liver). The same methodology was applied to compare these groups among postmenopausal women with NAFLD (n = 435). Furthermore, we constructed three logistic regression models by progressively including covariates in the analysis. Model 1 was adjusted for age, overweight and race/ethnicity. In Model 2, we adjusted for education level, physical activity, and smoking, in addition to the covariates included in Model 1. In Model 3, we adjusted for diabetes, uric acid levels, hypertension, and dietary cholesterol intake, in addition to the covariates included in Model 2. To ensure robustness of the regression model, we employed the variance inflation factor (VIF), which assesses the level of high correlation among independent variables in multiple regression analysis, to examine the presence of multicollinearity in the logistic regression model. In cases where high multicollinearity was detected, we removed the covariate with the highest VIF value. In Model 3 of the study investigating the relationships of B-vitamin intakes with hepatic steatosis, the covariate "overweight.group" was removed due to multicollinearity. In the study on the relationships of B-vitamin intakes with liver fibrosis, "race" was excluded in Model 2 due to multicollinearity, and both "race" and "smoking" were excluded as covariates in Model 3. We also conducted a stratified analysis based on ethnicity (including only non-Hispanic White, non-Hispanic Black, and Mexican American individuals) with a sample size of 334. Additionally, we conducted restricted cubic spline analyses to examine whether there were nonlinear relationships in the model (with four knots at 5, 35, 65, and 95%). If a nonlinear relationship was found to be statistically significant (p < 0.05), we illustrated the relationship between vitamin intake and the dependent variable using graphical plots. We also investigated the interactions between age (treated as a categorical variable) and B-vitamin intakes using the multiplication interaction approach. All statistical analyses were carried out utilizing R version 4.1.2, with a significance threshold set at $p \leq 0.05$ to establish statistical significance.

3. Results

3.1. Population characteristics

There were a total of 15,560 participants from March 2017 to 2020, with 2,208 of them being postmenopausal women. According to the exclusion criteria, we initially removed participants who lacked B-vitamin intake data (n = 129) and/or had insufficient or noncompliant transient elastography data (n = 248). Then, we excluded patients who might have had other liver diseases (n = 259), such as those with hepatitis B and C and those with excessive alcohol consumption, and those with missing covariate data (n = 784). Finally, a total of 668 subjects met the criteria for inclusion in this analysis. As shown in Table 1, the weighted prevalence of liver steatosis was 65.11%, while among patients with NAFLD, the weighted prevalence of liver fibrosis was 17.70%. In postmenopausal women, the prevalence of diabetes, obesity, hypertension, and hyperuricemia was higher among women with hepatic steatosis. Patients with hepatic steatosis (fatty liver) had lower intake of folate, choline, niacin, and vitamin B1. However, no such differences were observed in patients with liver fibrosis.

TABLE 1 Population characteristics by presence of non-alcoholic fatty liver disease and liver fibrosis in postmenopausal women.

		NAFLD group			Liver fibrosis group		
Characteristic	Overall, N = 668 (100%)[a]	NO, N = 233 (34.8%)[a]	YES, N = 435 (65.2%)a	*p*-value[b]	NO, N = 358 (82.3%)[a]	YES, N = 77 (17.7%)[a]	*p*-value[b]
age.group				0.8			0.8
18-60 years	255 (38.2%)	95 (40.8%)	160 (36.8%)		128 (35.8%)	32 (46%)	
60 + years	413 (61.8%)	138 (59.2%)	275 (63.2%)		230 (64.2%)	45 (54%)	
Weight.group				<0.001			0.025
Normal	142 (21.3%)	91 (39.1%)	51 (11.8%)		47 (13.1%)	4 (4.0%)	
Obesity	335 (50.1%)	61 (26.2%)	274 (63.0%)		211 (58.9%)	63 (84%)	
Overweight	191 (28.6%)	81 (34.8%)	110 (25.3%)		100 (27.9%)	10 (NA%)	
Race				0.5			0.5
Mexican American	60 (9.0%)	15 (6.4%)	45 (10.3%)		33 (9.2%)	12 (8.6%)	
Other Hispanic	64 (9.5%)	23 (9.9%)	41 (9.4%)		35 (9.8%)	6 (3.8%)	
Non-Hispanic White	264 (39.5%)	93 (39.9%)	171 (39.3%)		142 (39.7%)	29 (64%)	
Non-Hispanic Black	179 (36.8%)	61 (26.2%)	118 (27.1%)		94 (26.3%)	24 (12%)	
Others/multiracial	101 (15.1%)	41 (17.5%)	60 (13.8%)		54 (12%)	6 (11%)	
Smoke				0.2			0.2
Current	92 (13.8%)	36 (15.5%)	56(12.9%)		52 (14.5%)	4 (5.2%)	
Former	149 (21.4%)	45 (19.3%)	104 (23.9%)		87 (24.3%)	17 (22.1%)	
Never	426 (63.8%)	152 (65.2%)	275(63.2%)		219 (61.2%)	56 (72.7%)	
Hypertention	467 (69.9%)	147 (63.1%)	320 (73.6%)	0.002	257 (71.8%)	63 (86%)	0.002
Hyperuricemia	152 (22.8%)	32 (13.7%)	120 (27.6%)	0.019	92 (25.6%)	28 (36%)	0.087
Diabetes	188 (28.1%)	31 (13.3%)	157 (36.1%)	<0.001	114 (31.8%)	43 (63%)	<0.001
Exercise				0.009			0.089
No exercises	388 (58.1%)	127 (54.5%)	261 (60.0%)		209 (58.4%)	52 (67.5%)	
Moderate exercises	202 (30.2%)	68 (29.2%)	134 (30.8%)		111 (31.0%)	23 (29.9%)	
Vigorous exercises	78 (11.7%)	38 (16.3%)	40 (9.2%)		38 (10.6%)	2 (2.6%)	
Education				0.7			0.4
<=High school	284 (42.5%)	93 (39.9%)	191 (43.9%)		155 (43.3%)	36 (46.8%)	
>High school	384 (57.5%)	140 (60.1%)	244 (56.1%)		203 (56.7%)	41 (53.2%)	
Cholesterol(mg/d)				0.4			0.007
excessive	223 (33.4%)	66 (28.3%)	157 (36.1%)		122 (34.1%)	35 (45.5%)	
suitable	445 (66.6%)	167 (71.7%)	278 (63.9%)		236 (65.9%)	42 (54.5%)	
Choline(mcg/d)	272.54 (131.16)	309.92 (124.96)	227.51 (124.37)	0.10	256.52 (118.13)	354.30 (195.29)	0.012
Folate(mcg/d)	526.12 (397.09)	666.27 (469.24)	452.19 (330.56)	0.001	457.32 (334.37)	425.52 (310.70)	0.6
Niacin(mg/d)	34.30 (69.30)	32.67 (20.50)	35.16 (84.37)	0.023	36.30 (90.77)	29.21 (35.66)	>0.9
Vitamin 6(mg/d)	5.96 (19.33)	9.73 (28.64)	3.97 (11.30)	0.008	3.44 (5.22)	6.74 (25.48)	0.5
Vitamin 12(mcg/d)	89.40 (264.43)	60.01 (199.06)	124.82 (323.02)	0.2	137.32 (336.70)	38.25 (184.33)	0.12
Vitamin 1(mg/d)	2.27 (4.02)	2.94 (6.36)	1.95 (2.03)	0.3	1.93 (2.17)	2.04 (1.06)	0.2
Vitamin 2(mg/d)	5.69 (18.00)	9.18 (24.37)	3.85 (13.15)	0.005	3.86 (13.26)	3.78 (12.63)	0.5
RBC Folate(nmol/L)	1,353.71 (568.00)	1,368.74 (516.89)	1,345.79 (593.61)	0.3	1,345.87 (598.39)	1,345.37 (571.99)	>0.9

[a]*n* (unweighted) (%); Mean (SD).
[b]chi-squared test with Rao & Scott's second-order correction; Wilcoxon rank-sum test for complex survey samples. NAFLD: non-alcoholic fatty liver disease.

3.2. Associations with liver steatosis

In general, the results regarding the associations of dietary B-vitamin intakes with hepatic steatosis in postmenopausal women were similar between Model 1 and Model 2. Additionally, Model 3 showed similar results to Model 1 and Model 2, except for the associations involving the following B vitamins: folate, vitamin B6, choline, and niacin. In Model 2, comparing the highest and lowest

B-vitamin intake levels, a higher intake of vitamin B6 [OR (95% CI): 0.27 (0.10–0.74)] was negatively associated with hepatic steatosis. However, in Model 3, a higher intake of vitamin B6 was only weakly negatively associated with hepatic steatosis [0.33 (0.11–1.02)]. Similar to previous findings, in all three models, the highest intake level of folate was negatively associated with hepatic steatosis when compared with the lowest intake level [Model 3: 0.30 (0.10–0.88)]. This suggests that a higher intake of folate is a protective factor against NAFLD in postmenopausal women. However, no similar results were observed for RBC folate levels compared with those of dietary folate intake. For choline, we did not find statistically significant results in Models 1 or 2. However, in Model 3, after further adjustment for covariates, including diabetes, hypertension, cholesterol intake, and hyperuricemia, and the exclusion of obesity as a covariate due to collinearity, a higher intake of choline was shown to have a statistically significant negative association with hepatic steatosis [0.26 (0.07–0.95)]. Based on this result, we continued our research by gradually adding the aforementioned four covariates to Model 2, and it was found that including the covariate of cholesterol intake resulted in the aforementioned results. In addition, we found a negative association between a higher niacin intake and hepatic steatosis in Model 1. However, this relationship disappeared in Models 2 and 3. No links were observed between liver steatosis and other vitamins (see Table 2).

3.3. Associations with liver fibrosis

In patients with NAFLD, when comparing the intakes of the different B vitamins using quartiles, there was a significant variation in intakes between Model 3 and Models 1 and 2 (Supplementary Table S1). This may be due to the limited number of patients with liver fibrosis ($n = 77$) and the extremely uneven distribution of liver fibrosis patients among the four quartile groups. We also conducted a baseline analysis on four groups categorized by quartiles of B-vitamin intake, and we found no significant differences in their baseline characteristics (refer to Supplementary Table S3). Therefore, in this logistic regression model, we adopted the third quartile (Table 3) for analysis. Similar to the regression model for hepatic steatosis, the results of the liver fibrosis regression models in Models 1 and 2 showed fluctuations but no significant changes. Comparing the highest tertile to the lowest tertile in Model 2, a higher dietary intake of choline showed a positive association with liver fibrosis, with an odds ratio of 4.05 (95% confidence interval: 1.24–13.2). However, In Model 3, the aforementioned association became nonsignificant. There were no significant associations observed between intakes of the other B vitamins and liver fibrosis (Table 3). Due to the potential associations of race with dietary and genetic backgrounds, we also conducted a stratified analysis based on race (refer to Supplementary Table S2). In this analysis, we only included non-Hispanic White, non-Hispanic Black, and Mexican American individuals, resulting in a sample size of 334. The results showed significant fluctuations compared to those in Table 3; however, the overall trend remained consistent. In this stratified analysis, we did not observe any significant relationship between the intakes of B vitamins and liver fibrosis.

3.4. Nonlinear relationships

We used restricted cubic splines in all of the Model 3 analyses to test for nonlinear relationships between the independent variables and the response variable and found that dietary intakes of vitamin B1, vitamin B2, and niacin exhibited nonlinear relationships with NAFLD. As shown in Figure 1, there was an inverted U-shaped association between niacin and NAFLD in menopausal females. When niacin intake was 15.8 mg, the odds ratio (OR) reached its highest point of 1.04 (95% confidence interval: 1.02–1.07). Subsequently, as the intake of niacin increased, the prevalence of NAFLD decreased. When the niacin intake was approximately 40 mg, the prevalence of NAFLD reached a plateau. We also investigated the relationship between niacin intake and NAFLD in the general population during the NHANES cycle from 2017 to March 2020 ($n = 3{,}088$) using restricted cubic splines, and the results revealed that there was no significant association between niacin intake and the prevalence of NAFLD (Supplementary Figure S2A). The associations of the dietary intakes of vitamin B2 and vitamin B1 with NAFLD followed a decreasing curve (Figures 2B,C). As shown in Figure 2B, a decrease in the risk of developing NAFLD was observed with an increasing intake of vitamin B1 when it exceeded 1.42 mg, and once the dietary intake of vitamin B1 exceeded 15 mg, the change in OR with a further increased intake became negligible. In Figure 2C, when vitamin B2 was greater than 1.75 mg (OR = 1), the risk of developing NAFLD decreased with the increasing intake of vitamin B2. When the dietary intake of vitamin B2 exceeded 10 mg, further increases in the intake resulted in minimal changes in the OR. These findings indicated that the protective effect against NAFLD does not continue to improve beyond dietary niacin intake of 40 mg, dietary vitamin B1 intake of 15 mg, and dietary vitamin B2 intake of 10 mg. We did not observe nonlinear relationships in other models.

3.5. The interaction effect in the model

In order to ensure the robustness of the results, we applied the Rao-Scott chi-square test or the likelihood ratio to explore the multiplicative interaction of age group and diabetes with B-vitamin intakes for all three models. The results indicated no significant multiplicative interaction between these variables. Additionally, we included the interaction term between dietary intake of vitamin B12 and folate in Models 1 and 3 to investigate whether there was a multiplicative interaction effect on NAFLD. Notably, due to the inclusion of the interaction term with quartiles, the model degrees of freedom were limited. Therefore, we categorized the intake of folate and vitamin B12 into tertiles instead of quartiles. As shown in Table 4, the p value for interaction was not statistically significant, but when we applied the Wald t test, the result was statistically significant ($p = 0.012$). The difference in results between the Wald t test and the Rao-Scott chi-square test can be attributed to their underlying assumptions and methodologies. The Wald t test assumes a normal distribution and is suitable for simple random samples. However, in the case of the NHANES study, which is based on complex survey sampling, the Rao-Scott chi-square test is more appropriate. This test accounts for the complex sampling design and uses weighted estimates to provide valid statistical inference. We also presented the results of the stratified interaction effect in the Table 4.

TABLE 2 Associations between B vitamin and non-alcoholic fatty liver disease in postmenopausal women.

Exposure	Model 1			Model 2			Model 3		
	Q2 vs. Q1 OR (95%CI)	Q3 vs. Q1 OR (95%CI)	Q4 vs. Q1 OR (95%CI)	Q2 vs. Q1 OR (95%CI)	Q3 vs. Q1 OR (95%CI)	Q4 vs. Q1 OR (95%CI)	Q2 vs. Q1 OR (95%CI)	Q3 vs. Q1 OR (95%CI)	Q4 vs. Q1 OR (95%CI)
Vitamin B1	0.76(0.34–1.68)	0.71(0.34–1.50)	0.46(0.18–1.13)	0.78(0.34–1.80)	0.70(0.30–1.80)	0.48(0.19–1.24)	0.60(0.22–1.63)	0.60(0.19–1.86)	0.47(0.16–1.39)
Vitamin B2	0.79(0.38–1.64)	0.81(0.31–2.10)	0.51(0.26–1.00)	0.76(0.35–1.66)	0.79(0.32–1.96)	0.52(0.26–1.01)	0.58(0.27–1.25)	0.61(0.23–1.66)	0.53(0.24–1.16)
Vitamin B6	0.88(0.42–1.83)	0.87(0.1–1.86)	0.26(0.10–0.70)*	0.91(0.41–1.22)	0.85(0.39–1.85)	0.27(0.10–0.74)*	0.92(0.38–2.22)	0.77(0.30–1.97)	0.33(0.11–1.02)
Vitamin B12	1.15(0.46–2.84)	0.59(0.22–1.57)	0.64(0.40–1.03)	1.23(0.47–3.23)	0.60(0.22–1.65)	0.67(0.41–1.09)	1.02(0.41–2.55)	0.62(0.19–1.97)	0.69(0.41–1.16)
Choline	0.85(0.30–2.42)	1.13(0.51–2.53)	0.74(0.34–1.61)	0.85(0.30–2.46)	1.22(0.50–3.01)	0.70(0.29–1.71)	0.61(0.21–1.77)	0.79(0.25–2.53)	0.26(0.07–0.95)*
Folate, DFE	1.35(0.54–3.37)	0.80(0.33–1.95)	0.28(0.10–0.78)*	1.48(0.53–4.17)	0.80(0.31–2.11)	0.30(0.10–0.89)*	0.93(0.38–2.27)	0.62(0.19–2.0)	0.30(0.10–0.88)*
Niacin	1.17(0.50–2.72)	0.94(0.40–2.18)	0.42(0.17–0.99)*	1.24(0.51–2.97)	0.94(0.38–2.31)	0.45(0.18–1.13)	0.98(0.38–2.54)	0.77(0.28–2.12)	0.48(0.17–1.36)
RBC folate	0.68(0.28–1.66)	0.56(0.21–1.46)	0.58(0.23–1.43)	0.72(0.27–1.89)	0.63(0.23–1.71)	0.60(0.24–1.51)	0.49(0.21–1.18)	0.59(0.23–1.49)	0.56(0.19–1.65)

Q1, quantiles 1; Q2, quantiles 2; Q3, quantiles 3; Q4 quantiles 4.
OR (95% CI), odds ratio (95% confidence interval).
*$P < 0.05$, **$p < 0.01$.
DFE, dietary folate equivalents.
Model 1 was adjusted for age.group, weight.group, and race/ethnicity.
Model 2 was adjusted for covariates in model 1, and also education, physical activity, smoking.
Model 3 was adjusted for covariates in model 2, and also hypertension, diabetes, and dietary intakes of cholesterol and hyperuricemia, while eliminate weight.group because of collinearity.

TABLE 3 Associations between B vitamin and liver fibrosis in postmenopausal women.

Exposure	Model 1		Model 2		Model 3	
	T2 vs. T1 OR (95%CI)	T3 vs. T1 OR (95%CI)	T2 vs. T1 OR (95%CI)	T3 vs. T1 OR (95%CI)	T2 vs. Q1 OR (95%CI)	T3 vs. T1 OR (95%CI)
Vitamin B1	1.58(0.71–3.52)	0.82(0.33–2.04)	1.89(0.82–4.37)	0.85(0.42–1.71)	1.84(0.86–3.93)	0.87(0.35–2.15)
Vitamin B2	1.61(0.54–4.85)	1.50(0.51–4.43)	1.96(0.59–6.49)	1.74(0.82–3.67)	1.70(0.57–5.05)	1.47(0.57–3.82)
Vitamin B6	1.06(0.33–3.40)	1.45(0.53–4.01)	1.14(0.32–4.03)	1.69(0.72–3.94)	1.05(0.34–3.28)	1.57(0.62–3.95)
Vitamin B12	1.36(0.50–3.72)	1.56(0.40–6.04)	1.23(0.37–4.07)	1.38(0.52–3.66)	1.03(0.37–2.89)	1.01(0.41–2.46)
Choline	2.47(0.76–8.05)	4.32(1.43–13.1)*	2.25(0.90–5.65)	4.05(1.24–13.2)*	1.69(0.45–6.40)	1.82(0.32–10.3)
Folate, DFE	1.09(0.39–3.01)	0.83(0.33–2.13)	1.21(0.50–2.96)	0.90(0.35–2.33)	1.04(0.39–2.81)	0.85(0.34–2.12)
Niacin	2.28(0.90–5.75)	1.04(0.28–3.82)	2.31(0.92–5.81)	1.09(0.40–2.98)	1.79(0.80–4.02)	0.80(0.26–2.45)
RBC folate	0.97(0.59–1.58)	0.97(0.63–1.49)	0.98(0.59–1.61)	0.98(0.62–1.53)	0.97(0.57–1.65)	0.97(0.59–1.59)

T1, tertiles 1; T2, tertiles 2; T3, tertiles 3.
OR (95% CI), odds ratio (95% confidence interval).
*$P < 0.05$, **$P < 0.01$.
DFE, dietary folate equivalents.
Model 1 was adjusted for age.group, weight.group, and race/ethnicity.
Model 2 was adjusted for covariates in model 1, and also education, physical activity, smoke, while eliminate race because of collinearity.
Model 3 was adjusted for covariates in model 2, and also hypertension, diabetes, and dietary intakes of cholesterol and hyperuricemia, while eliminate smoke because of collinearity.

4. Discussion

The results of this nationwide survey among postmenopausal women suggest a negative potential association between the dietary intakes of folate, choline and vitamin B6 and hepatic steatosis. Additionally, restricted cubic spline analysis indicated a nonlinear relationship between vitamin B1, vitamin B2, niacin, and hepatic steatosis. In addition, there was no evidence of an interaction between age grouping and dietary intake of B vitamins, diabetes and dietary intake of B vitamins, or dietary intake of vitamin B12 and folate.

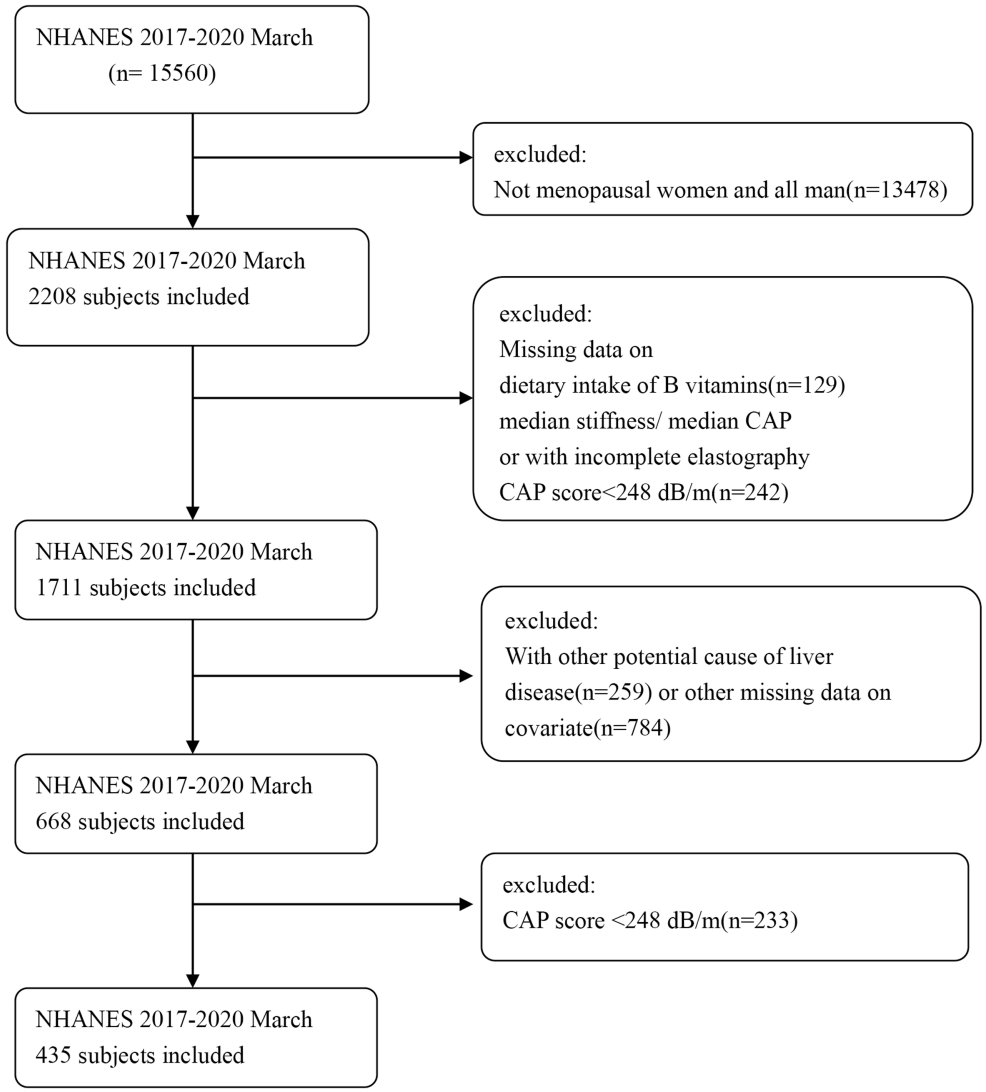

FIGURE 1
Flowchart of the study population.

Several mouse models related to choline have demonstrated the association between choline-deficient diets and NAFLD (22, 23). These diets can disrupt the gut microbiota by reducing diversity and altering the microbial community, potentially activating the Nod-like receptor protein 3 (NLRP3) inflammasome (24). Additionally, choline-deficient diets can impair the intestinal barrier, allowing increased bacterial penetration from the gastrointestinal tract into organs like the liver, leading to hepatic steatosis and lipid accumulation (25, 26). The accumulation of lipids may be linked to abnormal fatty acid metabolism, inflammatory responses, and activation of the NLRP3 inflammasome. These research findings demonstrate the impact influence of insufficient choline on the progression of NAFLD. Cholesterol and choline were often found together in the same food. In our study, after adjusting for the effects of cholesterol, the relationship between choline and NAFLD became significant. In a recent study, it was found that an increased intake of eggs and choline in elderly women in the United States was significantly associated with an increased risk of diabetes. However, when cholesterol consumption was accounted for and adjusted for, the aforementioned relationship disappeared (27). This suggests that cholesterol intake may mask the relationship between choline and NAFLD. However, further research is needed to better understand these relationships and optimize dietary intakes of these nutrients to reduce the risk of this disease.

Folate plays a role in methylation processes, and folate deficiency has been linked to triglyceride (TG) accumulation in the liver and a lower risk of NAFLD (OR: 0.75; 95% CI: 0.58, 0.99) (15, 28). Additionally, dietary folate deficiency can lead to reduced levels of choline and phosphatidylcholine (PC) in the liver (29). As mentioned above, in a recent study, dietary folate was reported to promote the conversion of homocysteine to methionine, stimulate fatty acid β-oxidation, and improve liver histology in mouse models (18). However, the relationship between folate intake and NAFLD risk remains a topic of debate, as some studies have not observed an association with folate intake (30, 31). The Institute of Medicine (IOM) recommends a Recommended Dietary Allowance (RDA) of

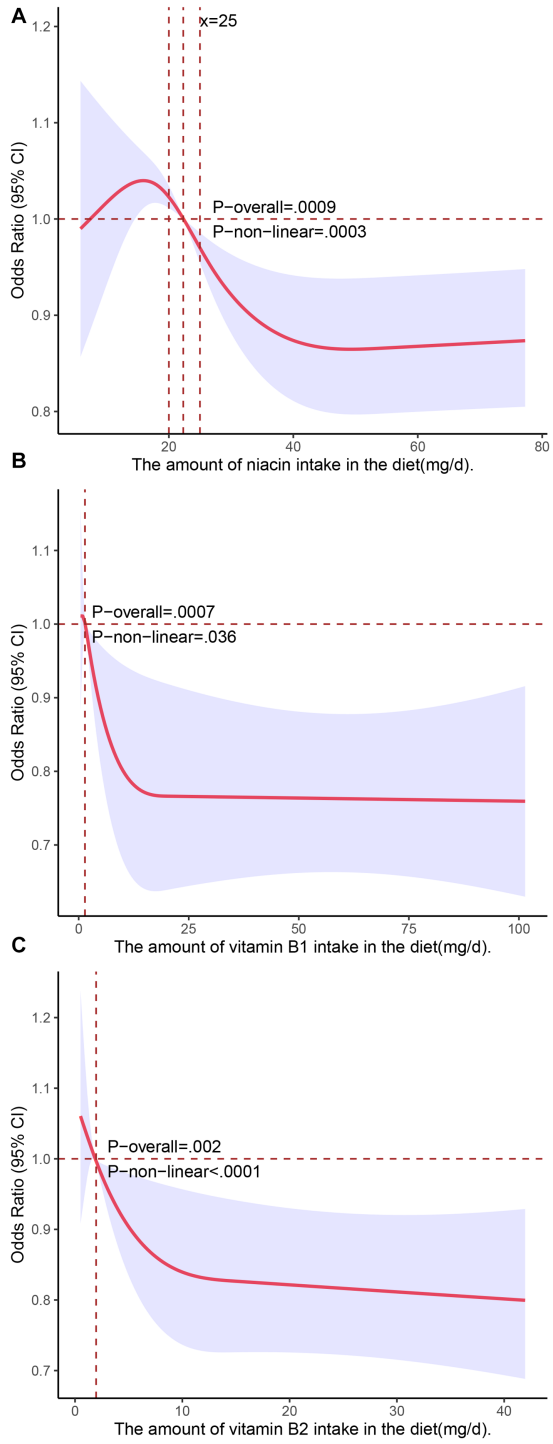

FIGURE 2
Associations of dietary intakes of niacin **(A)**, vitamin B1 **(B)**, and vitamin B2 **(C)** with the risk of NAFLD (presented as odds ratios) in postmenopausal women using a restricted cubic spline regression model.

400 micrograms of folate for adults aged 18 and older, with a Tolerable Upper Intake Level (UL) of 1,000 micrograms (32). In our study, we observed that higher folate concentrations (>698 mcg) in the Q4 group were negatively associated with the incidence of NAFLD compared to the Q1 group. The aforementioned findings suggest that

TABLE 4 Combined effect of dietary intake of folate and vitamin B12 on NAFLD.

Variable 1	Variable 2		
Folate intake	Vitamin B12 intake	Adjusted OR (95%CI)	P for interaction
T1	T1	1.00(reference)	0.192
T1	T2	0.84(0.18–3.85)	
T1	T3	9.25(0.72–119)	
T2	T1	0.91(0.20–4.10)	
T2	T2	1.58(0.25–10.1)	
T2	T3	1.20(0.02–23.1)	
T3	T1	0.57(0.07–4.88)	
T3	T2	0.13(0.01–2.65)	
T3	T3	0.07(0.00–1.13)	

folate supplementation in postmenopausal women may have the potential to reduce the risk of developing NAFLD. However, unmetabolized folic acid has been reported to reduce the cytotoxicity of natural killer cells, thereby compromising the immune system in the human body (33). Therefore, the intake of folate in elderly individuals needs to be considered comprehensively. In contrast to previous studies, our research did not find a relationship between RBC folate levels and NAFLD. This discrepancy may be because only one participant among the included postmenopausal women had a lower plasma RBC folate concentration.

Preclinical studies have shown that niacin inhibits the activity of hepatic cell DGAT2 and NADPH oxidase, leading to a reduction in hepatic lipid accumulation and ROS generation in the liver (34, 35). In an uncontrolled clinical trial involving 39 patients with hypertriglyceridemia and concurrent hepatic steatosis, treatment with extended-release niacin (ER) for 6 months resulted in a significant 47% reduction in liver fat (13). Nevertheless, it should be noted that there is still a lack of sufficient clinical evidence in this area. However, in our study, a protective effect was observed when the intake of niacin exceeded 22.3 mg (Figure 2A), which contributes to the clinical evidence supporting the above notion to some extent.

The mechanisms of vitamin B1 and vitamin B2 in NAFLD are not fully understood, and there is limited clinical research available on this topic. However, related animal experiments have revealed the potential of vitamin B1 supplementation in preventing NAFLD. In its active form thiamine pyrophosphate (TPP), thiamine (vitamin B1) may enhance carbohydrate metabolism by maximizing the activity of TPP-dependent enzymes, potentially reducing the rate of hepatic lipid accumulation. Recent animal experiments have demonstrated that thiamine supplementation can markedly reduce hepatic fat content in animal models consuming a high-calorie diet (36). In our study, we found nonlinear relationships of dietary intakes of vitamin B1 and vitamin B2 with NAFLD. Specifically, higher intakes of vitamin B1 and vitamin B2 were negatively associated with the risk of NAFLD. Consequently, providing adequate levels of Vitamin B1 and Vitamin B2 in the daily diets of postmenopausal women could potentially serve as a preventive measure against NAFLD. However, more clinical studies are needed to confirm and further understand this phenomenon.

Our study focused on postmenopausal women in the United States, which is a relatively understudied population. This study provides valuable information for understanding the relationship between dietary intake of B vitamins and NAFLD in postmenopausal women. Moreover, we found nonlinear associations of the intakes of niacin, vitamin B1 and vitamin B2 with NAFLD, with higher intakes being negatively associated with the risk of NAFLD. These findings offer new insights into the potential role of niacin, vitamin B1, vitamin B2, folate and choline in the prevention of NAFLD and can contribute to guiding dietary recommendations and strategies for NAFLD prevention in postmenopausal women, with practical implications for public health and clinical practice. However, there are some limitations to consider. First, our study was cross-sectional, and since the dietary data was collected for only 1–2 days, and due to limitations in data and software, we did not apply the NCI model or the SPADE model for dietary intake estimation, which could potentially introduce bias and might not fully represent the long-term dietary habits of the participants. Nonetheless, dietary data collection is inherently challenging in dietary research. Additionally, the observational nature of the study limits our ability to establish causality, which would require prospective studies for confirmation. Second, importantly, our study had a relatively small sample size, and the grouping based on quartiles may have resulted in a highly imbalanced distribution of the liver fibrosis ($n = 77$) across each group, potentially leading to erroneous results. However, we validated the results by testing different grouping methods (e.g., using tertiles) to minimize such errors. Last, our assessment of hepatic steatosis relied on ultrasound imaging rather than the gold standard of liver biopsy.

In conclusion, the findings of this study targeting postmenopausal women suggest that dietary intakes of folate, choline, vitamin B1, vitamin B2, and niacin may be negatively associated with NAFLD in this population. Specifically, folate and choline intakes showed a linear relationship with NAFLD, while the associations of vitamin B1, vitamin B2, and niacin with hepatic steatosis may be nonlinear. However, further prospective research is needed to confirm these observations and establish a clearer understanding of the relationships between these nutrients and NAFLD in postmenopausal women.

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 the National Center for Health Statistics Research 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

JL: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. JH: Supervision, Writing – original draft, Formal analysis. YL: Writing – original draft, Validation, Writing – review & editing. HJ: Supervision, Conceptualization, 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.2023.1272321/full#supplementary-material

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The relationship between skeletal muscle mass to visceral fat area ratio and metabolic dysfunction-associated fatty liver disease subtypes in middle-aged and elderly population: a single-center retrospective study

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Background: It has been reported that decreased muscle mass combined with excessive visceral adipose tissue are significantly correlated with the risk of non-alcoholic fatty liver disease (NAFLD). However, it has not been explored among populations with metabolic dysfunction-associated fatty liver disease (MAFLD) subtypes. We aimed to investigate whether appendicular skeletal muscle mass to visceral fat area ratio (SVR), an indicator of sarcopenic obesity, influences on the risk of MAFLD subtypes and its hepatic condition in middle-aged and elderly population.

Methods: A total of 4,003 middle-aged and elderly subjects were finally enrolled in this single-center retrospective study. Abdominal ultrasonography was employed for hepatic steatosis diagnosis. Participants were divided into four groups: diabetes-MAFLD, overweight/obese-MAFLD, lean-MAFLD and no MAFLD. Appendicular skeletal muscle mass as well as visceral fat area (VAF) was estimated by bioimpedance analysis measurements. Liver fibrosis was defined as a Fibrosis-4 index (FIB-4) and the NAFLD Fibrosis Score (NFS). Multivariate logistic regression analysis was performed to estimate the odds ratio and 95% confidence interval between SVR and MAFLD subtypes/hepatic condition stratified by sex.

Results: Participants with MAFLD subtypes had a significant lower value of SVR compared with those without MAFLD (P<0.001), while high quartiles of FIB-4 and NFS also showed a decreasing value of SVR in comparison with its lower quartiles ($P_{\text{for trend}}$<0.001). The lowest quartile of SVR increased the prevalence of MAFLD subtypes [adjusted OR (95%CI): 2.96 (1.48 ~ 5.93) $_{\text{male}}$ /3.30(1.46 ~ 7.46) $_{\text{female}}$ for diabetes-MAFLD, 1.91(1.26 ~ 2.88) $_{\text{male}}$ /4.48(1.91 ~ 10.49) $_{\text{female}}$ for overweight/obese-MAFLD and 4.01(1.46 ~ 10.98) $_{\text{male}}$/2.53(1.19 ~ 5.37) $_{\text{female}}$ for lean-MAFLD groups] compared with the highest quartile of SVR (all $P_{\text{for trend}}$<0.001). Besides, the interaction effect of gender on the relationship between SVR and MAFLD subtypes

was statistically significant (all $P_{\text{for interaction}}<0.001$).Restricted cubic spline indicated an inverse association between SVR and the risk of MAFLD subtypes with linearity (all P for non-linearity>0.05). The lowest quartile of SVR also increases the risk of MAFLD fibrosis in both males and females.

Conclusion: Our study concluded that a decrease in SVR (appendicular skeletal muscle mass divided by visceral fat area) is significantly associated with an increased prevalence of developing MAFLD subtypes and liver fibrosis in middle-aged and older persons of both genders.

KEYWORDS

skeletal muscle mass to visceral fat area ratio, metabolic dysfunction-associated fatty liver disease subtypes, gender, fibrosis, middle-aged, elderly

Introduction

Metabolic associated fatty liver disease (MAFLD), previously known as NAFLD, is a liver disease related to metabolic dysfunction and is the most common chronic liver disease worldwide. It is estimated that 24% of adults are affected by NAFLD, posing a serious threat to human health (1). In contrast to NAFLD, MAFLD does not necessitate the exclusion of other sources of liver disease, like overindulging in alcohol or viral hepatitis (2, 3). An international expert consensus recently concluded that "MAFLD" was a more suitable term to describe liver disease caused by metabolic disorders, and subsequently released a set of diagnostic criteria to facilitate the accurate, comprehensive and straightforward diagnosis of MAFLD (3). MAFLD is a multi-system disorder that increases the risk of liver-specific complications, as well as other health concerns such as cardio-metabolic morbidity and mortality (4–7). To gain a better comprehension of how to identify those at high risk and create successful treatments for the illness, Further investigation is necessary to comprehend the diverse elements that contribute to the etiology and pathogenesis of this complex liver condition.

Excess weight, particularly abdominal obesity, is a major risk factor for MAFLD (8, 9). VFA (Visceral Fat Area) is a reliable and reproducible measure of abdominal obesity, and is linked to a greater risk of metabolic syndrome (MetS) and MAFLD than BMI (Body Mass Index) and WC (Waist Circumference) as indicators of adiposity (10). Furthermore, decreased muscle mass, known as sarcopenia, has also been identified as a risk factor for MAFLD, as skeletal muscle influences glucose disposal and insulin resistance (11–13). Sarcopenic obesity, a combination of low muscle mass and high visceral fat, has a substantial influence on metabolism and increases the risk of MAFLD. It has been suggested that alterations in body composition, such as an increase in visceral fat and a decrease in skeletal muscles due to muscular protein degradation in the context of obesity-related chronic inflammation, may be linked to the onset and progression of NAFLD. Furthermore, abnormal body composition has been found to be a significant factor influencing the pathophysiology and prognosis of hepatic conditions (14). By using bioelectrical impedance, it is possible to measure the appendicular skeletal muscle mass and intra-abdominal visceral fat area, and the SVR index, which is an indicator of sarcopenic obesity, can be derived from these measurements.

It has been established that SVR has a strong correlation with the probability of having NAFLD and the worsening of hepatic conditions. To date, few studies have investigated the impact of SVR on the risk of developing different MAFLD subtypes and its hepatic condition among the health check-up population. Therefore, this study aimed to explore the gender-specific associations between SVR and the risk of MAFLD subtypes and its hepatic condition in middle-aged and elderly populations.

Materials and methods

Study population

Individuals aged 45 years or above who attended annual health examinations in the health check-up center of Wuxi People's Hospital Affiliated to Nanjing Medical University were included. This retrospective study originally enrolled 4,811 middle-aged and elderly population. Participants were excluded if they had incomplete medical information mainly including bioelectrical impedance analyzer (BIA) measurements and abdominal ultrasound. Excluding the participants mentioned (Figure 1), 4,003 individuals were finally included in the study, comprising of 2,420 males and 1,583 females aged between 45 and 91 years. Demographic data comprised of age, gender, and cigarette/alcohol use; smoking was classified as smoking three or more cigarettes daily in a year, and alcohol consumption of at least three times a week for at least 12 months.

This retrospective study was performed in compliance with the Declaration of Helsinki and was permitted by the Health Examination Center of Wuxi People's Hospital Ethics and Research Committee (approval number KY23150). Personal data was anonymized in order to protect the privacy of patients; statistical analysis was conducted in a confidential manner and was only utilized for scientific objectives. Therefore, the necessity for informed consent was waived.

Body composition and anthropometric measurements

Assessment of the body composition of all participants was done using the segmental multifrequency bioelectrical impedance analysis system (InBody 4.0, InBody Co., South Korea). The validated equation

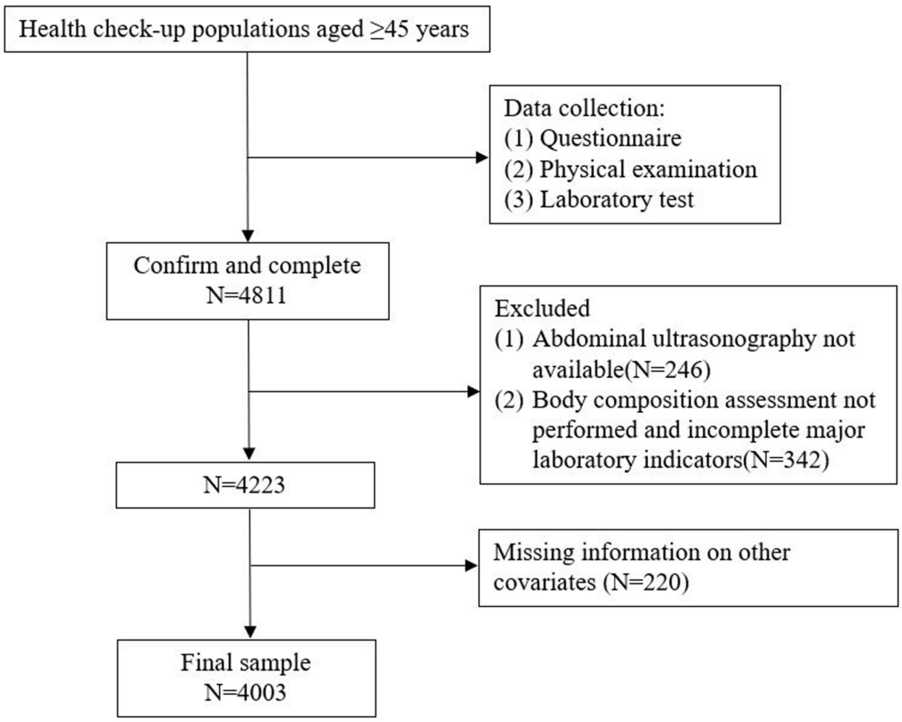

FIGURE 1
Study flow chart illustrated the flow of the participants included in this study.

of Janssen et al. was used to calculate the skeletal muscle mass of each subject, which is calculated as (15): skeletal muscle mass (kg) = [(height^2/BIA resistance * 0.401) + (gender*3.825) + (age * −0.071)] + 5.102, where height is in centimeters, BIA resistance is in ohms, gender is coded as 1 for male and 0 for female, and age is in years. Appendicular skeletal mass (ASM) is the sum of lean muscle mass in the upper and lower limbs. The skeletal muscle mass to visceral fat area ratio (SVR) is calculated by dividing ASM by the visceral fat area. Body weight, height, and waist circumference (WC) were measured by trained nurses, and Body Mass Index (BMI) was calculated using the formula: BMI = body weight (kg)/height (m)2. WC was measured to the nearest 0.1 cm around the horizontal level at the high point of the iliac crest.

Laboratory and clinical measurements

Blood specimens (10–15 mL) were collected from the antecubital vein of participants after at 12-h overnight fast. The serum was placed at room temperature for 30 min and centrifuged at 3000 rpm for 10 min. Laboratory measurements included fasting blood glucose (FBG), triglycerides (TGs), total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-C), high-density-lipoprotein cholesterol (HDL-C), serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), white blood cells (WBC), neutrophils (NE), and lymphocytes (LY). All blood samples were tested within 24 h in medical laboratory center of Wuxi People's Hospital Affiliated to Nanjing Medical University.

Diabetes mellitus was defined as fasting blood glucose ≥ 100 mg/dL or currently receiving antidiabetic medication therapy (16), while hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or receiving anti-hypertension treatment at present (17). dyslipidemia defined as two or more of these four criteria: fasting serum triglyceride ≥ 1.70 mmol/L, TC ≥ 5.20 mmol/L, LDL cholesterol ≥ 3.12 mmol/L, or HDL cholesterol ≤ 0.91 mmol/L.

Assessment of MAFLD and liver fibrosis

MAFLD was identified when hepatic steatosis was present, and at least one of the following criteria (3): being overweight or obese (BMI ≥ 23 kg/m^2), having type 2 diabetes mellitus, or exhibiting metabolic dysregulation. Metabolic dysregulation was defined by the occurrence of at least two of the following risks for metabolic disorders (18): ① waist circumference ≥ 90/80 cm in Asian men and women; ② blood pressure ≥ 130/85 mmHg or specific drug treatment for controlling blood pressure; ③ plasma triglyceride ≥ 1.7 mmol/L or specific drug treatment; ④ plasma high-density lipoprotein cholesterol < 1.0 mmol/L for men and < 1.3 mmol/L for women or specific drug treatment; ⑤ prediabetes with fasting glucose levels between 5.6 to 6.9 mmol/L; ⑥ homeostasis model assessment of insulin resistance (HOMA-IR) score ≥ 2.5.

Hepatic steatosis was measured using abdominal high-resolution ultrasonography (SIMENS ACUSON S2000 ABVS) after 12 h fasting. Well-trained radiologists conducted the ultrasonography and diagnosed the hepatic steatosis according to the characteristics of ultrasonic diagnosis satisfied the following abnormal abdominal ultrasound images (19–21): The near field echo of liver increased diffusely ("bright liver "), and the echo of liver was larger than that of kidney or spleen; vascular blurring; Poor visibility of the posterior right lobe due to deep attenuation.

In this research, participants with MAFLD were categorized into three groups (22, 23). To begin with, we identified the diabetes-MAFLD group depending on the presence of DM regardless of BMI. Subsequently, in individuals without DM, we classified MAFLD subgroups based on BMI: ①overweight/obese (BMI ≥ 23 kg/m^2) or ②lean or normal weight (BMI < 23 kg/m^2). In the end, the study population was divided into four subgroups: no MAFLD, diabetes-MAFLD, overweight/obese-MAFLD, and lean-MAFLD.

The fibrotic burden of the liver was evaluated using the fibrosis-4 index (FIB-4) or NAFLD fibrosis score (NFS). Previous research has demonstrated that the FIB-4 index has a similar overall diagnostic performance for diagnosing MAFLD fibrosis, and it has also been shown to have a better diagnostic performance for fibrosis in all MAFLD subtypes (24). In this study, both FIB-4 index and NFS were used to assess the liver fibrosis by the following equation (25): FIB-4 index = age (years)*AST(U/L)/ [PLT (10^9/L) * ALT(U/L)$^{1/2}$], NFS = −1.675 + 0.037 * age + 0.094 * BMI + 1.13 * Prediabetes/diabetes + 0.99 * (AST / ALT) - 0.013 * PLT - 0.66 * Alb. The cut-off values of FIB-4 index and NFS in diagnosing fibrosis were at least 1.3 and −1.455, respectively (26).

Statistical analysis

All statistical analyzes were performed using SPSS 26.0 software and STATA 17.0 software. Continuous variables are reported as mean ± SD or median with interquartile ranges according to the evaluation of the normal distribution by Shapiro–Wilk test, and categorical variables are presented as the numbers(n) with percentages (%). Comparisons of baseline characteristics and laboratory parameters specified by gender were conducted using the Student's *t*-test or Mann–Whitney *U*-test for continuous variables, and the chi-square test for categorical variables as appropriate. Restricted cubic spline model was performed to estimate the association between SVR and MAFLD subtypes among gender in a fully adjusted model. The knots were located at the 5th, 35th, 65th, and 95th percentiles. Additionally, a multivariable regression model was used to evaluate the relationship between SVR quartiles (based on gender) and MAFLD subtypes and its fibrosis by sex, with the fourth quartile as the reference group, adjusting for potential confounders. Model 1 adjusted age, smoking, drinking, WC, and BMI. Model 2 adjusted Model 1 plus FBG, hypertension and dyslipidemia. Model 3 adjusted model 2 plus ALT, AST, WBC, NE and LY. Adjusted odds ratios (ORs) were presented with 95% confidence interval (CI). *P* for trend was evaluated for linear trend test using the median value of SVR as a continuous variable in the adjusted models. The interactions effect of gender on the relationship between SVR and MAFLD subtypes were assessed by including stratification analysis and interaction tests in the regression model ($P_{\text{for interaction}}$). A two-tailed *p*-value < 0.05 was considered statistically significant.

Results

Baseline characteristics of participants

Baseline characteristics of 4,003 participants were presented in Table 1, Prevalence of lean-MAFLD, overweight/obese-MAFLD and diabetes-MAFLD stratified by gender was 4.4% (106), 30.2% (730) and 7.8% (188) for males, and 5.6% (88), 19.0% (301) and 7.3% (115) for females. Significant differences were existed in different variables including age, smoking, drinking, waist circumference, BMI, VFA, ASM, SBP, DBP, hypertension, FPG, diabetes, TG, TC, HDL-C, LDL-C, AST, ALT, FIB-4 index, NFS, WBC, NE, LY among groups (all *p*-value <0.05). Moreover, baseline characteristics of the participants were also compared according to SVR quartiles (Table 2), both males and females in the lowest quartile (Q1) of SVR tended to be older, and had the highest level of WC, BMI, ALT, AST, FIB-4, NFS, WBC, NE and LY than those in another three quartiles ($P_{\text{for trend}}$<0.05). Furthermore, there were a higher proportion of subjects of hypertension, diabetes and dyslipidemia in the first quartile of SVR compared to the higher SVR quartile($P_{\text{for trend}}$<0.05). Besides, different MAFLD subtypes had a higher SVR value than those without MAFLD (*P*<0.001; Figure 2). Participants with lean-MAFLD had an increasing value than those with overweight/obese MAFLD or diabetes-MAFLD (*P*<0.001). there was no significant difference between overweight/obese MAFLD group and diabetes-MAFLD group in SVR values (*P*>0.05). Additionally, both males and females had a decreasing value of SVR in the higher quartile of FIB-4 index and NFS ($P_{\text{for trend}}$<0.001).

Dose–response relationship between SVR values and MAFLD subtypes risk in male and female subjects

The results of a multivariable adjusted restricted cubic spline analysis showed that a decreasing SVR value significantly increased the risk of developing MAFLD subtypes in both males and females when SVR value was below its cut-off point. The cut-off points for males were 0.26 kg/cm^2, 0.19 kg/cm^2, and 0.24 kg/cm^2, while for females they were 0.14 kg/cm^2, 0.18 kg/cm^2 and 0.15 kg/cm^2. Above these cut-off points, a decreasing SVR value had a protective effect. Figure 3 illustrates the gender-specific dose–response relationships between SVR values and MAFLD subtypes risk, with the horizontal line representing the 5th, 35th, 65th, and 95th percentiles of SVR, and the red line representing the multivariable adjusted ORs for MAFLD with four knots located at the 5th, 35th, 65th, and 95th percentiles of SVR. We did not find a non-linear relationship between SVR and MAFLD subtypes (all $P_{\text{for nonlinearity}} > 0.05$).

Association between SVR quartiles and risk of developing MAFLD subtypes and its fibrosis by gender

Table 3 demonstrates that, after adjusting for potential confounding factors such as age, sex, smoking, drinking, waist circumference, BMI, hypertension, dyslipidemia, ALT, AST, WBC, NE and LY, the first quartile of SVR significantly increased the prevalence of diabetes-MAFLD [2.15(1.25 ~ 3.71)], overweight-obese MAFLD [1.73(1.19 ~ 2.49)], and lean-MAFLD [2.38(1.49 ~ 3.80)] among all participants compared to the highest quartile of SVR ($P_{\text{for trend}}$<0.001). Besides, we further explored the sex-specific association of SVR and MAFLD subtypes, the results indicated that middle-aged and elderly males with the lowest quartile of SVR had the highest risk of developing diabetes-MAFLD

TABLE 1 Baseline characteristics of the study population with different MAFLD subtypes according to sex (n = 4,003).

Characteristics	Male (n = 2,420)				P-value	Female (n = 1,583)				P-value
	No MAFLD (n = 1,396)	Lean-MAFLD (n = 106)	Overweight/obese–MAFLD (n = 730)	Diabetes-MAFLD (n = 188)		No MAFLD (n = 1,079)	Lean-MAFLD (n = 88)	Overweight/obese -MAFLD (n = 301)	Diabetes-MAFLD (n = 115)	
Age (years)	58.68 ± 9.77	58.04 ± 6.80	55.94 ± 7.39	58.11 ± 7.42	<0.001	55.06 ± 8.22	57.35 ± 7.65	56.69 ± 7.37	63.39 ± 9.68	<0.001
Smoking (n, %)	738 (52.9)	67 (63.2)	450 (61.6)	126 (67.0)	<0.001	9 (0.8)	6 (6.8)	7 (2.3)	11 (9.6)	<0.001
Drinking (n, %)	464 (33.2)	62 (58.5)	252 (34.5)	69 (36.7)	<0.001	23 (2.1)	6 (6.8)	21 (7.0)	8 (7.0)	<0.001
WC (cm)	87.08 ± 7.12	84.33 ± 4.72	95.78 ± 7.51	93.95 ± 7.87	<0.001	80.97 ± 6.81	81.63 ± 3.21	89.84 ± 6.99	88.15 ± 7.76	<0.001
BMI (kg/m^2)	24.20 ± 2.43	21.99 ± 0.79	27.16 ± 2.45	26.67 ± 2.81	<0.001	22.81 ± 2.48	22.11 ± 0.76	26.19 ± 2.45	25.82 ± 2.86	<0.001
VFA (cm^2)	80.09 ± 24.69	72.92 ± 15.54	104.38 ± 27.99	102.21 ± 27.22	<0.001	90.45 ± 30.42	89.17 ± 15.31	126.78 ± 29.71	124.74 ± 34.96	<0.001
ASM (kg)	22.13 ± 2.78	19.83 ± 2.05	23.78 ± 2.81	23.19 ± 2.95	<0.001	15.56 ± 2.01	15.62 ± 1.63	16.81 ± 2.41	15.89 ± 2.27	<0.001
SBP (mmHg)	127.11 ± 15.19	127.99 ± 13.07	129.50 ± 14.93	134.43 ± 15.99	<0.001	120.20 ± 17.22	126.83 ± 17.23	130.73 ± 17.42	134.63 ± 15.97	<0.001
DBP (mmHg)	75.43 ± 9.81	78.51 ± 7.65	79.02 ± 10.21	79.56 ± 10.79	<0.001	72.06 ± 10.29	73.30 ± 9.65	77.44 ± 9.96	76.29 ± 9.66	<0.001
Hypertension (n, %)	268 (19.2)	24 (22.6)	156 (21.4)	66 (35.1)	<0.001	123 (11.4%)	23 (26.1%)	83 (27.6%)	47 (40.9%)	<0.001
FBG (mmol/L)	5.35 (5.03 ~ 5.76)	5.74(5.40 ~ 6.10)	5.49 (5.14 ~ 5.94)	8.27 (7.43 ~ 9.47)	<0.001	5.12 (4.85 ~ 5.42)	5.50 (5.06 ~ 5.90)	5.39 (5.09 ~ 5.80)	8.10 (7.54 ~ 8.86)	<0.001
Diabetes (n, %)	108 (7.7)	0 (0)	0 (0)	188 (100.0)	<0.001	21 (1.9)	0 (0)	0 (0)	115 (100.0)	<0.001
TG (mmol/L)	1.27 (0.94 ~ 1.77)	2.02 (1.73 ~ 2.57)	1.87 (1.34 ~ 2.61)	2.18 (1.59 ~ 3.49)	<0.001	1.00 (0.78 ~ 1.36)	1.68 (1.13 ~ 2.15)	1.54 (1.27 ~ 2.16)	1.98 (0.97 ~ 3.03)	<0.001
TC (mmol/L)	4.77 (4.22 ~ 5.35)	4.92 (4.61 ~ 5.96)	4.92 (4.32 ~ 5.48)	4.83 (3.99 ~ 5.46)	<0.001	5.03 (4.52 ~ 5.68)	4.99 (4.64 ~ 5.69)	5.26 (4.57 ~ 5.88)	5.17 (4.11 ~ 5.97)	0.040
HDL-C(mmol/L)	1.22 (1.06 ~ 1.43)	1.10 (0.96 ~ 1.30)	1.07 (0.93 ~ 1.22)	1.02 (0.87 ~ 1.18)	<0.001	1.52 (1.32 ~ 1.77)	1.22 (1.16 ~ 1.41)	1.25 (1.13 ~ 1.41)	1.26 (1.07 ~ 1.42)	<0.001
LDL-C(mmol/L)	3.10 (2.54 ~ 3.64)	3.35 (3.14 ~ 4.11)	3.21 (2.65 ~ 3.74)	3.21 (2.65 ~ 3.74)	<0.001	3.22 (2.69 ~ 3.80)	3.14 (2.88 ~ 3.80)	3.43 (2.88 ~ 4.14)	3.32 (2.59 ~ 4.08)	0.005
Dyslipidemia (n, %)	598 (42.8)	101 (95.3)	456 (62.5)	134 (71.3)	<0.001	546 (50.6)	63 (71.6)	216 (71.8)	73 (63.5)	<0.001
ALT(IU/L)	17.00 (13.00 ~ 23.00)	17.00 (14.50 ~ 29.00)	26.00 (19.00 ~ 36.00)	26.00 (19.00 ~ 36.00)	<0.001	15.00 (11.00 ~ 19.00)	18.00 (13.00 ~ 24.00)	20.00 (16.00 ~ 28.00)	21.00 (17.00 ~ 34.00)	<0.001
AST(IU/L)	18.00 (15.00 ~ 21.00)	18.00 (15.00 ~ 23.00)	20.00(16.00 ~ 24.00)	20.00 (16.00 ~ 24.00)	<0.001	17.00 (14.00 ~ 19.70)	16.00 (15.00 ~ 20.00)	18.00 (15.00 ~ 21.00)	19.00(16.00 ~ 22.00)	<0.001
FIB-4 index	1.43 (1.08 ~ 1.83)	1.49 (1.26 ~ 1.56)	1.45 (1.16 ~ 2.27)	1.51 (1.03 ~ 2.00)	<0.001	1.06 (0.87 ~ 1.30)	1.05 (0.92 ~ 1.26)	1.13 (0.94 ~ 1.63)	1.19 (0.84 ~ 1.57)	0.004
NFS	−2.18 (−2.63 ~ −1.36)	−1.95 (−2.56 ~ −1.51)	−1.54 (−2.08 ~ −0.91)	−1.25(−1.73 ~ −0.58)	<0.001	−2.15 (−2.68 ~ −1.48)	−1.73 (−2.50 ~ −1.56)	−1.74 (−2.37 ~ −1.13)	−0.92 (−1.82 ~ −0.20)	<0.001
WBC (×10^9/L)	5.96 (5.07 ~ 7.00)	6.46 (5.49 ~ 7.44)	6.44 (5.59 ~ 7.48)	6.44 (5.59 ~ 7.48)	<0.001	5.21 (4.47 ~ 6.24)	5.37 (4.72 ~ 6.03)	5.68 (4.91 ~ 6.60)	6.01 (5.55 ~ 6.76)	<0.001
NE (×10^9/L)	3.29 (2.63 ~ 3.92)	3.35 (2.97 ~ 4.17)	3.48 (2.93 ~ 4.26)	3.48 (2.93 ~ 4.26)	<0.001	2.81 (2.25 ~ 3.48)	2.93 (2.57 ~ 3.74)	3.08 (2.48 ~ 3.80)	3.08 (2.89 ~ 3.69)	<0.001
LY(×10^9/L)	2.04 (1.68 ~ 2.48)	2.38 (1.98 ~ 2.56)	2.32 (1.91 ~ 2.77)	2.32 (1.91 ~ 2.77)	<0.001	1.94 (1.63 ~ 2.28)	2.07 (1.91 ~ 2.28)	2.10 (1.75 ~ 2.41)	2.31 (1.83 ~ 3.00)	<0.001

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TABLE 2 Baseline characteristics of the study population by SVR quartiles according to sex (n = 4,003).

Characteristics	Male (n = 2,420)				P for trend	Female (n = 1,583)				P for trend
	Q1 (n = 612)	Q2 (n = 596)	Q3 (n = 606)	Q4 (n = 606)		Q1 (n = 416)	Q2 (n = 378)	Q3 (n = 421)	Q4 (n = 368)	
Age (years)	61.52 ± 10.47	58.08 ± 8.04	56.42 ± 7.96	55.06 ± 7.48	<0.001	60.73 ± 9.25	56.92 ± 7.68	54.64 ± 7.83	51.72 ± 5.68	<0.001
Smoking (n, %)	337 (55.1)	359 (60.2)	378 (62.4)	307 (50.7)	0.224	11 (2.6)	10 (2.6)	2 (0.5)	10 (2.7)	0.482
Drinking (n, %)	192 (31.4)	226 (37.9)	240 (39.6)	189 (31.2)	0.882	31 (7.5)	12 (3.2)	9 (2.1)	6 (1.6)	<0.001
WC (cm)	97.47 ± 8.17	92.26 ± 5.81	88.32 ± 5.52	82.37 ± 4.96	<0.001	89.64 ± 7.83	84.69 ± 5.76	81.51 ± 5.24	76.38 ± 4.75	<0.001
BMI (kg/m^2)	27.45 ± 2.82	25.69 ± 2.28	24.64 ± 2.16	22.95 ± 2.07	<0.001	26.12 ± 2.91	24.17 ± 2.07	22.76 ± 1.85	21.26 ± 1.83	<0.001
ASM (kg)	22.20 ± 3.26	22.76 ± 2.74	22.90 ± 2.98	22.59 ± 2.68	0.014	15.34 ± 2.24	15.90 ± 2.08	16.00 ± 2.15	16.10 ± 2.02	<0.001
VFA (cm^2)	123.05 ± 25.21	92.81 ± 11.81	79.75 ± 10.88	59.41 ± 11.90	<0.001	140.32 ± 26.40	107.93 ± 15.32	85.33 ± 13.22	62.11 ± 11.27	<0.001
SBP (mmHg)	130.48 ± 14.89	129.90 ± 16.46	128.10 ± 14.62	125.28 ± 14.33	<0.001	130.39 ± 18.73	123.69 ± 17.38	123.27 ± 16.01	116.30 ± 16.71	<0.001
DBP (mmHg)	77.97 ± 10.28	78.26 ± 10.06	77.08 ± 9.57	74.58 ± 10.04	<0.001	76.41 ± 10.13	73.56 ± 10.60	73.42 ± 9.94	70.07 ± 9.93	<0.001
Hypertension (n, %)	139 (22.7)	147 (24.7)	125 (20.6)	103 (17.0)	0.004	117 (28.1)	68 (18.0)	58 (13.8)	33 (9.0)	<0.001
FPG (mmol/L)	5.62 (5.22 ~ 6.46)	5.56(5.14 ~ 6.15)	5.47 (5.06 ~ 6.02)	5.29 (5.02 ~ 5.69)	<0.001	5.45 (5.03 ~ 6.00)	5.27 (4.98 ~ 5.69)	5.24 (4.93 ~ 5.64)	5.04 (4.81 ~ 5.33)	<0.001
Diabetes (n, %)	96 (15.7)	81 (13.6)	61 (10.1)	58 (9.6)	<0.001	66 (15.9)	35 (9.3)	21 (5.0)	14 (3.8)	<0.001
MAFLD (n, %)	391 (65.6)	358 (58.5)	170 (28.1)	105 (17.3)	<0.001	206 (49.5)	160 (42.3)	94 (22.3)	44 (12.0)	<0.001
TG (mmol/L)	1.66 (1.18 ~ 2.22)	1.63 (1.18 ~ 2.30)	1.59 (1.09 ~ 2.40)	1.32 (0.91 ~ 1.94)	<0.001	1.28 (0.98 ~ 1.69)	1.25 (0.94 ~ 1.81)	1.17 (0.85 ~ 1.64)	0.94 (0.67 ~ 1.35)	<0.001
TC (mmol/L)	4.86 (4.14 ~ 5.32)	4.90 (4.30 ~ 5.51)	4.80 (4.26 ~ 5.42)	4.80 (4.25 ~ 5.38)	0.074	5.16 (4.62 ~ 5.77)	5.14 (4.54 ~ 5.71)	5.04 (4.47 ~ 5.75)	5.01 (4.49 ~ 5.62)	0.027
HDL-C(mmol/L)	1.10 (0.96 ~ 1.25)	1.10 (0.97 ~ 1.27)	1.16 (0.99 ~ 1.34)	1.27 (1.05 ~ 1.52)	<0.001	1.39 (1.22 ~ 1.62)	1.38 (1.23 ~ 1.59)	1.40 (1.18 ~ 1.67)	1.56 (1.31 ~ 1.87)	<0.001
LDL-C(mmol/L)	3.14 (2.53 ~ 3.68)	3.26 (2.65 ~ 3.76)	3.12 (2.59 ~ 3.75)	3.13 (2.46 ~ 3.60)	0.210	3.33 (2.79 ~ 4.00)	3.33 (2.72 ~ 3.84)	3.25 (2.82 ~ 3.80)	3.09 (2.58 ~ 3.71)	0.001
Dyslipidemia (n, %)	326 (53.3)	344 (57.7)	325 (53.6)	294 (48.5)	0.044	252 (60.6)	224 (59.3)	250 (59.4)	172 (46.7)	<0.001
ALT(IU/L)	21.00 (15.00 ~ 32.00)	21.00 (15.00 ~ 29.00)	20.00 (15.00 ~ 28.00)	17.00 (13.00 ~ 23.00)	<0.001	18.00 (13.25 ~ 24.00)	16.00 (13.00 ~ 21.00)	15.00 (12.00 ~ 20.00)	15.00 (11.00 ~ 19.75)	<0.001
AST(IU/L)	19.00 (16.00 ~ 23.00)	18.00 (15.00 ~ 22.00)	18.00 (15.00 ~ 23.00)	18.00 (15.00 ~ 21.00)	0.015	18.00 (15.00 ~ 21.00)	17.00 (14.00 ~ 20.00)	17.00 (14.00 ~ 20.00)	17.00 (14.00 ~ 20.00)	0.002
FIB-4 index	1.54 (1.24 ~ 2.16)	1.41 (1.09 ~ 1.74)	1.42 (1.04 ~ 1.86)	1.42 (1.06 ~ 1.90)	<0.001	1.19 (0.95 ~ 1.54)	1.05 (0.91 ~ 1.35)	1.05 (0.86 ~ 1.31)	1.02 (0.82 ~ 1.22)	<0.001
NFS	−1.21 (−1.83 ~ −0.59)	−1.24 (−1.87 ~ −0.64)	−1.55 (−2.20 ~ −0.88)	−1.60 (−2.15 ~ −1.13)	<0.001	−1.41 (−2.08 ~ −0.66)	−1.82 (−2.53 ~ −1.26)	−2.09 (−2.63 ~ −1.51)	−2.42 (−2.91 ~ −1.82)	<0.001
WBC (×10^9/L)	6.27 (5.38 ~ 7.28)	6.39 (5.52 ~ 7.44)	6.19 (5.35 ~ 7.24)	5.75 (5.04 ~ 6.99)	<0.001	5.57 (4.93 ~ 6.67)	5.56 (4.75 ~ 6.54)	5.37 (4.60 ~ 6.31)	4.99 (4.37 ~ 5.89)	<0.001
NE (×10^9/L)	3.46 (2.85 ~ 4.22)	3.36 (2.93 ~ 4.13)	3.37 (2.75 ~ 4.12)	3.24 (2.60 ~ 3.88)	<0.001	2.95 (2.47 ~ 3.59)	3.01 (2.46 ~ 3.65)	2.85 (2.29 ~ 3.64)	2.69 (2.18 ~ 3.36)	<0.001
LY(×10^9/L)	2.27 (1.87 ~ 2.70)	2.21 (1.77 ~ 2.63)	2.11 (1.80 ~ 2.56)	2.04 (1.68 ~ 2.49)	<0.001	2.11 (1.75 ~ 2.55)	2.01 (1.72 ~ 2.35)	1.99 (1.67 ~ 2.29)	1.85 (1.56 ~ 2.13)	0.037

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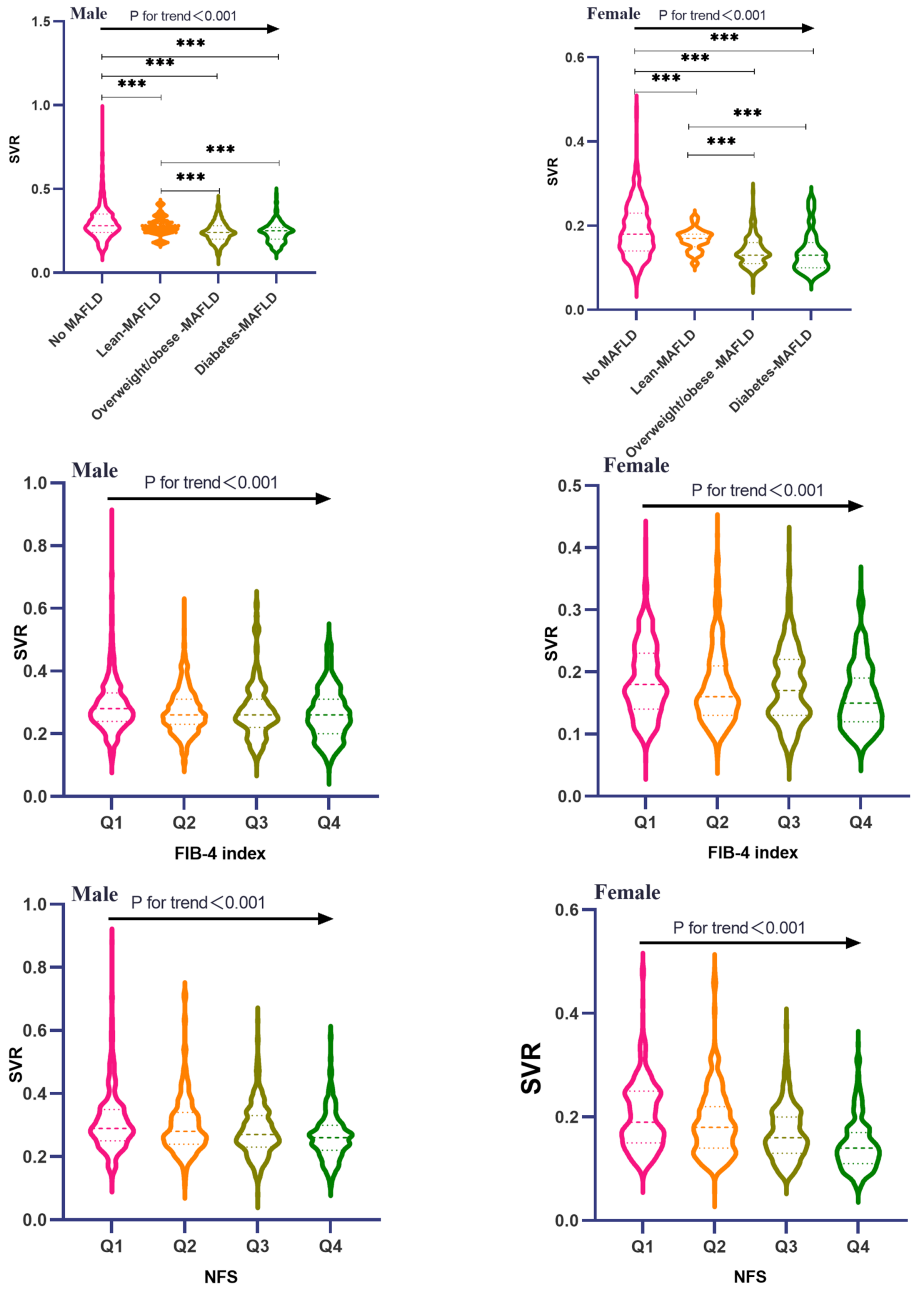

FIGURE 2
Comparison of SVR among all MAFLD subtypes and FIB-4/NFS quartiles stratified by sex.

[2.96(1.48 ~ 5.93)], overweight/obese-MAFLD [1.91(1.26 ~ 2.88)], and lean-MAFLD [4.01(1.46 ~ 10.98)] compared to the fourth quartile as the reference group (P for trend<0.001). Additionally, females with the first quartile of SVR had a significantly higher risk of diabetes-MAFLD, overweight/obese-MAFLD, and lean-MAFLD with ORs [95% confidence interval (CI)] of 3.30(1.46 ~ 7.46), 4.48(1.91 ~ 10.49) and 2.53(1.19 ~ 5.37) respectively ($P_{\text{for trend}}$<0.001). Additionally, the interaction effect of gender on the relationship between SVR and MAFLD subtypes was statistically significant (all $P_{\text{for interaction}}$<0.001). Moreover, Tables 4, 5 showed that the lowest quartile of SVR is linked to an increased prevalence of MAFLD fibrosis in both males and females compared to the highest quartile when liver fibrosis is defined by either FIB-4 index or NFS ($P_{\text{for trend}}$ < 0.001). Specifically, the first quartile of SVR was associated with a higher risk of MAFLD fibrosis in males (OR = 2.66, 95% CI: 1.88 ~ 3.76) and females (OR = 3.31, 95% CI: 2.08 ~ 5.26) when defined by FIB-4 index, and in males (OR = 1.97, 95% CI: 1.32 ~ 2.94) and females (OR = 3.84, 95% CI: 1.36 ~ 10.85) when defined by NFS. Besides, the association between SVR and MAFLD fibrosis was significantly different by gender when liver fibrosis was defined by NFS ($P_{\text{for interaction}}$<0.001); however, no significant sex-specific difference was observed in the association between SVR and MAFLD fibrosis when liver fibrosis was defined by FIB-4 index ($P_{\text{for interaction}}$>0.05).

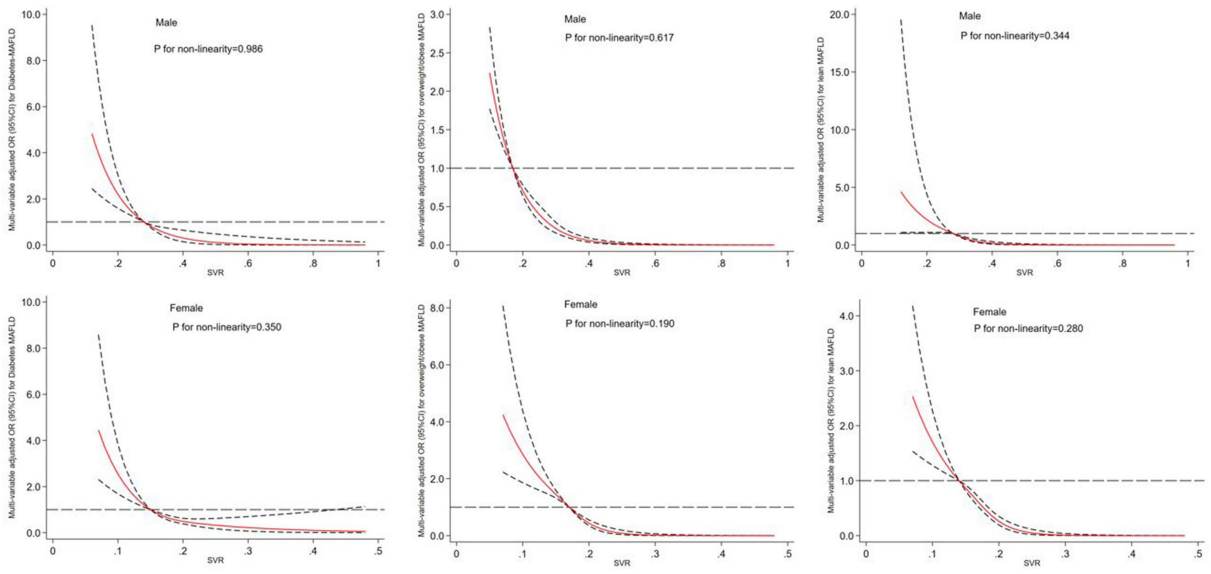

FIGURE 3
Dose–response relationship between SVR and risk of MAFLD subtypes in male and female. The restricted cubic spline regression analysis was adjusted for age, smoking, drinking, WC, BMI, FBG, hypertension, TG, TC, HDL-c, LDL-c, ALT, AST, WBC, NE and LY. The long dashed line represents OR is equal to 1, red line and the area between the short dashed lines means ORs and their 95%CI.

Discussion

Our findings demonstrate that in this single-center retrospective study of 4,003 middle-aged participants, all participants with MAFLD subtypes had a significantly lower SVR than those without MAFLD, and increasing quartiles of FIB-4 and NFS were also linked with a lower SVR, regardless of gender. Moreover, multivariable regression analysis indicated that low SVR significantly increased the prevalence of MAFLD subtypes including diabetes-MAFLD, overweight/obese-MAFLD and lean-MAFLD in both males and females. Moreover, we observed a close relationship between decreasing SVR and the risk of MAFLD with the presence of fibrosis. To our best knowledge, this is the first research to date that has examined the sex-specific associations of SVR and MAFLD subtypes and its hepatic condition.

Previous research has suggested that obesity is a risk factor which can exacerbate chronic liver disease, and that reduced skeletal muscle mass can increase the risk of developing NAFLD, as well as having an impact on the post-surgical outcome of those who have had a liver transplant (11, 27–30). Furthermore, a decrease in muscle mass can lead to an increase in visceral fat, which plays a role in the onset and progression of MAFLD. Recent study has highlighted a relationship between low skeletal muscle mass and the risk of NAFLD, adjusting for BMI or body weight, but not taking visceral fat into consideration (27). SVR, which considers both muscle mass and visceral fat, has been used to identify sarcopenic visceral obesity, and has been associated with NAFLD for both sexes (14). In contrast to a cross-sectional study of Chinese patients with type 2 diabetes which revealed a connection between SVR and ultrasonography-defined NAFLD, but only for women (31), our research concentrated on the association between SVR and MAFLD subtypes in middle-aged and older individuals, who possess a greater risk of developing chronic liver disease than younger individuals. In line with our study, another recent study also demonstrated that decreased muscle mass coupled with excessive visceral adipose is closely related to an increased risk of exacerbating NAFLD pathophysiology referring to moderate-to-severe steatosis and that of advanced fibrosis (14). In our research, the effects of SVR on the risk of diabetes-MAFLD and overweight-MAFLD were more prominent in women than in men, even though the absolute occurrence of MAFLD was much lower in women than in men. Research on the prevalence of fibrotic non-alcoholic steatohepatitis (NASH) in the United States population recently revealed that probable fibrotic NASH was present in 8.4% of the population (95% CI 8.0–8.8), with more cases found in males and Hispanic individuals (32). Women, particularly premenopausal women, tend to have fat distributed more beneficially, such as in the gluteofemoral region and subcutaneous area, as opposed to men who store fat mainly in the visceral area. Middle-aged and elderly females in our study were almost in postmenopausal state, and were thereby at a higher risk of MAFLD. Previous study also demonstrated that, independently from BMI, an android fat deposition pattern is associated with increased prevalence of NAFLD in both sexes (33). Our study also showed that women had less appendicular skeletal muscle mass than men (15.64 ± 1.85 kg vs. 21.86 ± 2.89 kg, P <0.001), indicating that BMI may not be a reliable measure of metabolic risk in women. We suggest that sarcopenic visceral obesity may be a better indicator of metabolic risk in women. To gain a better understanding of the different effects of SVR on MAFLD risk between genders, further research is necessary to accurately assess fat distribution and skeletal muscle mass. A cohort study revealed that the relationship between SVR and NAFLD was more prominent in non-obese people than in obese individuals, and suggested that low SVR is a supplementary index to traditional measures of obesity when assessing the risk of NAFLD (34).

It is known that when skeletal muscle mass is reduced and visceral adipose tissue accumulates, the insulin-mediated ability of skeletal muscle and adipose tissue to use or store blood glucose is impaired.

TABLE 3 Adjusted associations between SVR quartiles and MAFLD subtypes by gender.

	Diabetes-MAFLD			Overweight/obese-MAFLD			Lean-MAFLD		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
All participants									
Q1	2.17 (1.28 ~ 3.66)	2.19 (1.29 ~ 3.73)	**2.15 (1.25 ~ 3.71)**	1.75 (1.23 ~ 2.49)	1.74 (1.22 ~ 2.49)	**1.73 (1.19 ~ 2.49)**	2.66 (1.69 ~ 4.18)	2.30 (1.45 ~ 3.65)	**2.38 (1.49 ~ 3.80)**
Q2	2.02 (1.26 ~ 3.26)	1.97 (1.22 ~ 3.20)	1.69 (1.03 ~ 2.76)	2.13 (1.57 ~ 2.90)	2.09 (1.53 ~ 2.85)	1.86 (1.35 ~ 2.57)	2.15 (1.57 ~ 3.73)	2.46 (1.39 ~ 3.73)	2.27 (1.17 ~ 3.45)
Q3	1.09 (0.65 ~ 1.83)	1.04 (0.61 ~ 1.75)	0.92 (0.54 ~ 1.57)	1.46 (1.07 ~ 2.00)	1.44 (1.05 ~ 1.98)	1.31 (0.94 ~ 1.82)	2.16 (1.09 ~ 4.31)	1.91 (1.13 ~ 3.69)	1.95 (0.96 ~ 3.97)
Q4	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
P for trend	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Male									
Q1	3.08 (1.57 ~ 6.02)	3.23 (1.64 ~ 6.35)	**2.96 (1.48 ~ 5.93)**	2.16 (1.46 ~ 3.20)	2.12 (1.43 ~ 3.15)	**1.91 (1.26 ~ 2.88)**	3.52 (1.37 ~ 9.01)	4.01 (1.48 ~ 10.88)	**4.01 (1.46 ~ 10.98)**
Q2	3.01 (1.61 ~ 5.63)	2.88 (1.54 ~ 5.41)	2.39 (1.26 ~ 4.54)	2.39 (1.69 ~ 3.38)	2.32 (1.63 ~ 3.29)	1.93 (1.34 ~ 2.78)	4.34 (2.18 ~ 8.65)	3.76 (1.80 ~ 7.85)	3.52 (1.66 ~ 7.46)
Q3	2.20 (1.18 ~ 4.09)	2.16 (1.15 ~ 4.06)	1.83 (0.96 ~ 3.48)	1.49 (1.06 ~ 2.10)	1.50 (1.06 ~ 2.12)	1.30 (0.91 ~ 1.86)	3.78 (2.15 ~ 6.66)	3.57 (1.96 ~ 6.51)	3.62 (1.96 ~ 6.68)
Q4	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
P for trend	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Female									
Q1	5.77 (2.67 ~ 12.43)	5.85 (2.67 ~ 12.80)	**3.30 (1.46 ~ 7.46)**	5.34 (2.38 ~ 12.00)	6.11 (2.69 ~ 13.86)	**4.48 (1.91 ~ 10.49)**	2.45 (1.19 ~ 5.05)	2.76 (1.33 ~ 5.74)	**2.53 (1.19 ~ 5.37)**
Q2	2.94 (1.35 ~ 6.41)	3.11 (1.42 ~ 6.83)	1.97 (0.85 ~ 4.55)	6.56 (3.02 ~ 14.22)	7.16 (3.27 ~ 15.68)	5.62 (2.48 ~ 12.72)	2.04 (1.10 ~ 3.78)	2.15 (1.15 ~ 4.02)	2.04 (1.08 ~ 3.89)
Q3	1.28 (0.54 ~ 3.01)	1.32 (0.55 ~ 3.14)	0.90 (0.36 ~ 2.28)	3.78 (1.73 ~ 8.24)	3.83 (1.74 ~ 8.44)	3.01 (1.32 ~ 6.84)	1.62 (0.66 ~ 3.97)	1.96 (0.79 ~ 4.85)	1.62 (0.65 ~ 4.04)
Q4	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
P for trend	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P for interaction	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Model 1: Age, smoking, drinking, WC and BMI. Model 2: Model 1 + FBG, hypertension and dyslipidemia. Model 3: Model 2 + ALT, AST, WBC, NE and LY.

This decrease in whole-body insulin-mediated glucose uptake and the associated insulin resistance can cause persistent muscle loss, and may even be a factor in the development of MAFLD (35). Our research showed that middle-aged and older males with low SVR were more likely to develop MAFLD than females. We also found that males in the lowest quartile of SVR had the highest odds of developing lean-MAFLD compared to other MAFLD subtypes. Additionally, the predominant obesity phenotype in the Asian population is abdominal obesity, which is characterized by an excess of visceral adipose tissue deposition. Our study revealed that middle-aged and older females also had a higher level of visceral fat area than males ($99.78 \pm 27.62\,cm^2$ vs. $86.06 \pm 25.12\,cm^2$, $P<0.001$), and were more likely to develop diabetes-MAFLD or overweight/obese MAFLD. A study has demonstrated that the prevalence of NAFLD is significantly higher in postmenopausal females than premenopausal females (36). This suggests that estrogen may act as a protective biomarker against the development of hepatic steatosis (37, 38). This hypothesis is supported by a cohort study in patients with NAFLD, which reported a higher risk of severe fibrosis in males than premenopausal females, but similar risk levels between males and postmenopausal females, which is in line with our findings. This indicates that estrogen may have a protective effect against liver fibrosis in NAFLD (39).

Our research has several limitations which should be considered. This single-center retrospective study design does not provide sufficient evidence to determine the causal relationship between SVR and MAFLD subtypes and its hepatic condition. To confirm our findings, more studies with larger samples and a wider range of participants in multi-centers are required in a longitudinal manner. Second, Sarcopenia is not only characterized by a decrease in muscle mass, but also involves muscle function including reduced hand-grip strength and slower gait speed.

TABLE 4 Logistic regression analysis to identify the association between SVR quartiles and MAFLD fibrosis (FIB-4 index) by gender.

	Total (*n*, %)	MAFLD with Fibrosis (*n*, %)	Model 1		Model 2		Model 3	
Liver fibrosis defined by FIB-4 index			OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Male	*n* = 2,420	*n* = 546						
Q1	612 (25.3)	208 (38.1)	2.48 (1.76 ~ 3.48)	<0.001	2.49 (1.77 ~ 3.49)	<0.001	**2.66 (1.88 ~ 3.76)**	<0.001
Q2	596 (24.6)	201 (36.8)	1.95 (1.47 ~ 2.58)	<0.001	1.91 (1.44 ~ 2.53)	<0.001	2.13 (1.60 ~ 2.84)	<0.001
Q3	606 (25.1)	86 (15.8)	1.32 (1.03 ~ 1.70)	0.029	1.31 (1.02 ~ 1.69)	0.034	1.42 (1.10 ~ 1.84)	0.008
Q4	606 (25.0)	51 (9.3)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
P for trend			<0.001		<0.001		<0.001	
Female	*n* = 1,583	*n* = 229						
Q1	416 (26.3)	116 (50.7)	2.32 (1.50 ~ 3.59)	<0.001	2.53 (1.63 ~ 3.93)	<0.001	**3.31 (2.08 ~ 5.26)**	<0.001
Q2	378 (23.9)	76 (33.2)	2.19 (1.50 ~ 3.20)	<0.001	2.33 (1.59 ~ 3.41)	<0.001	2.96 (1.98 ~ 4.42)	<0.001
Q3	421 (26.6)	31 (13.5)	1.48 (1.04 ~ 2.12)	0.030	1.54 (1.08 ~ 2.21)	0.018	1.80 (1.24 ~ 2.61)	0.002
Q4	368 (23.2)	6 (2.6)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
P for trend			<0.001		<0.001		<0.001	
P for interaction			0.315		0.255		0.236	

Model 1: Age, smoking, drinking, WC and BMI. Model 2: Model 1 + FBG, hypertension and dyslipidemia. Model 3: Model 2 + ALT, AST, WBC, NE and LY.

TABLE 5 Logistic regression analysis to identify the association between SVR quartiles and MAFLD fibrosis (NFS) by gender.

	Total (*n*, %)	MAFLD with Fibrosis (*n*, %)	Model 1		Model 2		Model 3	
Liver fibrosis defined by NFS			OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Male	*n* = 2,420	*n* = 528						
Q1	612 (25.3)	196 (37.1)	1.82 (1.27 ~ 2.63)	0.001	1.74 (1.20 ~ 2.52)	0.004	**1.97 (1.32 ~ 2.94)**	0.001
Q2	596 (24.6)	187 (35.4)	1.50 (1.05 ~ 2.16)	0.027	1.57 (1.09 ~ 2.27)	0.016	1.76 (1.18 ~ 2.62)	0.006
Q3	606 (25.1)	84 (15.9)	0.88 (0.61 ~ 1.27)	0.492	0.91 (0.63 ~ 1.32)	0.607	1.07 (0.72 ~ 1.59)	0.750
Q4	606 (25.0)	61 (11.6)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
P for trend			<0.001		<0.001		<0.001	
Female	*n* = 1,583	*n* = 203						
Q1	416 (26.3)	123 (60.6)	4.08 (1.46 ~ 11.35)	0.007	4.58 (1.64 ~ 12.84)	0.004	**3.84 (1.36 ~ 10.85)**	0.011
Q2	378 (23.9)	59 (29.1)	3.78 (1.42 ~ 10.07)	0.008	4.00 (1.49 ~ 10.72)	0.006	3.28 (1.21 ~ 8.90)	0.020
Q3	421 (26.6)	16 (7.9)	1.65 (0.58 ~ 4.69)	0.348	1.64 (0.57 ~ 4.67)	0.358	1.38 (0.48 ~ 4.01)	0.550
Q4	368 (23.2)	5 (2.5)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
P for trend			<0.001		<0.001		<0.001	
P for interaction			<0.001		<0.001		<0.001	

Model 1: Age, smoking, drinking, WC and BMI. Model 2: Model 1 + FBG, hypertension and dyslipidemia. Model 3: Model 2 + ALT, AST, WBC, NE and LY.

However, the diagnosis of sarcopenic obesity necessitates combining SVR and muscle function, but the latter was not measured in our study. Besides, we did not include any specific data regarding physical activities, menopausal status, sex hormone levels, or the utilization of estrogen and progestogen medications.

Finally, A study recently investigated the epidemiological effect of the definition of steatotic liver disease (SLD) proposed by a multi-society Delphi consensus statement, and found that there was a high degree of agreement between metabolic dysfunction-associated steatotic liver disease (MASLD) and the definition of MAFLD previously proposed. However, due to the lack of data including controlled attenuation parameter and liver stiffness measurement, the study could not determine if SVR is still significantly associated with MASLD risk by gender (40).

Conclusion

Our study concluded that a decrease in SVR (appendicular skeletal muscle mass divided by visceral fat area) is significantly associated with an increased prevalence of MAFLD subtypes and liver fibrosis in middle-aged and older persons of both genders. Notably, there was a moderating effect of gender on the relationship between SVR and diabetes-MAFLD as well as overweight/obese MAFLD. Compared to females, males with low SVR had a greater chance of having lean-MAFLD. Furthermore, because of the lower SVR in females, middle-aged and elderly females were more likely to have diabetes-MAFLD and overweight-obese MAFLD than males. Additionally, females with the lowest quartile of SVR also had a higher probability of having MAFLD with fibrosis than males. To address this issue, healthcare professionals should promote hepatic rehabilitation through diet and exercise therapies to reduce abnormal body composition. To gain a better understanding of the relationship between SVR and the risk and progression of MAFLD subtypes, future research should include larger sample sizes and different age and ethnic groups, both from a prospective and mechanistic perspective.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethics and Research Committee of Health Examination Center of Wuxi People's Hospital Affiliated to Nanjing Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because personal data was anonymized in order to protect the privacy of patients; statistical analysis was conducted in a confidential manner and was only utilized for scientific objectives.

Author contributions

XY conceived and designed the study and supervised and critically reviewed the original manuscript and contributed to statistical analyzes. MX and YN collected the clinical and demographic data. XZ and YZ were responsible for data validation and reviewed and designed visualizations (tables and figures). MX drafted the original manuscript with YN. YZ contributed to project administration and funding acquisition. 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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Feeding soy protein concentrates with low or high isoflavone decreases liver inflammation by reducing lipopolysaccharide translocation

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Lipopolysaccharide (LPS) translocation and inflammation contribute to the increased risk of chronic diseases, including non-alcoholic fatty liver disease (NAFLD), associated with obesity. Previously, we reported that feeding soy protein with high or low (negligible) isoflavone reduces liver steatosis in obese Zucker rats, and the reduced steatosis is accompanied by decreased serum C-reactive protein levels. The current study investigated the effect of feeding soy protein concentrate (SPC) with high or low isoflavone (HIF or LIF) on liver inflammation and LPS translocation in obese Zucker rats. Six-week-old male lean (L, $n = 21$) and obese (O, $n = 21$) Zucker rats were fed casein control, SPC-LIF, or SPC-HIF diets for 18 weeks. At the end of 18 weeks, the expression levels of tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), inducible nitric oxide synthase (iNOS), arginase 1 (ARG1), lipopolysaccharide binding protein (LBP), myeloperoxidase (MPO), and sterol regulatory element-binding protein 1 (SREBP-1) were significantly higher in obese rats compared to lean rats. Compared to the casein control diet, both the SPC-LIF and SPC-HIF diets significantly decreased TNF-α, MCP-1, iNOS, and LBP expression in obese rats, which is accompanied by significantly less LPS staining in liver slides from SPC-LIF-and SPC-HIF-fed obese rats compared to the casein control diet-fed obese rats. Taken together, the SPC-LIF and SPC-HIF diets attenuated liver inflammation in obese Zucker rats, likely by decreasing LPS translocation.

KEYWORDS

obesity, Zucker rats, liver, LPS translocation, inflammation, soy protein concentrate, isoflavone

1 Introduction

Low-grade inflammation contributes to obesity-associated chronic diseases such as cardiovascular disease, type 2 diabetes, certain types of cancer, and non-alcoholic fatty liver disease (NAFLD). In obesity, increased pro-inflammatory cytokine and chemokine secretion from adipose tissue affects organs/tissues such as the liver and skeletal muscle, leading to insulin resistance, ectopic fat deposition, and a wide range of metabolic disturbances (1–5). Traditionally, inflammatory signals that arise from the lipid-laden adipose tissue were considered the main source of systemic inflammation. However, this cannot fully explain the observation that

diseases such as NAFLD are associated with increased inflammation after adjusting for confounding variables such as obesity and metabolic syndrome (6, 7). In recent years, aided by a better understanding of the role of gut microbiota in health and diseases, there has been a paradigm shift regarding the origin(s) of obesity-associated inflammation that brings the gastrointestinal (GI) tract into the spotlight (8, 9). GI tract commensal bacteria-derived components, particularly LPS, traverse the portal vein to encounter the liver and enter systemic circulation or are transported by lipoproteins, collectively known as LPS translocation (10). Obesity is associated with altered gut microbiota and increased intestinal permeability, both of which may contribute to increased LPS translocation and subsequent systemic inflammation (11). Elevated blood LPS levels are associated with obesity in humans and animals (12).

LPS translocation is particularly relevant to liver inflammation and liver diseases because the liver is exposed to LPS that traverses the portal vein (13). For example, patients with diagnosed NAFLD have a higher LPS concentration in their circulation compared to healthy controls (14). Levels of LPS in blood and liver biopsy samples also correlate with severity of the disease in NAFLD patients (15). Because NAFLD has no symptoms in most cases and diagnoses rely on imaging or histology, human studies on the relationship between LPS translocation and NAFLD are limited by relatively small sample sizes. The observational nature of human studies reveals only correlations rather than causality. On the other hand, a causal relationship between LPS translocation and NAFLD has been revealed by animal studies. In obese (*fa/fa*) Zucker rats, intraperitoneal LPS injection exacerbates hepatic steatosis (16). Low-dose subcutaneous LPS injection also worsens high-fat diet-induced NAFLD in C57BL/6 mice (17). LPS leads to liver inflammation by triggering macrophages to release inflammatory cytokines, including TNF-α (18, 19). The contribution of TNF-α to the development of NAFLD is supported by the resistance to liver steatosis in TNF-α−/− mice and mice treated with a TNF-α receptor antagonist (20, 21).

In our previous study, we reported that feeding obese Zucker rats SPC-LIF and SPC-HIF diets reduces liver steatosis compared to a casein control diet (22, 23). The reduced hepatic steatosis in obese rats is accompanied by decreased systemic inflammation [i.e., serum C-reactive protein (CRP)] (24). This study was designed to investigate whether there are differences in liver inflammation between lean and obese rats fed casein control, SPC-LIF, and SPC-HIF diets, and, if so, whether different levels of LPS translocation may be a contributing factor. We hypothesized that the SPC-LIF and SPC-HIF diets would reduce liver inflammation and LPS translocation in obese Zucker rats compared to the casein control diet.

2 Materials and methods

2.1 Ethics statement

The University of Arkansas for Medical Sciences/Arkansas Children's Research Institute Institutional Animal Care and Use Committee approved the animal care protocol and procedures used in the study. We adhered to the institutional regulations (Protocol code no. 3968; approved on December 20, 2019) and the guidelines of the United States Department of Agriculture (USDA, Washington, DC, United States) Animal Welfare Act.

2.2 Experimental design

Six-week-old male lean and obese *(fa/fa)* Zucker rats were purchased from Charles River Laboratories (Wilmington, MA, United States). All rats were acclimated to the AIN-93G rodent diet for a week before the start of the experiment. Lean and obese rats were randomly assigned to one of the three dietary groups with 7 rats to each group and fed a semi-purified diet similar to the AIN-93G diet with dietary protein supplied in the form of casein (control), soy protein concentrate with low isoflavone (SPC-LIF) (Arcon SJ; ADM; Decatur, IL), or soy protein concentrate with high isoflavone (SPC-HIF) (Arcon SM; ADM; Decatur, IL). Rats had *ad libitum* access to water and diets during the experiment. The SPC-LIF diet contained 0.154 mg isoflavone/g protein with an aglycone component of approximately 0.16 mg/g protein (genistein, 0.15 mg/g protein; daidzein, 0.011 mg/g protein; and glycitin, below level of detection). The SPC-HIF diet had 2.153 mg isoflavone/g protein with an aglycone component of approximately 1.72 mg/g protein (genistein, 0.382 mg/g protein; daidzein, 0.216 mg/g protein; glycitin, 0.005 mg/g protein). The casein-based diet does not have a detectable level of isoflavones. L-cystine was added at 3 g/kg to the casein diet and 1.2 g/kg to the SPC-LIF and SPC-HIF diets. L-methionine was added at 2.2 g/kg to the SPC-LIF and SPC-HIF diets to match the L-methionine level in the casein diet. All three diets were made to be isocaloric and isonitrogenous, with detailed compositions reported previously (23). Three identical dietary groups (control, SPC-LIF, and SPC-HIF) were created in lean rats and in obese rats. During week 18, rats were anesthetized with carbon dioxide and euthanized by decapitation. Liver samples were snap frozen in liquid nitrogen and stored at −80°C for subsequent RNA extraction. Two 3-mm sections of each liver lobe were fixed in tissue cassettes and stored in 10% buffered formalin until histological examination and immunohistochemistry.

2.3 Quantitation of liver mRNA expression

Total RNA was isolated from frozen liver samples using TRIzol Reagent (15596026, ThermoFisher Scientific, Waltham, MA) according to the protocol provided by the manufacturer. cDNA was synthesized using SuperScript™ First-Strand Synthesis System for RT-PCR (11904018, ThermoFisher Scientific, Waltham, MA). qPCR was performed using the validated TaqMan assays (ThermoFisher Scientific, Waltham, MA) (Table 1) on a QuantStudio 6 Real-time PCR system (Applied Biosystems, Foster City, CA). 18 s rRNA was used as endogenous control, and relative gene expression was calculated using the delta delta Ct (ΔΔCt) method.

2.4 Immunohistochemistry and image analysis

Formalin-fixed, paraffin-embedded liver samples were microtome-sectioned. Slides were pretreated with Dako Target Retrieval High pH (pH 9.0) buffer (S236784-2, Agilent Technologies, Santa Clara, CA). Liver sections were stained with a primary antibody against LPS (ab35654, Abcam, Cambridge, MA) or a primary antibody against the pan-macrophage marker CD68 (ab283654, Abcam,

TABLE 1 Quantitative PCR assay list.

Gene Name	Species	Assay ID (Thermo Fisher Scientific)
Tnfa	Rat	Rn01525859_g1
Ccl2/Mcp1	Rat	Rn00580555_m1
Il1b	Rat	Rn00580432_m1
Il10	Rat	Rn01483988_g1
iNos/Nos2	Rat	Rn00561646_m1
Arg1	Rat	Rn00691090_m1
Lbp	Rat	Rn00567985_m1
Mpo	Rat	Rn01460205_m1
Srebp1c	Rat	Rn01495769_m1
18 s rRna	Rat	Rn03928990_g1

Cambridge, MA). Biotinylated secondary antibodies, Vector ABC standard Elite HRP Kit (PK-6100, Vector Laboratories, Newark, CA), and Dako DAB (GV82511-2, Agilent Technologies, Santa Clara, CA) were used to visualize antigen staining. Richard-Allan Hematoxylin was used for counterstaining. Slides were scanned at 40× magnification using Aperio Scanscope CS2 (Leica Biosystems, Nußloch, Germany) and subsequently analyzed by Aperio Imagescope software using total pixel count and cytoplasmic algorithms (Leica Biosystems, Nußloch, Germany).

2.5 Statistical methods

Quantitative PCR data were analyzed by a two-way ANOVA on obesity status, diet, and the interactions between obesity status and diet, followed by the Fisher's LSD *post-hoc* tests. Statistical significance was determined at the level of p of <0.05. Immunohistochemistry data were analyzed by a one-way ANOVA on diet. Statistical significance was determined at the level of p of <0.05. Analyses were performed in SPSS Statistics for Windows, version 28.0.0.0 (IBM Corp., Armonk, NY, United States).

3 Results

3.1 The effect of soy protein on liver gene expression

After lean and obese Zucker rats were fed a casein control diet, SPC-LIF diet, or SPC-HIF diet for 18 weeks, liver TNF-α, MCP-1, iNOS, and LBP expression levels had significant interactions between diet and obesity status. The expression of TNF-α, MCP-1, iNOS, and LBP was significantly higher in casein control diet-fed obese rats compared to casein control diet-fed lean rats. In obese rats, both the SPC-HIF and SPC-LIF diets significantly decreased TNF-α, MCP-1, iNOS, and LBP expression compared to the casein control diet. In lean rats, the SPC-HIF diet significantly decreased LBP expression compared to the casein control diet (Figures 1A,B,E,G). Liver IL-1β and IL-10 expression did not differ between obese and lean rats or between any dietary groups in obese or lean rats (Figure 1D). There is an effect of obesity status but not diet on liver ARG-1, MPO, and SREBP-1 expression. Obese rats had significantly increased expression of ARG-1, MPO, and SREBP-1 compared to lean rats (Figures 1F,H,I).

3.2 The effect of soy protein on liver LPS level In obese rats

Because the liver expression levels of pro-inflammatory genes TNF-α, MCP-1, iNOS, and LBP were decreased by feeding SPC-LIF and SPC-HIF diets in obese rats, we analyzed liver samples from obese rats fed the casein control diet, the SPC-LIF diet, and the SPC-HIF diet for LPS translocation by immunohistochemistry. Representative images from the casein control group (Figure 2A), the SPC-LIF group (Figure 2B), and the SPC-HIF group (Figure 2C) all showed positive LPS staining, while a negative control sample had minimum to zero staining (Figure 2D). When the immunohistochemistry images were quantified by a positive pixel count algorithm (Aperio ImageScope, Leica) and compared between groups, liver sample LPS positivity was significantly lower in the SPC-LIF and SPC-HIF diet-fed obese rats compared to the casein control diet-fed obese rats (Figure 2E). Because the LPS-induced inflammatory response requires endocytosis of the LPS TLR4 complex (25), we quantified cytoplasmic LPS in the immunohistochemistry images using a cytoplasmic algorithm (Aperio ImageScope, Leica). In the pseudo-color-coded representative images from a casein control diet-fed obese rat (Figure 3A), a SPC-LIF diet-fed obese rat (Figure 3B), and a SPC-HIF diet-fed obese rat (Figure 3C), both SPC-LIF and SPC-HIF samples appeared to have a lower frequency of cells staining strongly positive for cytoplasmic LPS (orange pseudo-color). When data from all slides were analyzed, LPS cytoplasm H-scores were significantly lower in SPC-LIF and SPC-HIF diet-fed obese rats compared to casein control diet-fed obese rats (Figure 3D).

3.3 The effect of soy protein on liver macrophage marker CD68

When obese rat liver samples were stained for the pan-macrophage marker CD68 by immunohistochemistry, samples from the casein control group (Figure 4A), the SPC-LIF group (Figure 4B), and the SPC-HIF group (Figure 4C) had positive CD68 staining. When the immunohistochemistry images were quantified by a positive pixel count algorithm (Aperio ImageScope, Leica), there were no significant differences in CD68 positivity between any groups (Figure 4D).

4 Discussion

Soy foods have anti-inflammatory properties that may mediate their health benefits by reducing the risk of chronic diseases (26–28). In previous studies, we reported that feeding obese Zucker rats SPC-LIF and SPC-HIF diets protected the liver against steatosis and reduced systemic inflammation compared to a casein control diet (23, 24). In the current study, we investigated whether feeding obese Zucker rats SPC-LIF and SPC-HIF diets would reduce liver inflammation and LPS translocation. We found that obese rats had increased expression of a number of pro-inflammatory genes, including TNF-α, MCP-1, and iNOS, in the liver compared to lean

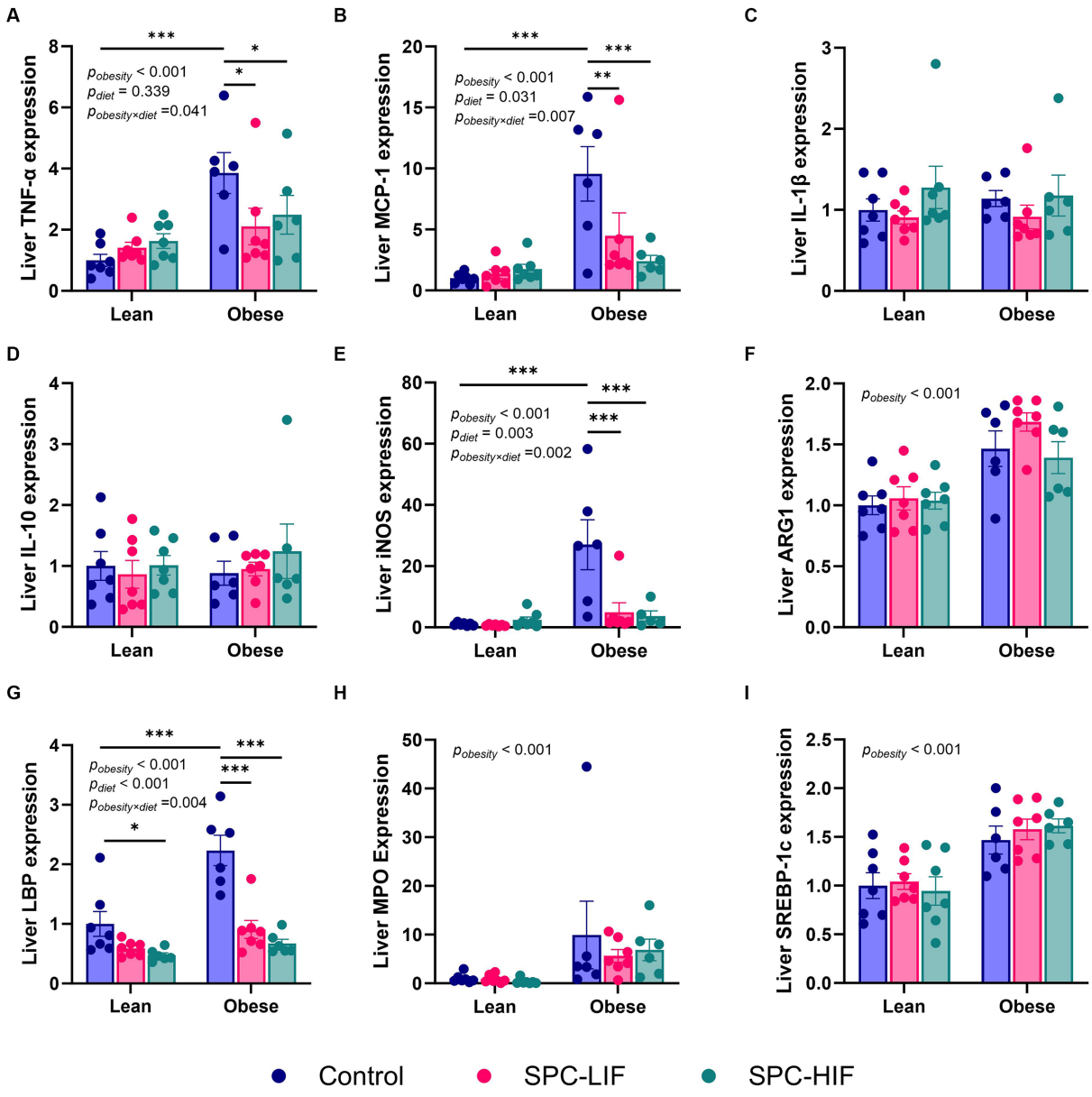

FIGURE 1
Hepatic TNF-α **(A)**, MCP-1 **(B)**, IL-1β **(C)**, IL-10 **(D)**, iNOS **(E)**, ARG1 **(F)**, LBP **(G)**, MPO **(H)**, and SREBP-1c **(I)** expression measured by TaqMan qPCR. The data are expressed as a ratio to the lean control group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, SPC-LIF, soy protein concentrate with low isoflavone; SPC-HIF, soy protein concentrate with high isoflavone.

rats, while their expression levels were decreased in obese rats by SPC-LIF and SPC-HIF diets. As a master pre-inflammatory cytokine, TNF-α is primarily produced by activated macrophages (29). The contribution of TNF-α to the development of NAFLD is supported by the resistance to liver steatosis in TNF-$\alpha^{-/-}$ mice and mice treated with a TNF-α receptor antagonist (20, 21). MCP-1 is a chemokine produced primarily by activated macrophages to recruit monocytes to the site of inflammation (30). iNOS is a hallmark marker of M1 (pro-inflammatory, classically activated) macrophages (31). The increased TNF-α, MCP-1, and iNOS expression in the liver of obese rats compared to lean rats and their decreased expression in the SPC-LIF and SPC-HIF diet-fed obese rats compared to the casein control diet-fed obese rats suggest that liver pro-inflammatory macrophage activity was increased by obesity, and the increase was attenuated by soy protein diets.

We did not detect any effect of obesity or diet on liver IL-10 or IL-1β expression. IL-10 is an anti-inflammatory cytokines produced by T cells, B cells, and macrophages (32). Our data indicate that IL-10 is not involved in the development of liver steatosis or the effect of soy protein on liver steatosis in obese Zucker rats. Although IL-1β, a macrophage-derived pro-inflammatory cytokine, did not differ between obese and lean rats or between any dietary groups, we speculate that it may be because the increased inflammatory stimuli in obesity were not sufficient to increase IL-1β expression.

Because it has been reported by others that neutrophils may contribute to the development of NAFLD (33), we also measured the

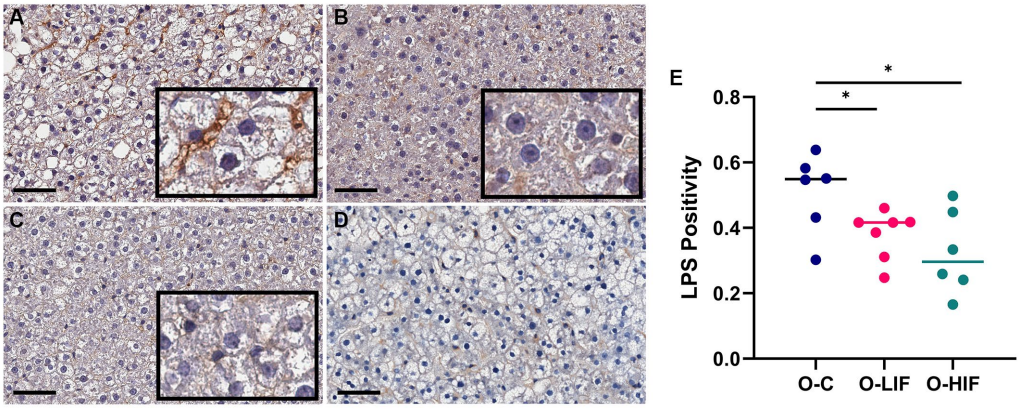

FIGURE 2
Immunohistochemistry staining for LPS in liver samples from obese (*fa/fa*) Zucker rats. Rats were fed **(A)** a casein control diet, **(B)** a soy protein diet with low isoflavone, and **(C)** a soy protein diet with high isoflavone. **(D)** Negative control for immunohistochemistry: a liver sample stained without primary anti-LPS antibody. **(E)**, LPS positivity (NPositive/NTotal) in all slides was analyzed by a positive pixel count (PPC) algorithm in all samples using Aperio ImageScope software (Leica biosystems). *$p < 0.05$, O, obese; C, control; LIF, low isoflavone; HIF, high isoflavone.

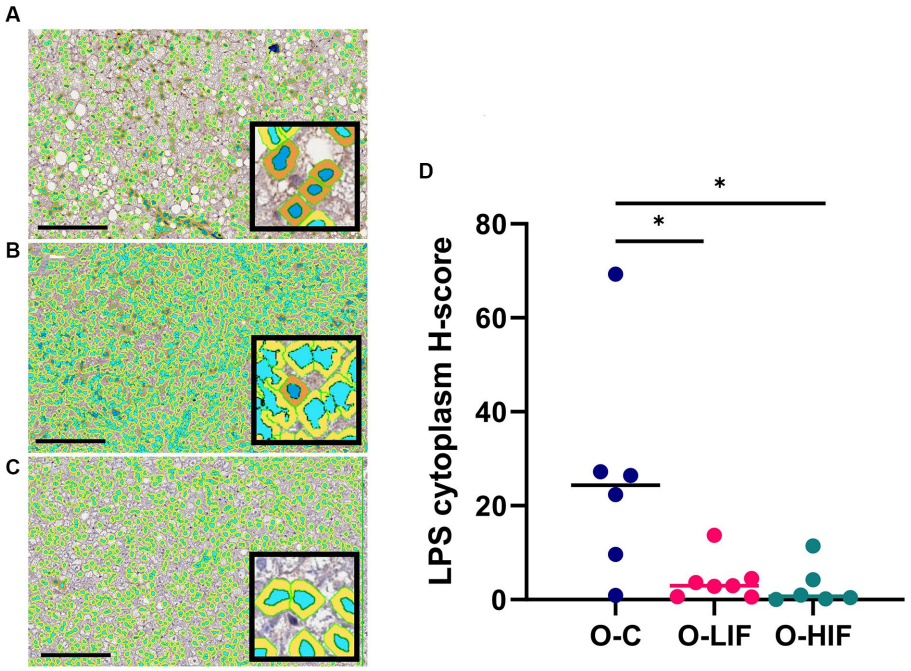

FIGURE 3
Immunohistochemistry staining for LPS in liver samples from obese (*fa/fa*) Zucker rats analyzed by a cytoplasm algorithm (Aperio ImageScope software, Leica Biosystems). Rats were fed **(A)** a casein control diet, **(B)** a soy protein diet with low isoflavone, and **(C)** a soy protein diet with high isoflavone. The pseudo-blue color defines nuclear areas, and darker cytoplasmic areas indicate greater LPS staining. **(D)** LPS cytoplasm H-score from all samples. *$p < 0.05$. O, obese; C, control; LIF, low isoflavone; HIF, high isoflavone.

expression of the neutrophil marker MPO in the liver samples. Obesity increased liver MPO expression, but there was no effect of any diet (Figure 1H), suggesting that the liver neutrophil activity was increased by obesity but changes in neutrophil activity did not mediate the protective effect of soy protein on liver steatosis. There was also an increase in ARG1 expression in obese rats. Initially, we chose to measure ARG1 as a marker for anti-inflammatory M2 type macrophages, and we expected that soy protein diets may increase ARG1 in obese rats compared to the casein control diet. However, ARG1 is a urea cycle enzyme that is abundant in hepatocytes. Its increased expression in the obese groups was most likely not an indication of M2 macrophage activity but rather an upregulation of urea cycle enzymes due to the increased food/protein intake by obese rats compared to lean controls. The high level of ARG1 expression by hepatocytes may have masked any potential difference in ARG1 expression in macrophages. Finally, SREBP-1c expression levels were also higher in obese rats but not affected by diets. The mammalian genome encodes three SREBP isoforms, namely, SREBP-1a, SREBP-2,

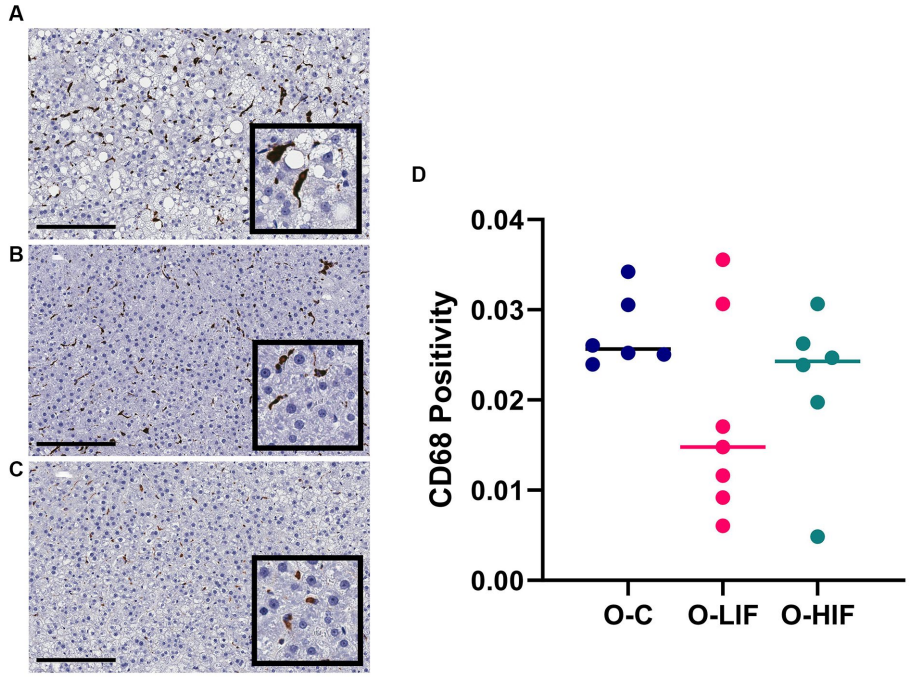

FIGURE 4
Immunohistochemistry staining for CD68 in liver samples from obese (fa/fa) Zucker rats. Rats were fed **(A)** a casein control diet, **(B)** a soy protein diet with low isoflavone, and **(C)** a soy protein diet with high isoflavone. **(D)** CD68 positivity (NPositive/NTotal) analyzed by a positive pixel count (PPC) algorithm in all samples using Aperio ImageScope software (Leica biosystems). O, obese; C, control; LIF, low isoflavone; HIF, high isoflavone.

and SREBP-1c. All SREBPs are activators of lipid synthesis, with SREBP-1c being the predominate form in the liver that activates fatty acid synthesis (34). Liver-specific expression of SREBP-1c is associated with fatty liver (35). Our results suggest that obese Zucker rats had increased liver lipogenesis compared to lean rats, but SREBP-1c expression did not mediate the effect of soy protein on liver steatosis.

Liver LBP expression was significantly higher in obese rats compared to lean rats and decreased by the SPC-LIF and SPC-HIF diets to levels similar to those of lean rats (Figure 1G). Because increased LBP is an indicator for LPS translocation (36, 37), we measured LPS levels in the liver of obese rats by immunohistochemistry and showed that the SPC-LIF and SPC-HIF diet-fed rats had significantly less liver LPS staining compared to casein control diet-fed obese rats (Figure 2). Similar to total LPS staining, the cytoplasmic LPS levels were also significantly lower in SPC-LIF and SPC-HIF diet-fed obese rats compared to casein control diet-fed obese rats (Figure 3). The differences in cytoplasmic LPS staining were likely due to differences in LBP expression. In aqueous environments such as plasma and interstitial fluids, LPS molecules form aggregates due to their amphipathic nature. LBP interacts with LPS aggregates and, together with CD14, extracts LPS molecules as monomers to facilitate their interaction with the TLR-4 receptor complex (25, 38). Endocytosis of LPS and the TLR-4 complex initiates a cascade of intracellular signaling events that lead to the activation of the NFκB pathway and increased expression of inflammation cytokines (25). The increased cytoplasmic LPS staining that we observed is consistent with the increased expression of LBP and its subsequent effect on LPS-TLR4 endocytosis.

Finally, we stained the liver slides of obese rats with the pan-macrophage marker CD68 to investigate whether the decrease in inflammation markers by the SPC-LIF and SPC-HIF diets was accompanied by a decreased number of macrophages. The results showed no difference in CD68 positive pixel counts between obese rats fed different diets (Figure 4D), indicating that the numbers of macrophages are similar between groups. However, macrophages in the liver of SPC-LIF and SPC-HIF diet-fed obese rats appeared to aggregate less compared to casein control diet-fed obese rats, as reflected by smaller foci of positive staining (Figures 4A–C).

Although both SPC-LIF and SPC-HIF diet-fed obese rats attenuated liver inflammation and LPS translocation compared to casein control diet-fed obese rats, there were no significant differences between the SPC-LIF and SPC-HIF groups. Soy isoflavones have a wide range of health benefits. The benefits of soy isoflavones are dependent on food sources that affect bioavailability and the interaction of isoflavones with the intestinal microbiota (39). For example, certain bacterial populations can produce equol, a beneficial bacterial metabolite derived from daidzein. There are equol producers and non-producers in the human population (40). Previously, we reported the bioavailability of isoflavones from SPC in lean and obese Zucker rats (24). Rats fed the SPC-HIF diet have significantly higher serum levels of genistein, daidzein, and equol compared to rats fed the SPC-LIF diet (24). When taken together with the lack of additional effects of the SPC-HIF diet compared to the SPC-LIF diet in the current study, we concluded that the anti-inflammatory effect of SPC and the reduction of LPS translocation by SPC in obese Zucker rats could mostly be attributed to the protein rather than the isoflavone components of SPC. The anti-inflammatory effects of some soy protein-derived peptides have been reported. Soy-derived angiotensin-converting enzyme (ACE)-inhibitory peptide inhibits inflammation in vascular smooth muscle

cells (41). Soy peptides also protect against LPS-induced inflammation in cultured intestinal cells by reducing nitric oxide and inflammatory cytokine expression and cultured macrophages by suppressing TLR4-mediated pathways such as NFκB activation (42, 43). Soy-derived tripeptide Phe-Leu-Val reduces TNF-α-induced inflammation and insulin resistance in adipocytes (44). Although most of these reports have come from *in vitro* experiments, soy-derived peptide can be absorbed into the circulation from the GI tract (45). For example, the soy peptide lunasin has been shown to have anticancer effects *in vivo* (46). The protective effects of soy bioactive peptides against chronic diseases have been reviewed in detail elsewhere, with many of their benefits attributed to their anti-inflammatory functions (47, 48).

There are two main potential mechanisms for soy protein's inhibition of LPS translocation in obese rats. First, soy protein may optimize the intestinal microbiota and therefore reduce the origin of LPS from its source. The effects of soy foods on intestinal microbiota composition, in particular the reduction of pathogenic bacterial populations by soy foods, have been reported in both animal and human studies (39). Second, soy protein may promote the barrier function of the intestinal epithelium to reduce its permeability to LPS. The effects of soy protein on intestinal permeability have been investigated in animal models with mixed results. In zebra fish, soybean meal increases intestinal permeability independent of the microbiota (49). In weaned piglets, a high dose of soybean agglutinin (SBA) increases intestinal permeability, while a low dose of SBA exerts no effect (50, 51). In mice, feeding SPC reduces colonic inflammation and prevents the loss of gut barrier function induced by dextran sulfate sodium (DSS) (52). The effects of soy protein on intestinal function and microbiota will form the basis of our future investigations.

5 Conclusion

Feeding the SPC-LIF and SPC-HIF diets to obese Zucker rats significantly reduced obesity-induced liver inflammation, likely by decreasing LPS translocation. Based on these results, we will design future studies to investigate the mechanisms of soy protein's inhibitory effect on LPS translocation. Because increased LPS translocation has been implicated in the development of other obesity-associated chronic diseases, including type 2 diabetes, cancer, and Alzheimer's disease (53, 54), an in-depth mechanistic understanding of soy protein's inhibition of LPS translocation during obesity may shed light on soy protein's wide range of health benefits.

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 animal study was approved by University of Arkansas for Medical Sciences/Arkansas Children's Research Institute Institutional Animal Care and Use Committee and adhered to the institutional regulations. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

WL: Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Resources. RH: Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration, Supervision, Resources.

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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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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The effects of weight loss and improved metabolic health status on the risk of non-alcoholic fatty liver disease—results from a prospective cohort in China

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Background: The impact of weight loss and/or improved metabolic status on the risk of non-alcoholic fatty liver disease (NAFLD) has yet to be determined.

Methods: A total of 35,322 participants without NAFLD were followed. NAFLD risk was compared between consistently metabolically healthy non-obese (MHNO) and non-MHNO who lost weight to become non-obese and/or improved their metabolic health, using Cox proportional hazards and logistic regression models.

Results: Following 148,186 person-years, 8,409 participants had onset NAFLD, with an incidence rate of 56.75 (95% CI: 55.57, 57.94) per 1,000 person-years. Metabolically healthy obese (MHO), metabolically unhealthy obese (MUO), and metabolically unhealthy non-obese (MUNO) at baseline were associated with increased NAFLD risk, with hazard ratios of 4.48 (95%CI:4.24, 4.73), 8.85 (95%CI:7.95, 9.84), and 10.70 (95%CI:9.73, 11.78). Weight loss and/or metabolic status improvements could significantly reduce NAFLD risk by 79.46 to 41.46%. Specifically, after weight loss from MHO to MHNO, the reduction in NAFLD risk [OR decreased from 12.01 (95%CI:9.40, 15.35) to 4.14 (95%CI:3.08, 5.57)] was greater than that of the MUNO subgroup whose metabolic status improved to MHNO [OR decreased from 5.53 (95%CI:5.15, 5.94) to 2.71 (95%CI:2.50, 3.93)]. In the MUO subgroup, the group with the greatest risk reduction of NAFLD was the weight and metabolic state both improvement group [MUO to MHNO, OR decreased from 22.74 (95%CI:17.61, 29.37) to 4.67 (95%CI:3.05, 7.16)], followed by the weight loss only group [MUO to MUNO, OR decreased to 6.83 (95%CI:4.87, 9.57)], and finally the group with the least and insignificant risk reduction was the metabolic state improvement group [MUO to MHO, OR decreased to 13.38 (95%CI:9.17,19.53)]. NAFLD risk was negatively correlated with the duration of improvement ($p < 0.001$).

Conclusion: Individuals with non-MHNO were more likely to develop NAFLD than those with consistent MHNO, but metabolic improvements and weight loss can alleviate the risk. Their NAFLD risk was negatively correlated with improvement

duration. However, it remained higher than in individuals with consistent MHNO at an average follow-up of 4.2 years.

KEYWORDS

obesity, metabolic health, NAFLD, cohort study, loss weight

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease and a risk factor for cardiovascular disease and death worldwide (1–4). It is estimated that the global prevalence of NAFLD was approximately 25.2% (5). Obesity, insulin resistance, and sub-clinical inflammation are considered the major determinants of NAFLD. The presence of obesity, metabolic abnormalities, or inflammatory abnormalities alone or in combination can increase the risk of NAFLD (6–9). Additionally, obesity is frequently accompanied by metabolic syndrome (MetS), which refers to abdominal obesity, elevated blood pressure, fasting glucose and triglycerides, and low levels of high-density lipoprotein cholesterol (HDL-c). MetS and NAFLD are closely linked, and both contribute to each other. NAFLD is now recognized as a cause and consequence of MetS, especially diabetes. The relationship between NAFLD and diabetes is more complex than previously thought. First, NAFLD can help assess the risk of complications in diabetes based on new findings. Second, NAFLD should be included in disease management programs for diabetic patients. Third, NAFLD should be considered when personalizing diabetes treatment, as it shares its underlying causes with certain forms of prediabetes and diabetes (8, 10–13).

Depending on the presence or absence of MetS and obesity, the population could be classified into the following four phenotypes: metabolically healthy non-obese (MHNO); metabolically healthy obese (MHO); metabolically unhealthy non-obese (MUNO); and metabolically unhealthy obese (MUHO). Different phenotypes present different NAFLD risks (14–16), liver fibrosis progression (17), and mortality due to all causes (18). In addition, recent studies suggest that NAFLD risk may be affected by changes in phenotype. Chang et al. (15) followed 77,425 metabolic health participants for 4.5 years and found that increasing BMI was independently associated with an increased risk of NAFLD. Cho et al. (19) followed 14,779 metabolically healthy overweight or obese (BMI $\geq$ 23 kg/m^2) participants for 5.2 years and found that clinically relevant weight loss of >5% was associated with a lowered risk of NAFLD. Those prior studies, however, focused mainly on metabolically normal populations. It remains unclear whether subjects with unhealthy metabolic conditions, such as the MUNO or MUO phenotype, might benefit from weight loss and improved metabolic status, thereby reducing their risk of NAFLD.

Conducting studies on the association between metabolic status improvement, weight loss, and NAFLD risk among metabolically abnormal populations will help us better understand clinical expectations for lifestyle and weight interventions. According to our previous research (20), more than one-third of Chinese had metabolic abnormal or obesity problems in 2012, including 3.47% of MHO, 21.93% of MUNO, and 5.36% of MUO, which is a particularly prominent public health issue. We, therefore, have followed Chinese subjects without NAFLD since 2012 to investigate whether weight loss and/or improved metabolic status could reduce the risk of NAFLD in individuals with different obesity and metabolic health statuses.

Patients and methods

Study population

Participants in the cohort study were enrolled in the health management centers of Aerospace Center Hospital (in Beijing) and the Third Xiangya Hospital (in Hunan). It was common for people from local cities to visit these two hospitals, as well as people from nearby suburban counties. Those who underwent annual health check-ups in those two centers between January 2012 and December 2018 were eligible for selection. Aiming at exploring the association between transition in metabolic and obese phenotypes and NAFLD risk, subjects without data for exposure or outcome assessment were, therefore, excluded. The specific inclusion criteria were: (1) aged > 20 years, not pregnant at baseline and consent to participate; (2) having at least 2 follow-ups before December 2020; (3) with liver ultrasound results both at baseline and at follow-ups; (4) having enough data for metabolic and obesity status assessment at baseline and during follow-up periods; (5) free of NAFLD, hepatic fibrosis or cyst, or hepatic viral infection, and not using medication for the above listed diseases at baseline enrollment. To avoid selection bias resulting from missed diagnoses at baseline, those diagnosed with NAFLD, hepatic cysts, or fibrosis in the first 12 months following enrollment were further excluded. Approximately 80% of the participants in Hunan were from urban areas, while 95% of the participants in Beijing came from urban areas. All participants had signed informed consent forms, and the Third Xiangya Hospital Ethics Committee approved the study.

Measurement and definition

Typical annual health check-ups in those two health management centers consisted of a questionnaire, a series of physical examinations, and laboratory tests. Participants were asked to complete questionnaires detailing their age, gender, smoking history, alcohol consumption, current medications, and previous medical diagnoses. Physicians would confirm those details during the physical examination. Fasting venous blood samples were collected and analyzed at the clinical laboratory of the corresponding hospital for health check-ups. Methods used for physical and laboratory examinations were detailed in the online appendix, as was the definition of chronic diseases. Subjects with weight or metabolic health problems will receive general recommendations in their health

check-up report, including weight control, physical activity enhancement, and nutrition clinic visits. In the nutrition clinic of our health management centers, experienced experts will offer personalized dietary structure adjustments and physical exercise recommendations to help control weight and improve metabolic health. Those suspected of having certain kinds of diseases might be referred to outpatient clinics for further diagnosis or treatment. Most people would have an annual physical examination every 8–16 months. It is possible for some individuals to have their examination earlier or later than that time interval due to special problems that require re-examinations or collective payments from their employers.

The primary outcome of our study was the onset of NAFLD during the follow-up period. A NAFLD diagnosis was established when transabdominal ultrasound showed signs of hepatic steatosis without secondary causes of liver fat accumulation, such as excessive alcohol consumption, long-term steatogenic medication use, or monogenic hereditary disorders. The sign of steatosis refers to the increased echogenicity of the liver parenchyma compared to the cortex of the right kidney. Excessive alcohol consumption was defined as consuming more than 25 grams of ethanol per day for men and more than 15 grams per day for women. All the participants in this study were followed up from the baseline enrollment index data until NAFLD diagnosis, withdrawal from the study, or the end of 2020.

The body mass index (BMI) range in our population was 15 to 40. According to the standard weight criteria for adults in China, a BMI $\geq$ 28 kg/m^2 was defined as obesity. The MetS was defined as the presence of two or more of the following four conditions: (1) elevated triglyceridaemia (TG), TG $\geq$ 1.70 mmol/L or usage of lipid-lowering drugs; (2) low HDL-c, HDL-c < 1.04 mmol/L in male subjects or < 1.29 mmol/L in female subjects; (3) elevated blood pressure, systolic blood pressure (SBP) $\geq$ 130 mmHg, or diastolic blood pressure (DBP) $\geq$ 85 mmHg or usage of antihypertensive drugs; and (4) elevated fasting serum glucose (FSG), FSG $\geq$ 5.6 mmol/L, or usage of medications for diabetes. According to obesity and metabolic status, we divided participants into four phenotypes: MHNO, MUNO, MHO, and MUO. The phenotype classification was measured under the same definition at baseline and at all follow-up visits. During the follow-up period, one's phenotype may not remain constant and could vary several times. Accordingly, four types of variation patterns were identified: "throughout" was defined as exhibiting no phenotype variation during the follow-up period; "ever" was defined as a change in phenotype during the follow-up period; "intermittent" was defined as the change in phenotype at a specific follow-up and returning to the original condition; whereas "consistent" was defined as the continued change in phenotype after a specific follow-up. The purpose of our study was to determine whether weight loss or improved metabolism could reduce the risk of NAFLD in the non-MHNO population to the same level as in the MHNO population. We, therefore, further excluded 6,044 participants who were MHNO at baseline and deteriorated to any other phenotype during follow-up, which yielded a final sample size of 35,322.

The need for medication for MetS was defined as the presence of one or more of the following conditions: TG $\geq$ 5.60 mmol/L or usage of lipid-lowering drugs, SBP $\geq$ 140 mmHg, or DBP $\geq$ 90 mmHg or usage of antihypertensive drugs, FSG $\geq$ 7.1 mmol/L or usage of medications for diabetes.

Statistical analysis

The distributions of baseline characteristics were compared between different obesity and MetS phenotype groups. Cox proportional hazard models were used to estimate the associations between obesity and metabolic status at baseline and the risk of NAFLD and MHNO throughout the group as a reference. Potential confounding variables included age, sex, smoking, alcohol usage, and location of enrollment.

The exposures of interest in this study were improvements in obesity and metabolic phenotypes. In summary, the exposure categories included MUNO changes in MHNO, MHO changes in MHNO, MUO changes in MHO, MUO changes in MUNO, and MUO changes in MHNO. Meanwhile, MHNO throughout the group was considered non-exposed. The duration of improvement was calculated by adding half the interval between the time the improved phenotype was discovered and the time of the previous follow-up that was conducted, plus half the interval between the time when the improved phenotype was discovered and the time when the subsequent different phenotype appeared. For the "consistent" category, the length of exposure was calculated by adding half the time interval between the time when the improved phenotype was found and the time of the previous follow-up when no improvement occurred, plus the time between the time when the improved phenotype was found and the end of the subsequent follow-up period. For "intermittent" categories where improvements are observed at multiple intervals, cumulative exposure is calculated by adding the duration of each intermittent improvement. The improvement intervals are calculated by adding half the time between when a phenotype improved and the time when no improvement occurred in the previous follow-up, plus half the time between when the phenotype improved and when it changed. Logistic regression models were used to estimate the association between the risk of NAFLD and the exposure category, as well as the dose–response relationship. Sensitivity analysis was conducted by assessing the modification effect by age, sex, smoking, alcohol usage, location of enrollment, and need for medication for MetS and by using restricted cubic spline (RCS) logistic models to test the association between exposure duration and risk of NAFLD. Since the duration of the independent variable exposure in this model was measurement data, we used the RCS model to calculate the estimated dose–response relationship, in which the lowest dose reference group was those with no change in the phenotype from baseline to follow-up. A two-sided p-value of <0.05 was considered to be statistically significant. SAS version 9.4 (SAS Institute Inc.) was used for analyses (Figure 1).

Results

Overall, we followed 35,322 participants with an average age of 41.38 years old for 12 to 96 months. Demographic and medical factors differed significantly between the obesity and metabolic phenotype groups ($p < 0.001$), except for the location of enrollment ($p = 0.873$; Table 1).

During 148,186 person-years of follow-up, 8,409 participants had onset NAFLD, with an incidence rate of 56.75 (95%CI:55.57, 57.94) per 1,000 person-years. The median follow-up duration was 47 months for the overall subjects, 36 months for those with the onset of NAFLD, and 48 months for those without. Significantly different NAFLD risks

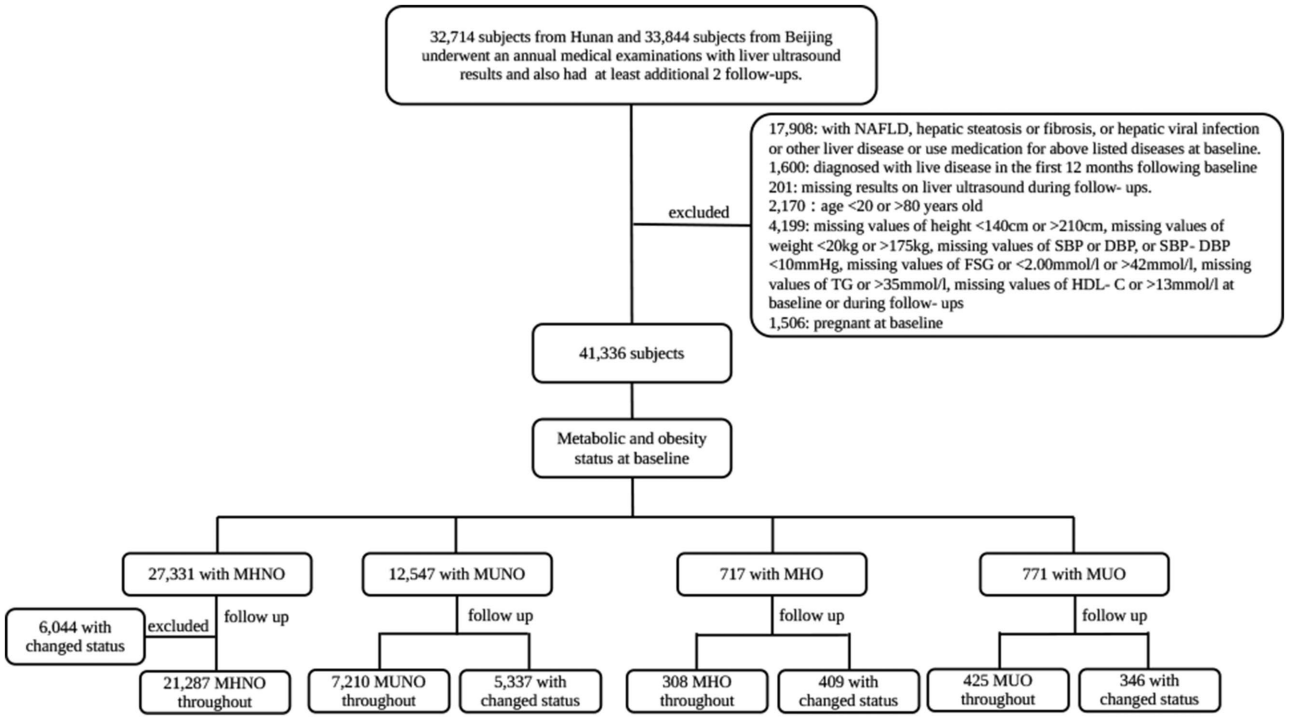

FIGURE 1
Enrolment flowchart. NAFLD, non-alcoholic fatty liver disease; MHNO, metabolic healthy non-obese; MUNO, metabolic unhealthy non-obese; MHO, metabolic healthy obese; MUO, metabolic unhealthy obese; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; FSG, fasting serum glucose.

were found between different baseline phenotype groups ($p < 0.001$; Figure 2A). In comparison with the MHNO at baseline group, the hazard ratios for MUNO at baseline, MHO at baseline, and MUO at baseline were still significantly higher after adjustment for confounding, respectively, at 4.48 (95%CI:4.24, 4.73), 8.85 (95%CI:7.95, 9.84), and 10.70 (95%CI:9.73, 11.78; Figure 2B).

The results of logistics models are listed in Tables 2, 3. For participants with MUNO at baseline, the risk of NAFLD decreased significantly for those who ever improved to the MHNO phenotype [OR = 5.53 (95%CI:5.15, 5.94) decreased to 2.71 (95%CI:2.50, 2.93)], and the reduction was significantly higher in the group that improved consistently to the MHNO phenotype compared to the group that improved intermittently ($p < 0.05$; Table 2). For participants with MUO at baseline, the improvement of metabolic health to the MHO phenotype could also reduce the risk of NAFLD, and the risk of consistently changing to the MHO group was statistically different from the risk of MUO throughout the group [OR = 3.85 (95%CI:1.19, 12.50) vs. 22.74 (95%CI:17.61, 29.37)]. The improvement in metabolic health among MUNO at baseline or MUO at baseline could not completely reduce the risk of NAFLD to the level of those with MHNO at baseline ($p < 0.05$; Tables 2, 3). However, the duration of improvement in metabolic health in the MUNO and MUO groups both showed a significantly linear association with lower odds of NAFLD (P for trend < 0.001; Table 3).

Population with improved obesity status, including changing from MHO to MHNO group and changing from MUO to MUNO group, also exhibited a reduced risk of NAFLD compared with the risk in the unchanged phenotype group ($p < 0.05$; Table 2), as well as a negative dose–response relationship between the duration of improvement and risk of NAFLD (P for trend < 0.001; Table 3).

Participants changing from MUO to MHNO involved simultaneous improvements in obesity and metabolic status, and a significantly lower NAFLD risk than the MUO throughout the group was observed ($p < 0.05$). The risk of NAFLD in the consistent improvement of the MUO group was not statistically different from that in the MHNO group, with OR = 1.16 (95%CI:0.24, 5.55). Less than five subjects had a cumulative improvement in MHNO greater than 24 months. Therefore, we only estimated the odds ratios for those with <12 months of cumulative improvement and ≥12 months, and no statistical difference was found between those two subgroups.

Sensitivity analyses

The RCS logistic model observed similarly significant associations between the duration of improvement in obesity and metabolic status and reduced risk of NAFLD ($p < 0.001$; Supplementary Figure S1). Except for changes from MUO to MUNO or MHO, those dose–response relationships were non-linear ($p < 0.05$). In male subjects, improvements in obesity or metabolic status resulted in lower risks of NAFLD compared to female subjects ($p < 0.01$), with the exception of changing from the MUO to the MHO group ($p = 0.449$). Among participants who changed from MUNO to MHNO, the risk of NAFLD was lower among those over 50 than among those <50 [OR = 1.92 (95%CI:1.66, 2.23) vs. 2.80 (95%CI:2.55, 3.08)]. We also find heterogeneity in the risk of NAFLD due to the need for MetS

TABLE 1 Characteristics of enrolled participants by obesity and metabolic status at baseline ($n = 35,322$).

Characterization	MHNO ($n = 21,287$)	MUNO ($n = 12,547$)	MHO ($n = 717$)	MUO ($n = 771$)	*P*
	Mean (SD)/*N* (%)	Mean (SD)/*N* (%)	Mean (SD)/*N* (%)	Mean (SD)/*N* (%)	
Age	36.78(11.22)	49.08(14.83)	39.10(12.07)	45.65(14.67)	<0.001
Female	13,993(65.73)	4,022(32.06)	284(39.61)	162(21.01)	<0.001
Enrolled from Beijing	10,627(49.92)	6,268(49.96)	351(48.95)	394(51.10)	0.873
Smoking	4,317(20.28)	4,074(32.47)	228(31.80)	344(44.62)	<0.001
Alcohol usage	6,197(29.11)	4,820(38.42)	298(41.56)	398(51.62)	<0.001
Hypertension	499(2.34)	3,938(31.39)	83(11.58)	318(41.25)	<0.001
Diabetes mellitus	39(0.18)	943(7.52)	4(0.56)	80(10.38)	<0.001
Dyslipidemia	1,231(5.78)	5,926(47.23)	121(16.88)	446(57.85)	<0.001
BMI, Kg/m^2	21.38(2.23)	23.62(2.20)	28.82(1.55)	29.22(1.54)	<0.001
SBP, mmHg	112.46(11.69)	130.91(15.55)	121.45(13.31)	134.72(15.41)	<0.001
DBP, mmHg	68.98(8.49)	79.61(10.70)	74.46(9.38)	82.83(11.24)	<0.001
Total cholesterol, mmol/L	4.61(0.82)	5.09(1.00)	4.84(0.87)	5.16(1.22)	<0.001
LDL-c, mmol/L	2.41(0.72)	2.72(0.87)	2.77(0.77)	2.77(0.91)	<0.001
HDL-c, mmol/L	1.76(0.38)	1.40(0.38)	1.46(0.32)	1.27(0.32)	<0.001
Triglycerides, mmol/L	0.95(0.43)	2.15(1.62)	1.31(0.67)	2.53(2.12)	<0.001
FSG, mmol/L	4.95(0.47)	5.72(1.33)	5.12(0.66)	5.89(1.72)	<0.001
≥1 component of the MetS requires medication	549(2.58)	4,697(37.44)	89(12.41)	373(48.38)	<0.001

MHNO, metabolic healthy non-obese; MUNO, metabolic unhealthy non-obese; MHO, metabolic healthy obese; MUO, metabolic unhealthy obese; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; FSG, fasting serum glucose; MetS, metabolic syndrome; SD, standard deviation.

medication at baseline. Participants with metabolic improvement who changed from MUNO to MHNO and required medication for MetS at baseline had a lower risk of NAFLD than those who did not need medication [OR = 1.76 (95%CI:1.36, 2.29) vs. 2.58 (95%CI:2.37, 2.81, $p < 0.001$); Figure 3].

Discussion

Our study compared NAFLD risk in populations with different metabolic health and obesity statuses. It also examined whether NAFLD risk changed with improvements in metabolic and obesity status. As expected, we found that individuals with MHO, MUNO, and MUO have a higher incidence of NAFLD than MHNO. In addition, we demonstrated for the first time that clinically intermittent or consistent improvements in BMI and/or metabolic status were significantly associated with a reduced risk of NAFLD.

In this large cohort study, metabolic unhealthy and obesity status at baseline were found to be associated with a higher risk of NAFLD. In a retrospective study, Wu et al. (21) reported that the prevalence of NAFLD was 7.14%, 27.92%, 34.80%, and 61.02% in MHNO, MUNO, MHO, and MUO in elderly Chinese adults, respectively. To our knowledge, this is the first cohort study reporting the incidence of NAFLD among Chinese adults. MUNO, MHO, and MUO groups had approximately 4-fold, 8-fold, and 10-fold higher risks of developing NAFLD than MHNO. Our results indicated that obesity has a greater impact on NAFLD incidence than metabolic abnormalities. Moreover, obesity combined with abnormal metabolism has the greatest impact on NAFLD incidence.

Emerging epidemiological evidence indicates that MHO increases NAFLD risk significantly. For example, in Vusirikala's study, MHO individuals had an approximately 7-fold higher risk of NAFLD than the MHNO population. This was a retrospective, population-based longitudinal cohort study conducted using over 4 million primary care patient records in the UK (16), and the results were similar to those of our own study. Moreover, BMI has shown a strong linear relationship with NAFLD incidence (15). These studies suggest obesity could significantly increase NAFLD risk, regardless of metabolic status. In metabolically healthy individuals, obesity increases the risk of fatty liver disease through the following mechanisms: (1) increased free fatty acids (FFAs), proinflammatory cytokines, ceramide, and dysregulated adipokine secretion from adipose tissue may contribute to hepatic lipid storage (22); and (2) MHO showed reduced cardiorespiratory fitness (CRF) compared to MHNO. And CRF was inversely associated with liver fat content and insulin resistance (23).

In the course of a person's life, the MHO phenotype might change and evolve. The proportion of metabolically healthy individuals who deteriorate varies depending on their ethnicity, gender, age, and follow-up period. Approximately 23%–74% of metabolically healthy individuals develop metabolic abnormalities at some point in their lives (24–27). However, few studies focus on the impact of MHO improvement on the risk of NAFLD. In our study, after an average of 4.2 years of follow-up, approximately 30% of individuals experienced improvement from MHO to MHNO, which provides novel evidence

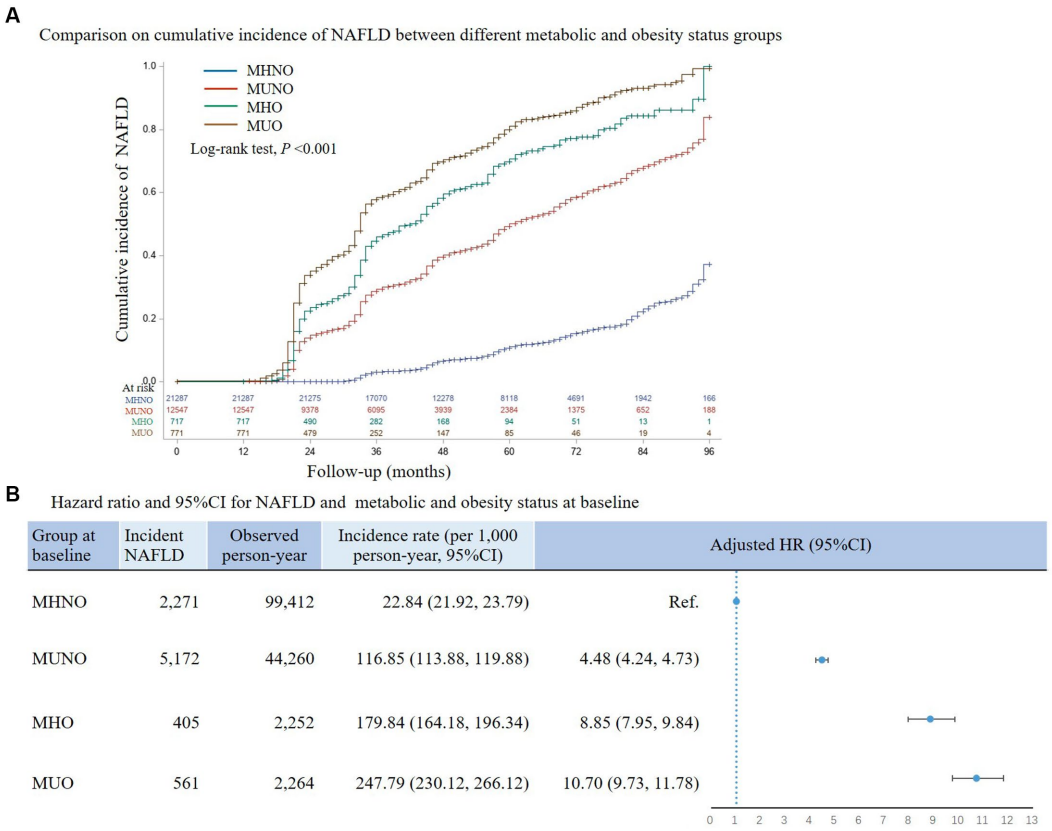

FIGURE 2
Association between risk of NAFLD and metabolic and obesity status at baseline. In panel **(A)** comparison between groups was based on Kaplan–Meier method; in panel **(B)** hazard ratio estimation was based on the cox proportion hazards model and adjustment variables included sex, age, alcohol usage, smoking, and location of enrollment. NAFLD, non-alcoholic fatty liver disease; MHNO, metabolic healthy non-obese; MUNO, metabolic unhealthy non-obese; MHO, metabolic healthy obese; MUO, metabolic unhealthy obese; HR, hazard ratio.

for an improving trend in obesity among the Chinese health check-up population. It is worth noting that individuals in this study may have attended nutrition clinics or been referred to outpatient clinics after their annual health check-up. In these clinics, patients could receive personalized dietary and exercise advice or medicine prescriptions. Consequently, the rate of obesity reduction and metabolic improvement in the current study may not be generalizable to the general population. Previous studies reported that the transition from normal weight to obesity was associated with a 2-fold increase in NAFLD risk among metabolically health individuals (15). Since there are many obese individuals who have not been diagnosed with NAFLD yet, it is crucial to determine how to prevent it. Our study found a significant reduction in NAFLD risk if obesity was improved. This supports their findings in another direction, which offers an effective prevention method. In addition, there was a linear dose–response relationship between the duration of improvement in MHO and MHNO and the reduced risk of NAFLD. The duration of improvement was grouped into four categories: 0 months, 1–12 months, 13–24 months, and over 25 months. NAFLD risk did not differ significantly between the MHO–MHNO consistent improvement group and the intermittent improvement group. This may be due to the small sample size of the group with consistent improvement. Indeed, previous studies have assessed the effect of weight loss on patients with NAFLD, and the guidelines recommend at least 3%–5% weight loss to improve hepatic steatosis in patients with NAFLD (6). In Cho's study, they first demonstrated that even weight loss of 1% may help reduce the risk of developing hepatic steatosis in MHO individuals without NAFLD (19). Our study suggests that obesity has a greater impact on NAFLD than metabolic abnormalities, supporting the importance of weight loss when an individual is obese without the presence of NAFLD. Unfortunately, our previous data indicate that obesity among the health check-up population has not decreased (20). Considering the fact that weight control is particularly prominent among Chinese, our findings have significant clinical implications.

As predicted, MUNO individuals are at a greater risk of NAFLD than MHNO individuals. In our study, approximately 40% of MUNO individuals improved to MHNO status, reducing the risk of NAFLD even with intermittent improvements. Furthermore, linear dose–response relationships were identified between improved health status from MUNO to MHNO and a reduced risk of NAFLD. It is noteworthy that men, older individuals, and those requiring MetS medication were observed to have a lower NAFLD risk among individuals who switched from MUNO to MHNO. Previous studies demonstrated that multiple molecular pathways and gene networks implicated in lipid metabolism, insulin signaling, and inflammation show sexual dimorphism (28, 29). Meanwhile, aging was generally correlated with increases in insulin resistance and the prevalence of

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TABLE 2 Association between NAFLD and improvement in metabolic health and weight loss during follow-up.

Exposure group	NAFLD, *n* (%)		Crude OR (95%CI)	Adjusted OR (95%CI)†
	Control	Case		
MHNO throughout (*n* = 21,287)	19,016(89.33)	2,271(10.67)	1.00	1.00
MUNO at baseline (n = 12,547)				
MUNO throughout (*n* = 7,210)	3,805(52.77)	3,405(47.23)	7.49(7.03,7.98)	5.53(5.15,5.94)
Ever MUNO to MHNO (*n* = 5,032)	3,514(69.83)	1,518(30.17)	3.62(3.36,3.90)	2.71(2.50,2.93)
Intermittent MUNO to MHNO (*n* = 3,924)	2,668(67.99)	1,256(32.01)	3.94(3.64,4.27)	2.95(2.71,3.21)
Consistent MUNO to MHNO (*n* = 1,108)	846(76.35)	262(23.65)	2.59(2.24,3.00)	1.97(1.70,2.28)
MHO at baseline (n = 717)				
MHO throughout (*n* = 308)	116(37.66)	192(62.34)	13.86(10.96,17.52)	12.01(9.40,15.35)
Ever MHO to MHNO (*n* = 214)	137(64.02)	77(35.98)	4.71(3.55,6.24)	4.14(3.08,5.57)
Intermittent MHO to MHNO (*n* = 161)	104(64.60)	57(35.40)	4.59(3.31,6.36)	3.79(2.69,5.33)
Consistent MHO to MHNO (*n* = 53)	33(62.26)	20(37.74)	5.08(2.91,8.86)	5.48(3.04,9.87)
MUO at baseline (n = 771)				
MUO throughout (*n* = 425)	79(18.59)	346(81.41)	36.67(28.61,47.01)	22.74(17.61,29.37)
Ever MUO to MHNO (*n* = 94)	51(54.26)	43(45.74)	7.06(4.69,10.62)	4.67(3.05,7.16)
Intermittent MUO to MHNO (*n* = 82)	41(50.00)	41(50.00)	8.37(5.42,12.94)	5.48(3.48,8.63)
Consistent MUO to MHNO (*n* = 12)	10(83.33)	2(16.67)	1.68(0.37,7.65)	1.16(0.24,5.55)
Ever MUO to MUNO (*n* = 154)	62(40.26)	92(59.74)	12.43(8.98,17.20)	6.83(4.87,9.57)
Intermittent MUO to MUNO (*n* = 118)	47(39.83)	71(60.17)	12.65(8.73,18.33)	6.77(4.61,9.94)
Consistent MUO to MUNO (*n* = 36)	15(41.67)	21(58.33)	11.72(6.04,22.77)	7.02(3.52,14.02)
Ever MUO to MHO (*n* = 140)	42(30.00)	98(70.00)	19.54(13.57,28.11)	13.38(9.17,19.53)
Intermittent MUO to MHO (*n* = 127)	34(26.77)	93(73.23)	22.90(15.43,34.01)	15.48(10.28,23.30)
Consistent MUO to MHO (*n* = 13)	8(61.54)	5(38.46)	5.23(1.71,16.01)	3.85(1.19,12.50)

†Adjustment variables included sex, age, alcohol usage, smoking, location of enrollment, and LDL cholesterol. NAFLD, non-alcoholic fatty liver disease; MHNO, metabolic healthy non-obese; MUNO, metabolic unhealthy non-obese; MHO, metabolic healthy obese; MUO, metabolic unhealthy obese; OR, odds ratio; CI, confidence interval.

the metabolic syndrome (30). In light of this difference, it may be explained why male subjects and older individuals may benefit more from the improvement of metabolic abnormalities.

We found that MUO is more susceptible to NAFLD than MHO. Compared to MHO, MUO has more abdominal and visceral fat accumulation, less physical activity, more sedentary activities, and poorer CRF, which will lead to an increased risk of NAFLD (23, 31–37). Therefore, when intervening in the MUO population, we should not only focus on weight loss but also improve fat distribution and promote cardiopulmonary function. A reduction in the risk of NAFLD can be achieved to a greater extent by improving both metabolic status and obesity. A linear dose–response relationship was found between the duration of improvement from MUO to MHNO and the reduction in NAFLD risk.

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TABLE 3 Association between NAFLD and duration of improvement in weight and metabolic health during follow-up.

Exposure duration	NAFLD, *n* (%)		Crude OR (95%CI)	*P* for trend	Adjusted OR (95%CI)[†]	*P* for trend
	Cases	Control				
MHNO throughout (*n* = 21,287)	2,271(10.67)	19,016(89.33)	1.00		1.00	
Changed from MUNO to MHNO						
0 months (*n* = 7,210)	3,805(52.77)	3,405(47.23)	7.49(7.03,7.98)	<0.001	5.53(5.15,5.94)	<0.001
1–12 months (*n* = 1,543)	495(32.08)	1,048(67.92)	3.96(3.52,4.44)		2.71(2.40,3.06)	
13–24 months (*n* = 1,793)	574(32.01)	1,219(67.99)	3.94(3.54,4.39)		2.62(2.33,2.94)	
25-months (*n* = 1,696)	449(26.47)	1,247(73.53)	3.02(2.68,3.39)		2.12(1.88,2.39)	
Changed from MHO to MHNO						
0 months (*n* = 308)	116(37.66)	192(62.34)	13.86(10.96,17.52)	<0.001	12.01(9.40,15.35)	<0.001
1–12 months (*n* = 71)	26(36.62)	45(63.38)	4.84(2.98,7.86)		4.33(2.61,7.19)	
13–24 months (*n* = 76)	27(35.53)	49(64.47)	4.61(2.88,7.40)		3.59(2.19,5.89)	
25-months (*n* = 67)	24(35.82)	43(64.18)	4.67(2.83,7.72)		4.67(2.75,7.93)	
Changed from MUO to MHNO						
0 months (*n* = 425)	79(18.59)	346(81.41)	36.67(28.61,47.01)	<0.001	22.74(17.61,29.37)	<0.001
≤12 months (*n* = 39)	17(43.59)	22(56.41)	6.47(3.43,12.20)		4.31(2.22,8.35)	
13-months (n = 55)	26(47.27)	29(52.73)	7.51(4.41,12.77)		4.89(2.80,8.54)	
Changed from MUO to MUNO						
0 months (*n* = 425)	79(18.59)	346(81.41)	36.67(28.61,47.01)	<0.001	22.74(17.61,29.37)	<0.001
≤12 months (*n* = 60)	36(60.00)	24(40.00)	12.56(7.48,21.09)		6.27(3.68,10.68)	
13–24 months (*n* = 62)	38(61.29)	24(38.71)	13.26(7.94,22.14)		8.45(4.94,14.46)	
25-months (*n* = 32)	18(56.25)	14(43.75)	10.77(5.35,21.68)		5.06(2.47,10.38)	
Changed from MUO to MHO						
0 months (*n* = 425)	79(18.59)	346(81.41)	36.67(28.61,47.01)	<0.001	22.74(17.61,29.37)	<0.001
≤12 months (*n* = 64)	51(79.69)	13(20.31)	32.85(17.84,60.49)		24.20(12.87,45.53)	
13–24 months (*n* = 52)	35(67.31)	17(32.69)	17.24(9.64,30.82)		11.01(6.03,20.11)	
25 months (*n* = 24)	12(50.00)	12(50.00)	8.37(3.76,18.66)		5.33(2.31,12.31)	

[†]Adjustment variables included sex, age, alcohol usage, smoking, location of enrollment location, and LDL cholesterol. NAFLD, non-alcoholic fatty liver disease; MHNO, metabolic healthy non-obese; MUNO, metabolic unhealthy non-obese; MHO, metabolic healthy obese; MUO, metabolic unhealthy obese; OR, odds ratio; CI, confidence interval.

Our study has several strengths, including the large population-based cohort, the longitudinal design, and follow-up over 148,186 person-years, allowing the assessment of NAFLD risk according to improvement in metabolic health and/or loss of weight in Chinese subjects without NAFLD at baseline. As far as we know, we were the first to report that improving metabolic abnormalities and/or losing weight in different subgroups have been beneficial. It is expected that these findings will have significant clinical implications for the prevention of NAFLD in the Chinese population. Moreover, our findings could pave the way for further research into the most effective ways to prevent NAFLD in other populations. It is our hope that our research will contribute to a better understanding of the underlying causes of NAFLD and ultimately lead to more effective treatments and preventive measures for this potentially life-threatening condition. Furthermore, we had extensive data on health behavior and sociodemographic information, allowing us to examine the relationship between metabolic health and/or body weight and NAFLD under the adjustment of a wide range of confounders.

Results should also be interpreted with consideration for limitations. Despite the large sample size, our study was not a community-based study but rather a cohort study based on the health checkup population. Moreover, the subjects enrolled in the present study are predominantly from urban areas. Due to these limitations, our subjects were not generalizable to the general Chinese population. More cohorts of a mixture of urban and rural populations should

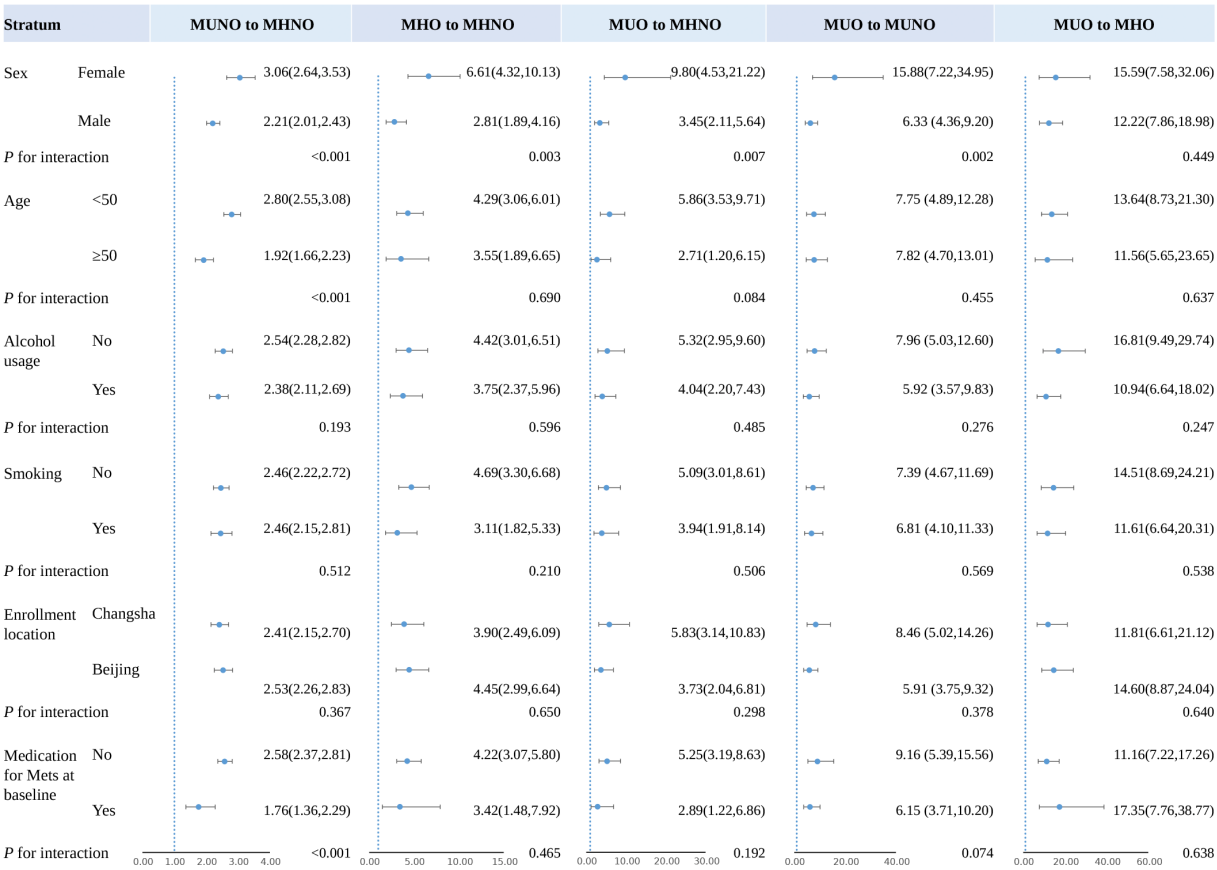

FIGURE 3
Sensitivity analysis for the association between NAFLD and improvement in weight and metabolic health during follow-up. All the adjusted odd ratios in the figure were calculated using MHNO throughout the group as the reference group. The adjustment variables included sex, age, alcohol usage, smoking, location of enrollment, and medication for metabolic syndrome components at baseline. When stratified by one of these factors, the stratification variable would not be included in the model. NAFLD, non-alcoholic fatty liver disease; MHNO, metabolic healthy non-obese; MUNO, metabolic unhealthy non-obese; MHO, metabolic healthy obese; MUO, metabolic unhealthy obese; MetS, metabolic syndromes; OR, odds ratio; CI, confidence interval.

be used to validate our findings. The results of this study may still be confounded by the fact that we did not collect all of the information that could have influenced outcomes, including information about healthcare and household income relating to NAFLD onset. In addition, insulin sensitivity is not routinely assessed during health check-ups, and C-reactive protein is not routinely measured. We did not use the combination of these two variables as a measure of metabolic health. This would make our measurement of changes in metabolic exposure less sensitive than when we used the metrics described above. However, we used exposure measurements in the same manner throughout the population, even if some misclassification was present, which would result in more conservative findings.

Conclusion

This study proves that MHO, MUNO, and MUO had a higher NAFLD risk than MHNO. Importantly, we also found that no matter whether the improvement in metabolic status and/or loss of weight was intermittent or persistent, it could reduce NAFLD risk, independent of traditional risk factors.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Third Xiangya Hospital. Written informed consent to participate in this study was provided by the patients/participants or patient/participants' legal guardian/next of kin.

Author contributions

XH: funding acquisition, methodology, and writing original draft preparation. WO: formal analysis, manuscript editing, and interpreting results. YHu, YHe, BT, PY, and KC: investigation. YHe:

supervision. HW, QL, and XL: experimental work. LY: formal analysis. JD: interpreting results. YL: supervision, funding acquisition, methodology, writing, reviewing, and interpreting results. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1239996/full#supplementary-material

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Diet quality indices and odds of metabolic dysfunction-associated fatty liver disease: a case-control study

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Objectives: There are only limited studies investigating the impact of dietary quality indicators, such as dietary quality index (DQI), dietary diversity score (DDS), and alternative healthy eating index (AHEI), on metabolic dysfunction-associated fatty liver disease (MASLD). Furthermore, these indicators may have different components that could lead to varying results. Therefore, this study aims to assess the nutritional quality indicators and their potential association with MASLD.

Methods: The study included 128 recently diagnosed MASLD patients and 256 controls aged between 20 and 60 years. The dietary intake of participants was evaluated using a validated semi-quantitative food frequency questionnaire that consisted of 168 items. In this study, the method used to evaluate dietary diversity was based on five main food groups, specifically bread and grains, vegetables, fruits, meat, and dairy. The AHEI-2010 was computed using data collected from the FFQ.

Results: After adjusting for confounders in the fully adjusted model, a significant negative correlation was observed between DDS and the risk of MASLD (OR 0.41, 95% CI 0.20, 0.97). Participants in the top quartile of AHEI had a 76% lower risk of MASLD compared with those in the bottom quartile after controlling for all potential confounders in the fully adjusted model (OR 0.24, 95% CI 0.12, 0.56).

Conclusion: The results of our study suggest that there is a significant association between adherence to a high-diversity diet and a reduced likelihood of developing MASLD. Similarly, we observed a similar association between adherence to the AHEI diet and a lower risk of MASLD.

KEYWORDS

alternative healthy eating index, AHEI, dietary diversity score, DDS, metabolic dysfunction-associated fatty liver disease, MASLD, dietary quality indices, DQI

Introduction

Metabolic dysfunction-associated fatty liver disease (MASLD) is a prevalent cause of liver abnormalities worldwide and has become increasingly common in Asia in recent years (1–3). Its global prevalence is estimated to be between 20 and 30% in Western countries and 5% and 18% in Asia (2). This disease can lead to several liver abnormalities, including non-alcoholic steatohepatitis (NASH), cirrhosis, liver failure, and liver cancer, and is believed to be the primary cause of cirrhosis of the liver (4, 5). The strong correlation between MASLD,

obesity, and metabolic syndrome is well established, and the disease is also an independent risk factor for cardiovascular diseases (5, 6). Therefore, identifying risk factors for fattyliver disease is crucial. The increasing incidence of MASLD in Asia makes it particularly important to understand the risk factors associated with the disease in this region. It is essential to continue researching this disease to develop effective prevention and treatment strategies, to reduce the burden of MASLD worldwide.

Lifestyle interventions, particularly a well-balanced diet, have been found to be significantly associated with preventing and managing MASLD (7). However, it is important to understand the complete composition of dietary intake as an alternative approach to establishing a link between diet and disease (8). This method considers the collaborative and opposing interactions of different dietary components and addresses the limitation of studying individual dietary components (8). By evaluating the overall dietary pattern, researchers can examine the combined effect of multiple nutrients on health outcomes, including MASLD. It can also provide a more comprehensive understanding of the role of diet in the development and progression of MASLD. Therefore, the assessment of dietary diversity and quality, as well as the identification of specific dietary patterns, is essential in the prevention and management of MASLD. A recent study showed that higher adherence to a nutritional pattern characterized by fructose, vitamin C, vitamin A, pyridoxine, and potassium, primarily from fruits, vegetables, and nuts, is inversely associated with MASLD risk (9). Another study indicated that healthy and Western dietary patterns may be associated with the risk of MASLD. It was stated that these results can be used for developing interventions in order to promote healthy eating for the prevention of MASLD (10).

Dietary Quality Indices (DQIs) such as alternative healthy eating index (AHEI) and dietary diversity score (DDS) have been developed to provide an overall picture of chronic nutritional intake, providing insight into the characteristics of diet-related diseases (11–13). The Dietary Diversity Score (DDS) is an index used to evaluate the overall dietary intake. It is associated with a higher consumption of macronutrients and micronutrients, better diet adequacy, and higher intake of fiber, antioxidants, and other nutrients (14). Previous research has shown that a higher DDS can help reduce certain risk factors associated with MASLD, such as diabetes (15), hypertension, and cardiovascular risk factors (16). These findings suggest that a diverse diet can provide the necessary nutrients to maintain good health and reduce the risk of chronic diseases. Therefore, evaluating dietary diversity is important in developing effective strategies for preventing and managing MASLD.

To the best of our knowledge, there are only limited studies investigating the impact of dietary quality indicators, such as DQI, DDS, and AHEI, on MASLD. Furthermore, these indicators may have different components that could lead to varying results. Therefore, this study aims to assess the nutritional quality indicators and their potential association with metabolic dysfunction-associated fatty liver disease in a case–control study. By analyzing the dietary habits of individuals with and without MASLD, this study provides a better understanding of the role of diet quality in the development and prevention of the disease in this population.

Patients and methods

Subjects

This was a cross-sectional study conducted at Umm Al-Qura University Medical Center, Makkah, Kingdom of Saudi Arabia, in patients attending Liver and Gastroenterology Clinic. The research study enrolled 128 individuals who had been recently diagnosed with MASLD and 256 healthy controls ranging in age from 20 to 60 years old. Individuals who were diagnosed with fatty liver based on laboratory tests and liver ultrasound indicating the presence of steatosis were included in the fatty liver group. The diagnosis of fatty liver was made by a specialist doctor. MASLD was defined as elevated liver enzymes, specifically alanine aminotransferase (ALT) levels >31 mg/dl and 41 mg/dl and aspartate aminotransferase (AST) levels >31 mg/dl and 47 g/dl in women and men, respectively. On the other hand, healthy individuals who had normal laboratory test results (ALT levels <31 UI/L and 41 UI/L and AST levels <31 UI/L and 37 UI/L in women and men, respectively) and normal liver sonography indicating no stages of hepatic steatosis were considered as the control group (17).

The study excluded participants who had specific dietary habits due to medical conditions or weight loss and those with certain medical conditions such as kidney and liver disease (e.g., viral infections, autoimmune liver disease, hemochromatosis, Wilson's disease, and alcoholic fatty liver disease), cardiovascular disease, diabetes mellitus and malignancies, thyroid disease, and autoimmune diseases. Additionally, individuals taking drugs that could harm the liver or promote weight gain were also excluded. The study also excluded participants who completed <35 items on the food frequency questionnaire or underreported/overreported their daily energy intake (<800 kcal or more than 4,500 kcal per day), but these participants were replaced with the new ones. All participants provided informed written consent before being included in the study.

Dietary assessment

The dietary intake of the participants was evaluated using a validated semi-quantitative food frequency questionnaire that consisted of 168 items (18). Participants were asked to report their average food consumption over the past few years by selecting one of the following options: never or less than once a month, 3–4 times a month, once a week, 2–4 times a week, 5–6 times a week, once a day, 2–3 times a day, 4–5 times a day, or 6 times or more a day. The amount of each food item was converted into grams using standard household measurements, and the frequencies of consumed foods were transformed into daily intakes. The nutrient composition of all foods was determined using modified Nutritionist IV software, which provided information on EPA, DHA, sodium levels, and other micronutrients (19). Additionally, the National Nutrient Database of the US Department of Agriculture (USDA) was used in other studies to obtain daily nutrient information such as EPA and DHA for each participant (20). The N4 software output was used to obtain the sodium content of the food. The FFQ questionnaire also included an item on the salt intake of participants. The use of

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TABLE 1 General characteristics and dietary intake among the study groups.

Variables	Controls ($n = 256$)	MASLD ($n = 128$)	*P*-value*
Age (years)	38.1 ± 7.9	37.6 ± 7.7	0.325
Male, *n* (%)	133 (51.8)	71 (55.6)	0.258
BMI (Kg/m^2)	25.3 ± 2.9	30.2 ± 3.7	<0.001
Smoking, *n* (%)	7 (2.7)	9 (7.1)	0.005
Physical activity (MET-min/week)	1590 ± 949	1119 ± 616	<0.001
Dietary intake			
Energy intake (kcal/day)	2117 ± 594	2325 ± 614	0.006
Carbohydrate (gr)	317 ± 27	387 ± 41	0.041
Protein (gr)	67 ± 15	63 ± 12	0.145
Fat (gr)	66 ± 17	82 ± 14	0.037
Fiber (gr/1000 Kcal)	16.4 ± 7.3	16.1 ± 6.2	0.164
Whole grains (gr/day)	58.0 ± 91.3	41.6 ± 58.2	0.041
Refined grains (gr/day)	322 ± 145	396 ± 188	<0.001
High fat dairy products (gr/day)	84.4 (38.9 – 167.5)	231.5 (109.6 – 310.8)	<0.001
Fruits (gr/day)	311 ± 244	293 ± 214	0.064
Vegetables (g/day)	302 ± 134	261 ± 125	0.001
Nuts and legume (gr/day)	15.4 (9.2 – 24.7)	14.8 (8.7 – 28.3)	0.412
Red and processed meat (gr/day)	21.2 ± 12.4	27.1 ± 16.8	0.001
Vitamin D (μg/day)	2.7 ± 2.4	2.0 ± 1.8	0.092
Vitamin C (mg/dar)	57.3 ± 24.2	42.8 ± 19.0	0.042
Calcium (mg/day)	1216.3 ± 323.3	993.1 ± 311.1	0.084
Zinc (mg/day)	246.3 ± 86.3	229.5 ± 84.1	0.088
Magnesium (mg/day)	8.9 ± 3.1	9.1 ± 4.4	0.127
Iron (mg/day)	9.4 ± 3.6	9.2 ± 3.5	0.732
Selenium (μg/day)	17.1 ± 8.6	10.0 ± 6.6	0.048
Vitamin E (IU/day)	2.24 ± 1.6	1.49 ± 1.4	0.031
Vitamin A (IU/day)	788.6 ± 351.2	541.4 ± 341.2	0.042
Vitamin B2 (IU/day)	2.1 ± 1.2	2.0 ± 1.2	0.113
Vitamin B6 (mg/day)	2.4 ± 1.2	2.3 ± 1.3	0.788
DDS scores	5.0 ± 1.7	4.7 ± 1.4	0.11
AHEI scores	50.4 ± 8.8	45.5 ± 9.6	<0.001

MET, metabolic equivalent task; DDS, dietary diversity score; AHEI, alternative healthy eating index; MASLD, metabolic dysfunction-associated fatty liver disease; IU, international unit; BMI, body mass index. Data are presented as mean ± SD for continuous variables and numbers and percentages for categorical variables. *Independent sample t-test and chi-square were used for continuous and categorical variables, respectively.

a standardized questionnaire and food composition tables allowed for the accurate assessment of the dietary intake of participants and ensured that the data obtained were reliable and comparable. The smoking status of the study subjects was obtained using the results of a general questionnaire that the subjects completed. Data on physical activity were obtained *via* a short form of the validated International Physical Activity Questionnaire (IPAQ), which was presented as metabolic equivalent task-minutes per week (MET-min/week).

Dietary diversity score

The method used to evaluate dietary diversity in this study was based on the approach developed by Kant et al. (21, 22). This study considered five main food groups, which were derived from the USDA Food Guide Pyramid, specifically bread and grains, vegetables, fruits, meat, and dairy. These food groups were further divided into 23 subgroups to capture the diversity within each group. To determine the diversity score for each group, the consumption of half a serving of each subgroup was considered. Each of the five food groups could receive a maximum diversity score of 2 out of 10 ($5 \times 2 = 10$). The total diversity score was calculated by summing up the scores from each of the five main food groups. The methodology facilitated a thorough evaluation of the extent of dietary variety and furnished significant perspectives on the general dietary patterns of the subjects.

Alternative healthy eating index

The AHEI-2010 was computed using data collected from the FFQ. This method incorporates various aspects of the original HEI developed by Kennedy et al. (23, 24) and considers eleven components, namely, fruits, vegetables, whole grains, nuts and legumes, long-chain n-3 fats (DHA and EPA), PUFA, wine consumption, sugar-sweetened drinks and fruit juice, red and processed meats, trans-fat, and Na intake. However, since our database lacked data on wine consumption and trans-fat, only nine components were evaluated in our study. Each individual was assigned a score for each component based on their decile ranking, with the highest decile receiving a score of 10 and the lowest decile receiving a score of 1. Individuals in other deciles were given corresponding scores. Conversely, individuals with the highest intake of sugar-sweetened drinks and fruit juice, red and processed meats, and Na were given a score of 1, while those with the lowest consumption of these components were given a score of 10. The total AHEI score for each participant was then calculated by summing up the scores for these ten components, which ranged from 9 to 81. This method allowed for the evaluation of dietary quality based on multiple components and provided a comprehensive assessment of the overall dietary habits of participants.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences software version 21 (SPSS Inc., Chicago, IL, USA). The significance level was determined as $P < 0.05$. The normality of variables was checked by Kolmogorov–Smirnov and histogram tests. The study compared the general characteristics and dietary intakes of cases and controls using appropriate statistical tests such as independent sample *t*-test and χ^2. After constructing the DDS and AHEI scores as described previously, energy-adjusted scores were obtained using the residual method (25). Participants were then categorized into quartiles based on the cutoff points obtained from the control group, and one-way ANOVA and χ^2 tests were used to assess continuous and categorical variables across these quartiles. The association of DDS and AHEI with MASLD was evaluated using binary logistic regression in different models, with the first quartile of DDS and AHEI serving as the reference category.

Results

The general characteristics and dietary intake of the participants are presented in Table 1. There was no significant difference between the two study groups in terms of age and gender ($p > 0.05$). However, compared with the control group, MASLD patients had a higher BMI, were more smokers, and had less physical activity ($p < 0.05$). In addition, MASLD patients had higher dietary intakes of calories, carbohydrates, fats, refined grains, high-fat dairy, and red and processed meats than controls ($p < 0.05$). The control group had higher dietary intakes of whole grains, vegetables, vitamin C, selenium, vitamin E, and vitamin A than MASLD patients ($p < 0.05$). There were no significant differences between the two groups in dietary intake.

Table 2 presents the dietary intakes of cases and controls in various quartiles of DDS and AHEI scores. Individuals in the top quartile of DDS had higher consumption of fruits, vegetables, carbohydrates, protein, fiber, whole grains, nuts, and legumes when compared with those in the bottom quartile. Moreover, those in the fourth quartile of AHEI had lower intakes of fat, refined grains, high-fat dairy products, and red and processed meat in comparison to participants in the first quartile. No other significant differences were observed in terms of dietary intakes across quartiles of DDS and AHEI scores.

Table 3 shows the multivariable-adjusted OR and 95% CI for MASLD across quartiles of DDS and AHEI. After adjusting for age, sex, body mass index, smoking, and physical activity, a significant negative correlation was observed between DDS and the risk of MASLD (OR 0.44, 95% CI 0.19, 0.94). This finding remained consistent after further controlling for dietary energy intake, with participants in the highest quartile of DDS being 59% less likely to have MASLD than those in the lowest quartile (OR 0.41, 95% CI 0.20, 0.97). Additionally, a significant inverse association was found between AHEI and the odds of MASLD. Participants in the top quartile of AHEI had a 76% lower risk of MASLD compared with those in the bottom quartile after controlling for all potential confounders in the fully adjusted model (OR 0.24, 95% CI 0.12, 0.56).

Discussion

Our study revealed that there is a negative relationship between the Dietary Diversity Score (DDS) and the risk of MASLD. We took into account several variables that could potentially affect this relationship, such as age, sex, body mass index, smoking, physical activity, and energy intake, and found an inverse association between DDS and MASLD. We also observed a similar inverse relationship between AHEI and MASLD, both before and after controlling for the aforementioned confounders.

Numerous studies have shown that an overall dietary pattern, rather than focusing on individual foods or nutrients, is a better predictor of overall diet quality and is associated with various diseases. In particular, DDS and AHEI have been used as indicators of diet quality in relation to non-communicable diseases such as cardiovascular disease, polycystic ovaries, and some cancers (26–29). In the present study, a high DDS was found to be associated with decreased odds of MASLD. This finding is consistent with previous research that has shown an inverse association between DDS and the risk of diabetic nephropathy (29) and bladder cancer (30). In another study, by emphasizing the higher diversity scores for vegetables and fewer diversity scores for meat and refined grains, high DDS is inversely correlated with the risk of MASLD (15).

One possible explanation for the protective association of a diverse diet against MASLD is its high nutrient content, including antioxidants. Several studies have demonstrated an inverse association between dietary intake of antioxidants and the risk of MASLD (31, 32). Additionally, diets with a high DDS tend to be rich in fruits and vegetables, which have also been shown to be protective against MASLD (33). Such diets are also typically high in fiber, which can reduce the risk of MASLD (34, 35). However, it should be noted that not all studies have found a significant association between dietary diversity and the risk of certain diseases, such as breast cancer (36). In some cases, confounding factors may not have been adequately adjusted to determine the independent association between dietary diversity and disease risk. Overall, increasing the diversity score of one's diet by emphasizing higher diversity scores for vegetables and fewer diversity scores for meat and refined grains may be beneficial to managing MASLD (15).

The current study found that a higher level of adherence to the AHEI was associated with a reduced likelihood of MASLD. Previous research has also shown that healthy dietary patterns are associated with a lower risk of MASLD, while Western dietary patterns are linked to an increased risk of this disease (10). In a large population-based study in Germany, individuals with the highest AHEI scores were less likely to develop colorectal cancer compared with those with the lowest scores (37). In a meta-analysis of observational studies, a high adherence to the AHEI dietary pattern was associated with a reduced risk of all-cause mortality and cardiovascular and cancer mortality (38).

Additionally, a study found that AHEI scores were significantly higher among individuals with high levels of sensitive C-reactive protein (Hs-CRP), which is a marker of inflammation in the body (39). A systematic review and meta-analysis of observational studies also showed that high adherence to the AHEI was associated with a reduced risk of cancer mortality (38). Fruit consumption

TABLE 2 Dietary and nutrient intakes of study participants across quartiles (Q) of DDS and AHEI scores.

	Quartiles of DDS					Quartiles of AHEI				
	Q1	Q2	Q3	Q4	*P*-value[†]	Q1	Q2	Q3	Q4	*P*-value[†]
Age (year)	36.9 ± 8.6	38.0 ± 8.5	39.4 ± 9.2	37.2 ± 7.3	0.322	37.8 ± 8.1	37.0 ± 7.9	38.2 ± 8.3	37.8 ± 7.5	0.147
Male (%)	53.8	51·4	49·3	56.3	0.188	54·4	52·5	56·9	53·4	0.454
BMI(Kg/m^2)	27.1 ± 4.1	26.7 ± 4.4	26.8 ± 4.4	26.8± 3.9	0.504	26.2 ± 3.1	27.5 ± 4.2	26.2 ± 3.4	27.4 ± 4.1	0.413
Smoking, (%)	4.9	5.1	4.7	5.2	0.522	4.7	4.8	5.6	5.1	0.427
Physical activity (MET-min/week)	1445 ± 848	1456 ± 811	1387 ± 885	1434 ± 874	0.565	1437 ± 878	1411 ± 871	1429 ± 805	1501 ± 985	0.139
Dietary intake										
Energy intake (kcal/day)	2323 ± 601	2198 ± 624	2287 ± 641	2317 ± 658	0.357	2273 ± 649	2235 ± 619	2316 ± 653	2246 ± 618	0.369
Carbohydrate (gr)	51.1 ± 7.7	57.7 ± 6.7	59.0 ± 6.3	60.1 ± 6.8	< 0.001	51.1 ± 7.2	52.1 ± 7.5	55.6 ± 6.4	60.2 ± 6.7	<0.001
Protein (gr)	13.1 ± 2.4	13.5 ± 2.1	13.8 ± 2.5	14.5 ± 2.5	< 0.001	13.3 ± 1.8	13.4 ± 2.3	14.0 ± 2.1	14.3 ± 2.5	<0.001
Fat (gr)	33.4 ± 7.9	31.2 ± 6.4	30.1 ± 5.8	29.8 ± 5.7	< 0.001	32.4 ± 7.2	31.5 ± 6.1	29.6 ± 5.8	28.8 ± 5.4	<0.001
Fiber (gr/1000 kcal)	14.8 ± 7.9	16.3 ± 6.7	17.3 ± 6.5	17.9 ± 6.8	< 0.001	13.2 ± 4.2	13.9 ± 5.8	14.8 ± 6.5	15.5 ± 6.9	0.031
Whole grains (gr/day)	22.7 ± 17.5	45.1 ± 34.9	84.0 ± 53.7	97.0 ± 73.7	< 0.001	31.7 ± 17.5	52.1 ± 34.9	96.0 ± 93.7	107.0 ± 83.4	<0.001
Refined grains (gr/day)	394 ± 194	341 ± 135	262 ± 144	241 ± 124	< 0.001	402 ± 188	352 ± 134	282 ± 137	253 ± 125	<0.001
High-fat dairy products (gr/day)	178 ± 154	151 ± 135	142 ± 114	138 ± 98	0.005	191 ± 149	177 ± 122	156 ± 147	152 ±102	0.008
Fruits (gr/day)	210 ± 146	332 ± 225	422 ± 247	453 ± 261	<0.001	194 ± 94	287 ± 118	320 ± 209	408 ± 219	<0.001
Vegetables (gr/day)	250 ± 133	301 ± 139	316 ± 156	341 ± 164	0.016	231 ± 121	252 ± 127	289 ± 148	305 ± 151	0.028
Nuts and legume (gr/day)	15.8 ± 12.4	20.5 ± 15.7	28.4 ± 25.8	33.1 ± 25.8	<0.001	18.8 ± 12.4	28.5 ± 15.7	31.4 ± 20.1	38.4 ± 21.8	<0.001
Red and processed meat (gr/day)	25.1 ± 19.3	23.7 ± 19.2	20.4 ± 15.2	19.8 ± 13.2	0.042	31.2 ± 21.3	28.4 ± 19.2	25.4 ± 14.2	22.4 ± 13.1	0.031

Data are presented as mean ± SD for continuous variables and numbers and percentages for categorical variables. [†]Obtained from ANOVA.

has also been shown to play a preventive role in the incidence of MASLD (33, 40). Furthermore, dietary intake of antioxidants is higher among individuals who have greater adherence to the AHEI (41). A high AHEI score is also associated with a lower intake of red and processed meats, which are known risk factors for MASLD (42, 43).

This study has several limitations that should be taken into consideration when interpreting the results. First, the study design was a case–control study, which is prone to several types of bias, including selection and recall bias. Although case–control studies are cost-effective and quick to conduct, they are highly vulnerable to bias. Recall bias is a particular concern in this study, as cases may recall their previous diet differently in light of their MASLD diagnosis. Additionally, dietary assessment occurred after diagnosis, which can further exacerbate recall bias. It is also possible that cases may have altered their diet before diagnosis due to early symptoms of the disease, leading to attenuation of the estimates. However, it is worth noting that MASLD-related symptoms may prompt patients to consume healthier foods rather than unhealthy ones. Furthermore, with all epidemiological studies that use food frequency questionnaire (FFQ), misclassification of study participants is unavoidable. Despite the efforts of the researchers to control for several confounding factors, the possibility of residual confounding cannot be entirely ruled out. It is important to acknowledge these limitations when interpreting the findings of this study and consider them in any future research in this area.

Conclusion

The results of our study suggest that there is a significant association between adherence to a high-diversity diet and a reduced likelihood of developing MASLD. Specifically, our findings indicate that individuals who closely followed a diverse diet had lower odds of developing MASLD. Similarly, we observed a similar association between adherence to the AHEI diet and a lower risk of MASLD. Participants who had greater adherence to the AHEI diet were found to have a lower likelihood of developing MASLD compared with those who had the lowest adherence to this dietary

TABLE 3 Risk for MASLD according to quartiles (Q) of DDS and AHEI scores (odds ratios and 95 % confidence intervals)[#].

	Quartiles of DDS					Quartiles of AHEI				
	Q1	Q2	Q3	Q4	P-trend	Q1	Q2	Q3	Q4	P-trend
Crude model	1	1.47 (0.84, 2.56)	0.73 (0.39, 1.36)	0.55 (0.28, 1.08)	0.021	1	0.55 (0.31, 0.96)	0.24 (0.12, 0.47)	0.41 (0.23, 0.74)	0.011
Model 1*	1	1.49 (0.85, 2.60)	0.72 (0.39, 1.35)	0.55 (0.28, 1.07)	0.034	1	0.55 (0.32, 0.96)	0.23 (0.12, 0.47)	0.41 (0.22, 0.74)	0.032
Model 2†	1	1.33 (0.69, 2.58)	0.67 (0.32, 1.37)	0.44 (0.19, 0.94)	0.018	1	0.47 (0.24, 0.91)	0.19 (0.08, 0.42)	0.25 (0.12, 0.55)	0.004
Model 3‡	1	1.36 (0.70, 2.64)	0.68 (0.33, 1.41)	0.41 (0.20, 0.97)	0.011	1	0.48 (0.25, 0.93)	0.19 (0.08, 0.43)	0.24 (0.12, 0.56)	<0.001

[#]Obtained from regression logistic. *Model 1: adjusted for age and sex. †Model 2: adjusted for model 1 and body mass index, smoking, and physical activity. ‡Model 3: adjusted for model 1 and body mass index, smoking, physical activity, and dietary intake of energy.

pattern. These results highlight the importance of consuming a diverse and healthy diet in reducing the risk of MASLD.

Data availability statement

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

Ethics statement

Ethical approval of this study was obtained from the Ethical Committee of Umm Al-Qura University (ID: HAPO-02-K-012-2023-02-345) Makkah, Kingdom of Saudi Arabia. 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

PR and KJ conceptualized and designed the study and supervised the project. SA participated in the data acquisition and literature review of the articles. GL analyzed and interpreted the data. PR, KJ, and LC drafted the initial manuscript. All authors approved the final 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.

Publisher's note

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Correlation between sarcopenia and cirrhosis: a meta-analysis

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Background: The relationship between sarcopenia and cirrhosis is unclear. In this research, our aim is to evaluate the prevalence of sarcopenia among individuals with liver cirrhosis and its correlation with survival and mortality risks.

Methods: We conducted searches on PubMed, Web of Science, EMBASE, and Cochrane for English articles published up to July 10, 2023, and additionally manually searched the bibliography of relevant articles. We incorporated research on sarcopenia in patients with cirrhosis to examine the connection between sarcopenia and the likelihood of survival and mortality. Statistical analyses were carried out utilizing the Stata version 15.1 software. Depending on the heterogeneity of the results, we employed either fixed-effects models or random-effects models for data synthesis. To assess publication bias, we employed funnel plots and conducted Egger's test.

Results: We included 40 studies involving 8,945 patients with cirrhosis. The overall prevalence of cirrhosis was 41% (95% CI 34%–48%). Male patients and those with liver cirrhosis and hepatic encephalopathy had a higher prevalence of sarcopenia (44% for male patients and 48% for hepatic encephalopathy patients). Sarcopenia emerged as a risk factor for both survival (HR = 2.57, 95% CI 2.02–3.27, $p < 0.001$) and mortality (HR = 2.13, 95% CI 1.86–2.44, $p < 0.001$) in patients with cirrhosis. Subgroup analyses consistently yielded the same results for study sites, whether HCC patients were excluded from the cohort, whether patients were from the liver transplant cohort or had undergone tips surgery, the definition of sarcopenia (L3-SMI or other methods), and the diagnostic criteria used by patients. The presence of sarcopenia was also a significant risk factor for hepatic encephalopathy [HR = 2.27, 95% CI (1.76–2.94), $p < 0.001$].

Conclusion: This systematic review and meta-analysis reveal that patients with cirrhosis have a prevalence of sarcopenia of 41% and is associated with survival rate and mortality rate. Therefore, we should attach importance to the screening of sarcopenia in patients with cirrhosis, early detection of susceptible populations, and appropriate measures to reduce the occurrence and adverse outcomes.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/#recordDetails.

KEYWORDS

sarcopenia, cirrhosis, hepatic encephalopathy, survival rate, mortality

1 Introduction

Cirrhosis is the advanced stage of chronic liver diseases and is mainly attributed to hepatitis B or C virus infection, alcohol consumption, non-alcoholic fatty liver disease, and autoimmune diseases (1). This disease has resulted in more than 1.3 million deaths, making it

one of the leading global causes of mortality (2). With disease progression, many complications follow, including ascites, variceal bleeding, and hepatic encephalopathy (HE), which considerably affect the prognosis of patients with cirrhosis (3). HE is defined as a spectrum of nonspecific neurological or psychiatric abnormalities ranging from subclinical alterations to coma. It is typically induced by liver failure and/or portal vein-systemic shunting. As one of the primary complications of late-stage cirrhosis, HE has an incidence rate of about 20% to 80% (4). In patients with cirrhosis, elevated ammonia concentrations, brain edema, and increased intracranial pressure can contribute to varying degrees of HE (5, 6). HE is associated with a poor prognosis, often necessitating frequent hospitalization, imposing socio-economic and psychological burdens on patients and their families, and ultimately reducing the overall survival rate (7). Therefore, it is pressingly urgent to find more risk factors for cirrhosis.

Sarcopenia is a prevalent concern in individuals diagnosed with liver cirrhosis (8). Characterized by the decline in muscle mass, strength, and physical performance (9), sarcopenia has been detected in 14% to 55% of cirrhosis patients, prompting growing interest among researchers (10). The liver holds a central position in nutrient metabolism, and the diminishment of liver functional reserves can result in a range of complications, including malnutrition and the development of sarcopenia (11). Previous studies have revealed that sarcopenia in cirrhosis patients may be attributed to liver metabolic dysfunction, reduced appetite, increased muscle autophagy, elevated serum myostatin levels, catabolic effects of systemic inflammation induced by intestinal bacterial translocation, and low testosterone levels (10). Sarcopenia in cirrhosis patients is associated with a grim prognosis (12), such as reduced quality of life, elevated hepatic venous pressure gradients, complications related to portal hypertension (such as ascites and upper gastrointestinal varices), infections (including urinary tract infections and spontaneous peritonitis) and HE (10). Reduced extrahepatic ammonia clearance in patients with sarcopenia may contribute to HE to some extent (13).

It has been demonstrated that sarcopenia affects both hepatic encephalopathy and mortality in cirrhosis patients (13), and the decline in muscle mass in cirrhosis patients for more than a year also carries unfavorable prognostic implications (14). A meta-analysis of 22 studies has confirmed that sarcopenia is an independent predictor of increased mortality in cirrhosis patients (15). The progressive and systemic loss of skeletal muscle mass and strength in patients with sarcopenia also indicates, to some extent, the poor prognosis of LC patients (16). Therefore, we have embarked on an up-to-date and more exhaustive meta-analysis to comprehensively investigate the consequences of sarcopenia in individuals with cirrhosis. We aim to appraise the survival rates and mortality rates in cirrhotic patients with sarcopenia. The secondary objectives include appraising the prevalence of sarcopenia in cirrhotic patients and examining the impact of sarcopenia on LC and HE.

2 Methods

This meta-analysis followed the updated PRISMA (2020) and MOOSE guidelines (17–19), and the study protocol was registered with PROSPERO (CRD42023458935).

2.1 Search strategy

We conducted a comprehensive search on PubMed, Embase, Cochrane, and Web of Science, spanning from the inception of these databases to July 10, 2023, with the language limited English. The combination of medical subject heading (MeSH) terms and their free-form words was used as the search strategy, such as sarcopenia [MeSH Terms] AND liver cirrhosis [MeSH Terms] OR hepatic encephalopathy [MeSH Terms]. Supplementary Table S1 provides detailed search strategies for all the included databases. We restricted our search to studies involving human subjects to maintain relevance. In our pursuit of thoroughness, we expanded our search efforts to include conference abstracts from major events such as the 2019–2020 American Association for the Study of Liver Diseases (AASLD), European Association for the Study of the Liver (EASL), Asia-Pacific Association for the Study of the Liver (APASL), Digestive Disease Week (DDW), and Asian Pacific Digestive Week (APDW) in the hopes of identifying additional research. Lastly, we conducted a manual examination of the reference lists of the studies included in our analysis and relevant systematic reviews and meta-analyses to identify any potential studies that may have been missed through our initial searches.

2.2 Inclusion and exclusion criteria

The research explored the association between sarcopenia and liver cirrhosis or liver cirrhosis accompanied by hepatic encephalopathy.

Study eligibility criteria were based on the following PICO format: P (population): people with sarcopenia and cirrhosis (patients with or without hepatic encephalopathy were recorded separately); I (intervention/predictors): NA; C (comparator): people without sarcopenia; O (outcome): correlation between sarcopenia and cirrhosis (i.e., prevalence of sarcopenia, risk of survival and death, impact of myasthenia gravis and hepatic encephalopathy); S (study design): Observational studies (i.e., longitudinal, cross-sectional, and case control studies).

The studies were excluded for the following reasons: (1) comments, editorials, letters, posters, case reports, reviews, meta-analyses, conference abstracts, guidelines, and animal experiments; (2) no clear diagnostic criteria for cirrhosis and/or sarcopenia; (3) without enough data or unavailability of full-texts; (4) not published in English.

2.3 Literature screening

The titles and abstracts were screened independently using a pre-planned list of inclusion and exclusion criteria. Information such as varying criteria for defining sarcopenia, the general health status of patients, country of origin, recruitment background (hospital or nursing home), or research environment (cohort or cross-section) was not excluded. Following the inclusion criteria, we included studies that involved the population diagnosed with liver cirrhosis (LC) and provided some original data related to sarcopenia (prevalence rate, survival rate, mortality rate, etc.). Further meta-analyses were

performed with patients stratified by the presence or absence of hepatocellular carcinoma as a covariate.

2.4 Quality evaluation

The quality of the included studies was assessed using the modified Newcastle–Ottawa scale (20). The scale consists of three sections with eight entries, which are as follows: (1) representativeness of the exposed cohort; (2) selection of the non-exposed cohort; (3) identification of exposure; (4) demonstration that no results of interest were found at the start of the study; (5) comparability of the cohorts based on design or analysis results controlled for confounders; (6) evaluation of results; (7) follow-up of sufficient duration to obtain results; and (8) appropriateness of follow-up to the population. The total score is 9 stars. Each study was independently assessed by two authors, and studies with NOS scores ≥6 were considered of high quality. Any disputed articles were referred to a third researcher, XW, for discussion and resolution of any disagreements.

2.5 Data extraction

We compiled data from each of the included studies utilizing a standardized table. The subsequent details were independently extracted by two assessors, YC and MZ: the primary author's name, publication year, study design, research location, the origin of the cirrhosis cohort (transplant waiting list or general population), the definition of sarcopenia, the methodology employed for muscle mass measurement, the number of participants, patient demographics, and clinical characteristics, including age, gender, cirrhosis etiology, hepatocellular carcinoma (HCC) presence, and pertinent outcome measures such as sarcopenia incidence, survival rate, and mortality in cirrhosis patients. When the required data were not readily accessible, we made contact with the authors to secure the essential study information.

2.6 Statistical analysis

We conducted the statistical analysis using Stata version 15.1. The forest plot illustrates the overall effect of the analysis. Heterogeneity among the studies was assessed using I^2. In general, $I^2 \geq 50\%$ indicated significant heterogeneity among the studies, leading us to adopt a random-effects model. We analyzed the source of heterogeneity through sensitivity analysis (one-by-one exclusion method). Additionally, pre-planned subgroup analyses were performed based on gender, age, etiology of cirrhosis, and study site. We employed meta-regression to determine the effects of sample size, mean age of participants, proportion of males, proportion of patients with alcohol-related liver disease, viral hepatitis, and the presence or absence of hepatocellular carcinoma (HCC) on the adjusted pooled hazard ratios (HR) for survival rate and mortality. Conversely, $I^2 < 50\%$ was considered indicative of small heterogeneity across studies. We used funnel plots and Egger's tests (21) to examine the possibility of publication bias. A p-value <0.05 (two-tailed test) was considered statistically significant.

3 Results

3.1 Literature screening process and results

Out of the 15,895 articles initially identified, we excluded 3,257 duplications and 4,339 articles classified as reviews, guidelines, letters, and animal experiments. An additional 8,199 papers were excluded based on a preliminary review of titles and abstracts. After carefully reviewing the full texts of the remaining 138 articles, we included 40 cohort studies with data from 8,945 patients (Figure 1).

3.2 Basic characteristics of the included literature

Table 1 summarizes the characteristics of all the studies encompassed in this meta-analysis. Out of the 40 studies, 18 were conducted in Asian regions (14, 22–24, 26, 29, 33, 35, 37, 38, 40, 44, 45, 48, 50, 53, 55, 59), and the remaining 22 studies were from non-Asian regions (13, 25, 27, 28, 30–32, 34, 36, 39, 41–43, 46, 47, 49, 51, 52, 54, 56–58). Among these 40 studies, 37 were cohort studies, 2 were cross-sectional studies (43, 57), and 1 was a case-control study (35). Specifically, 19 were retrospective cohort studies, and 18 were prospective cohort studies (13, 14, 27, 29, 30, 32, 33, 38–41, 49–57). The sample sizes in the included studies varied, ranging from 52 to 675 participants. In these studies, the mean age of patients ranged from 51 to 73 years. Notably, 24 studies (13, 14, 22–24, 27–29, 31, 33–35, 37, 38, 42, 44, 45, 48, 55, 57, 59) excluded all patients with hepatocellular carcinomas (HCC), while in 10 studies (28, 31, 37, 39, 46, 47, 49, 54, 55, 57), a proportion of patients ranging from 19 to 100% had HCC. Additionally, 8 studies (26, 39, 43, 46, 52, 54) neither explicitly excluded HCC patients nor reported the prevalence of HCC.

3.3 Quality evaluation

All studies were rated high quality with NOS scores ≥6. Among them, there were 5 studies (26, 28, 35, 55, 56) with 6 scores, 20 studies (13, 23, 24, 27, 32, 34, 36, 37, 39, 40, 43, 45, 47, 48, 51, 53, 54, 57–59) with 7 scores, and 15 studies (14, 22, 23, 29–31, 33, 38, 41, 42, 44, 46, 49, 50, 52) with 8 scores.

3.4 Meta-analysis

3.4.1 Association between sarcopenia and cirrhosis

Out of the 40 studies included, 34 studies (22–38, 40–45, 47–56, 58) with a total of 7,024 participants provided data on prevalence of sarcopenia, resulting in a pooled prevalence of 41% (RR = 41, 95% CI: 34%–48%, $p < 0.001$). Due to significant heterogeneity between studies ($I^2 = 97.8\%$), as depicted in Figure 2A, subgroup analyses were conducted based on gender, the definition of sarcopenia, diagnostic criteria, and etiology of cirrhosis. The results revealed variations in prevalence between these subgroups. In particular, male patients exhibited a greater overall prevalence of sarcopenia in contrast to female patients. Patients diagnosed with sarcopenia

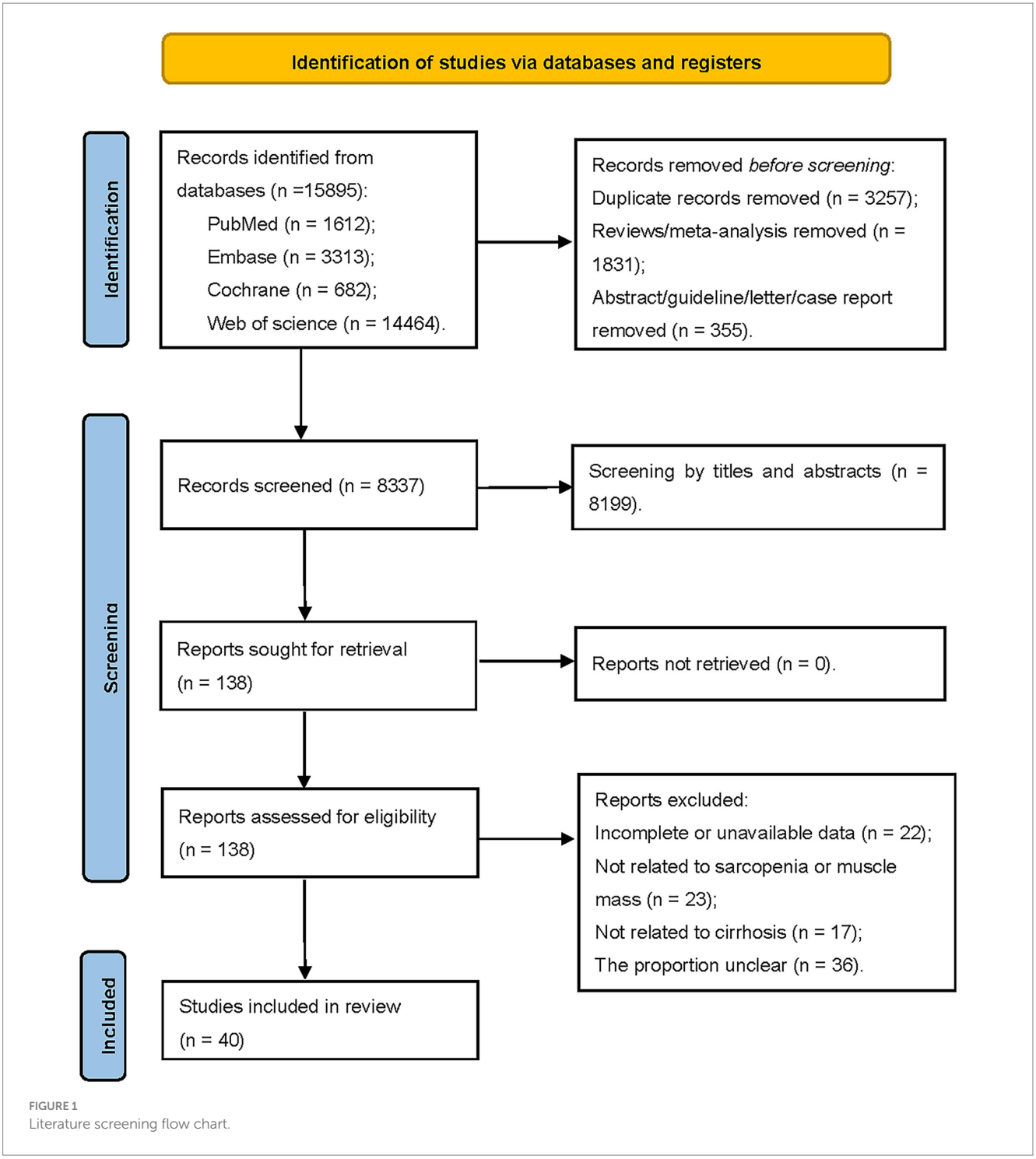

FIGURE 1
Literature screening flow chart.

according to the EWSOP2 criteria had a higher prevalence compared to those using other criteria (RR = 50, 95% CI: 44%–56%, $p < 0.001$). Furthermore, there was a higher prevalence of sarcopenia among patients in the liver transplant cohort compared to other patients (RR = 46, 95% CI: 17%–76%, $p < 0.001$). Notably, the prevalence of sarcopenia, as defined by the skeletal muscle area at the third lumbar spine (L3-SMA), was higher in the European population (RR = 67, 95% CI: 32%–100%, $p < 0.001$) compared to the Asian population (Europe, RR = 59, 95% CI: 38%–80%, $p < 0.001$; Asia, RR = 34, 95% CI: 25%–43%, $p < 0.001$). Surprisingly, the prevalence of patients with or without hepatocellular carcinoma was nearly the same (HCC, RR = 38, 95% CI: 26%–51%, $p < 0.001$; non-HCC, RR = 39, 95% CI: 31%–47%, $p < 0.001$). Furthermore, subgroup analyses indicated that factors such as gender, criteria for defining sarcopenia, methods of measuring muscle mass, patient origin, patient's country, and the inclusion of HCC patients in the cohort were not sources of heterogeneity (Table 2). The result of Egger's test indicated the presence of publication bias ($p = 0.001$).

TABLE 1 Basic characteristics of included studies.

Author (year)	Study design	Country	Source of the sample	Definition of sarcopenia	Measure muscle mass	Sample size	Age (years)	Male (%)	HCC	ALD *N* (%)	Viral hepatitis *N* (%)
Yin et al. 2023 (22)	Cohort	China	Patients underwent the TIPS	Others	CT scan/L3 TPMT	108	53.0 ± 10.8	78	N	N	74 (69)
Saeki et al. 2023 (23)	Cohort	Japan	General patients	JSH	BIA/SMI	104	72.8 ± 3.2	56	N	N	66 (64)
Saeki et al. 2021 (23)	Cohort	Japan	General patients	JSH	BIA/SMI	126	70.2 ± 3.5	61	N	47 (37)	49 (39)
Hanai et al. 2023 (24)	Cohort	Japan	General patients	JSH	CT scan/L3 SMI	239	67.9 ± 3.2	52	N	60 (25)	85 (36)
Dajti et al. 2023 (25)	Cohort	Italy	General patients	EASL	CT scan/L3 SMI	209	64.2 ± 3.5	73	40 (19)	N	121 (58)
Nardelli et al. 2022 (13)	Cohort	Italy	General patients	EASL	CT scan/L3 SMI	114	58	68	N	9 (8)	7 (6)
Kumar et al. 2022 (26)	Cohort	India	General patients	Others	CT scan L3 PMI	74	51	96	Y	7 (10)	10 (14)
Kim et al. 2022 (14)	Cohort	Korea	General patients	AASLD	CT scan/L3 SMI	595	55.4 ± 7.9	64	N	99 (21)	319 (54)
Iacob et al. 2022 (27)	Cohort	Romania	General patients	EWGSOP2	CT scan/L3 SMI	71	54.5 ± 12.6	68	N	23 (32)	42 (59)
Hentschel et al. 2022 (28)	Cohort	Germany	General patients	FLEXIT	CT scan/L3 PMI	65	60.4 ± 12.8	65	N	30 (46)	3 (5)
Zeng et al. 2021 (29)	Cohort	China	General patients	Others	CT scan/L3 SMI	480	54	60	N	60 (20)	153 (32)
Topan et al. 2021 (30)	Cohort	Romania	General patients	EWGSOP2	CT scan/L3 SMI	201	61.7 ± 9.5	63	65 (32)	111 (55)	68 (34)
Paternostro et al. 2021 (31)	Cohort	Austria	Patients underwent HVPG-measurement	Others	CT scan or MRI/L3 TPMT	203	55 ± 11	68	N	110 (54)	50 (25)
Miarka et al. 2021 (32)	Cohort	Poland	General patients	EWGSOP	CT scan/L3 SMI	98	55 ± 8	77	26 (27)	50 (51)	37 (38)
Kikuchi et al. 2021 (33)	Cohort	Japan	General patients	JSH	CT scan/L3 SMI	300	69.1 ± 11.3	58	N	74 (27)	152 (51)
Welch et al. 2020 (34)	Cohort	Ohio	General patients	Others	CT scan/L3 SMA	83	52.7 ± 9.5	71	N	N	N
Tateyama et al. 2020 (35)	Case control	Japan	General patients	JSH	CT scan/L3 SMI	99	69.5 ± 9.6	61	N	39 (39)	70 (71)
Mauro et al. 2020 (36)	Cohort	Argentina	Listed for LT (before LT)	EWGSOP2	CT scan or MRI/L3 SMI	180	58.9 ± 2.2	60	N	50 (28)	47 (26)
Feng et al. 2020 (37)	Cohort	China	General patients	EWGSOP2	CT scan/L3 SMI	492	51.1 ± 2.7	74	N	60 (12)	333 (68)
Sung et al. 2019 (38)	Cohort	Japan	General patients	JSH	CT scan/L3 SMI	166	68.0 ± 8.2	58	N	50 (30)	85 (51)
Praktiknjo et al. 2019 (39)	Cohort	Germany	Patients underwent the TIPS	Others	CT scan/ umbilicus-TPMT	186	55.5 ± 11.4	59	Y	129 (77)	24 (13)

(Continued)

TABLE 1 (Continued)

Author (year)	Study design	Country	Source of the sample	Definition of sarcopenia	Measure muscle mass	Sample size	Age (years)	Male (%)	HCC	ALD *N* (%)	Viral hepatitis *N* (%)
Ando et al. 2019 (40)	Cohort	Japan	General patients	JSH	CT scan/L3 SMI	88	67.3 ± 9.8	56	45 (51)	21 (24)	39 (44)
van Vugt et al. 2018 (41)	Cohort	Netherlands	Listed for LT (before LT)	Others	CT scan/L3 SMI	585	56.0 ± 2.3	69	193 (33)	91 (16)	52 (9)
Praktiknjo et al. 2018 (42)	Cohort	Germany	Patients underwent the tips	Others	MRI/FFMA	116	57.9 ± 12.0	59	N	73 (63)	18 (16)
Moctezuma-Velazquez et al. 2018 (43)	Cross-sectional	Canada	General patients	Others	CT scan/L3 SMI	210	57 ± 1	61	Y	54 (26)	74 (35)
Kang et al. 2018 (44)	Cohort	Korea	General patients	Others	CT scan/L3 SMI	452	51.8 ± 8.8	84	N	313 (69)	120 (27)
Jeong et al. 2018 (45)	Cohort	Korea	General patients	Others	CT scan/L3 SMA	131	53.7 ± 9.6	72	N	91 (70)	31 (24)
Bhanji et al. 2018 (46)	Cohort	Canada	General patients	Others	CT scan/L3 SMI	675	57 ± 1	67	Y	152 (22)	267 (40)
Begini et al. 2017 (47)	Cohort	Italy	General patients	Others	CT scan/L3 SMI	92	70.1 ± 11.3	71	92 (100)	22 (24)	51 (42)
Hanai et al. 2016 (48)	Cohort	Japan	General patients	Others	CT scan/L3 SMI	149	64.4 ± 11.4	55	N	34 (23)	89 (60)
Montano-Loza et al. 2015 (49)	Cohort	Canada	General patients	Others	CT scan/L3 SMI	669	57 ± 0.5	68	289 (43)	153 (23)	308 (46)
Hanai et al. 2015 (50)	Cohort	Japan	General patients	Others	CT scan/L3 SMI	130	65.4 ± 12.2	58	N	29 (22)	79 (61)
Tsien et al. 2014 (51)	Cohort	America	Listed for LT (before LT)	Others	CT scan	53	56.9 ± 7.5	77	34 (64)	12 (23)	34 (64)
Montano Loza et al. 2021 (52)	Cohort	Canada	Listed for LT (before LT)	Others	CT scan/L3 SMI	112	54 ± 1	70	Y	43 (38)	34 (30)
Anand et al. 2012 (53)	Cohort	India	General patients	Others	CT scan/L3 SMI	180	42.9 ± 9.9	79	N	47 (26)	N
Santos et al. 2019 (54)	Cohort	Brazil	General patients	ESPEN	DXA/ AMMI	261	57.0 ± 1.9	62	Y	N	N
Murata et al. 2022 (55)	Cohort	Japan	General patients	JSH	CT scan/L3 SMI	151	70 ± 10	63	N	37 (25)	88 (58)
Salman et al. 2020 (56)	Cohort	Egypt	General patients	Others	CT scan/L3 SMI	52	53.9 ± 5.0	73	52 (100)	N	28 (54)
Ebadi et al. 2020 (57)	Cross-sectional	Canada	General patients	Others	CT scan/L3 SMI	603	57 ± 0.4	68	N	73 (18)	207 (34)
Kappus et al. 2020 (58)	Cohort	America	Listed for LT (before LT)	Others	CT scan/L3 SMI	355	54.7 ± 10.8	65	95 (27)	14 (4)	209 (59)

JSH, Japan Society of Hepatology; EASL, European Association for the Study of the Liver; ESPEN, European Society of Clinical Nutrition and Metabolism; AASLD, American Association for the Study of Liver Diseases; FLEXIT, Each center is a member of the Fitness, Life Enhancement, and Exercise in Transplant; LT, liver transplant; HCC, hepatocellular carcinoma; EWGSOP2, 2019 European Working Group on Sarcopenia in Older Adults; EWGSOP, 2010 European Working Group on Sarcopenia in Older Adults; AWGS, Asian Working Group for Sarcopenia; BIA, bioimpedance analysis skeletal; SMD, muscle radiodensity; FFMA, fat-free muscle area; L3 SMA, skeletal muscle area at the L3 vertebra; SMI, muscle mass index at the L3 vertebra; L3-PMI, the psoas muscle index at the L3 vertebra; L3 TPMT, transversal psoas muscle thickness at the L3 vertebra; AMMI, appendicular muscle mass index; CT, computed tomography; L3, The third lumbar vertebra; N, There were no such patients in the study population; Y, There are such patients in the study population; TIPS, Transjugular intrahepatic portosystemic shunt.

3.4.2 Association between sarcopenia and risk of hepatic encephalopathy in patients with cirrhosis

The prevalence of sarcopenia was reported in seven studies (13, 22, 30, 35, 42, 46, 52) ($n = 426$). The results unraveled that the pooled prevalence of sarcopenia was 48% (95% CI: 27%–72%, $p < 0.001$) in patients with cirrhosis and hepatic encephalopathy (Figure 2B). The presence of hepatic encephalopathy increased the prevalence of sarcopenia. However, there was significant heterogeneity among the

studies ($I^2 = 97.6\%$). Egger's test showed no significant publication bias ($p = 0.217$).

The HR results from the other three studies (13, 22, 46) were combined, revealing that sarcopenia was a risk factor for hepatic encephalopathy (HR = 2.27, 95% CI: 1.76–2.94, $p < 0.001$). Slightly greater heterogeneity was detected between the studies ($I^2 = 53\%$) (Figure 3A). The result of Egger's test yielded a p-value of 0.323.

3.4.3 Impact of sarcopenia on the cumulative survival rate among individuals with liver cirrhosis

Ten studies (14, 23, 24, 44–46, 48, 49, 52, 59) ($n = 1{,}218$) were included in this analysis. The results revealed that sarcopenia in patients with cirrhosis was a risk factor for a 6-year survival rate (RR = 1.07, 95% CI: 1.04–1.09, $p < 0.001$), with low heterogeneity between studies ($I^2 = 45.2\%$) (Figure 3B). However, based on the result of Egger's test ($p < 0.001$), there was evidence of publication bias among the studies. Subgroup analysis by time (years) showed that sarcopenia had a more pronounced impact on survival rates in the first 4 years, which decreased with time (Figure 3C).

By analyzing the HR for cirrhosis survival and sarcopenia in seven of these studies (14, 22, 29, 42, 44, 47, 50) ($n = 1{,}973$), we found that sarcopenia was a barrier to survival (HR = 2.57, 95% CI: 2.02–3.27), with relatively low heterogeneity between studies ($I^2 = 48.7\%$) (Figure 4A).

When excluding the cohort that included HCC patients, a separate analysis of patients without HCC revealed a pooled HR of 2.44 (95% CI: 1.85–3.22). Furthermore, sarcopenia was associated with an over 2-fold reduction in survival rates in patients with liver cirrhosis, regardless of the study site, whether HCC patients were excluded from the cohort, whether patients underwent liver transplant or TIPS surgery, whether sarcopenia was defined by L3-SMI or other methods, and diagnostic criteria (Supplementary Figures S1–S3). The funnel plot is shown in Supplementary Figure S4.

3.4.4 Correlation between sarcopenia and mortality in patients with cirrhosis

The mortality in patients with cirrhosis across four studies (26, 30, 46, 56) was meta-analyzed, and the pooled mortality rate was 76% (95% CI: 62%–94%, $p = 0.011$). There was a high likelihood of heterogeneity between the studies ($I^2 = 80.7\%$) (Figure 4B). In the sensitivity analysis, we excluded one study (46) after careful examination, and the pooled corrected mortality risk ratio (RR) was similar to that of the main analysis, at 68% (95% CI: 61%–77%, $p < 0.001$). Heterogeneity was very low ($I^2 = 0\%$). The result of Egger's test was $p = 0.464$.

In a univariate analysis of data from 12 studies (13, 23, 26, 31, 33, 36, 39, 45, 46, 49, 52, 59) ($n = 1{,}781$), it was found that sarcopenia was a risk factor for mortality with a combined unadjusted hazard ratio (HR) of 2.13 (95% CI: 1.86–2.44, $p < 0.001$). The heterogeneity between studies was very low ($I^2 = 0\%$) (Figure 4C), and Egger's test revealed no publication bias in the studies and that the combined HR was reliable ($p = 0.959$). The funnel plot is shown in Supplementary Figure S5.

In all subgroups of the mortality analysis, including country, definition of sarcopenia, presence of HCC, and source of the sample, sarcopenia consistently emerged as a risk factor for mortality. Regardless of the patient's country and region, the definition standard of sarcopenia, and the method of measuring muscle mass, whether the cohort included ALD patients or excluded HCC patients, the HR for mortality in sarcopenia patients was consistently around 2 times higher (Supplementary Figures S6–S9).

4 Discussion

In this analysis of 40 studies involving 8,945 patients with cirrhosis, the prevalence of sarcopenia in the cirrhotic population was found to be higher (41%) than that (33%) reported in a previous study (60). A higher prevalence was observed in male patients (44%) and those with hepatic encephalopathy (48%). The findings also illustrated a robust correlation between sarcopenia and both the survival rate and mortality in cirrhosis patients. This correlation was substantiated by subgroup analyses based on study location, sample origin, diagnostic criteria, and methodologies. Furthermore, the analysis outcomes indicated a substantial connection between sarcopenia and hepatic encephalopathy in individuals with liver cirrhosis.

A previous systematic reviews and meta-analyses included four articles that assessed the relationship between sarcopenia and cirrhosis. However, one study only pooled retrospective cohorts of patients with sarcopenia and hepatic encephalopathy (61), another study did not include hepatic encephalopathy in the analysis (15), one study solely focused on the prevalence of sarcopenia in patients with cirrhosis (60). A different meta-analysis included a large number of patients after liver transplantation, but all included studies were retrospective observational cohort studies (62). In contrast, our study conducted a more comprehensive and extensive search, additionally analyzed hepatic encephalopathy in patients with liver cirrhosis, and incorporated approximately 50% prospective studies. To ensure the quality of the study, we rigorously controlled the inclusion and exclusion criteria for articles and performed subgroup and sensitivity analyses for all outcomes.

This research revealed that the general prevalence of sarcopenia in individuals with liver cirrhosis stood at 41%, with a notably elevated prevalence among male patients. This finding was in line with previous analyses (15, 60), which could be attributed to the predominance of males in the study samples (67.08%). Furthermore, we established a strong link between sarcopenia and an increased risk of death in cirrhotic patients. Patients with sarcopenia exhibited a higher mortality rate and a 2.13-fold elevated risk of death compared to those without sarcopenia. Sarcopenia posed a significant obstacle to survival. A previous study has demonstrated a higher incidence of complications and a reduced quality of life in patients with sarcopenia (27). The survival rate of patients with sarcopenia was 2.57 times lower than that of patients without sarcopenia. In a subgroup analysis of survival, the pooled HR was higher in patients receiving transjugular intrahepatic portocaval shunts (TIPS) than in the general population (3.71 vs. 2.35). This suggests that TIPS had a more significant impact on survival, and we hypothesized that sarcopenia played a role in worsening survival among cirrhotic patients receiving TIPS. The combined HR was also higher in the cohort containing hepatocellular carcinoma (HCC) patients compared to the cohort without HCC patients (3.38 vs. 2.31), indicating that HCC had a more pronounced effect on survival rates. This highlights the connection between sarcopenia and 5 years cumulative survival in cirrhotic patients.

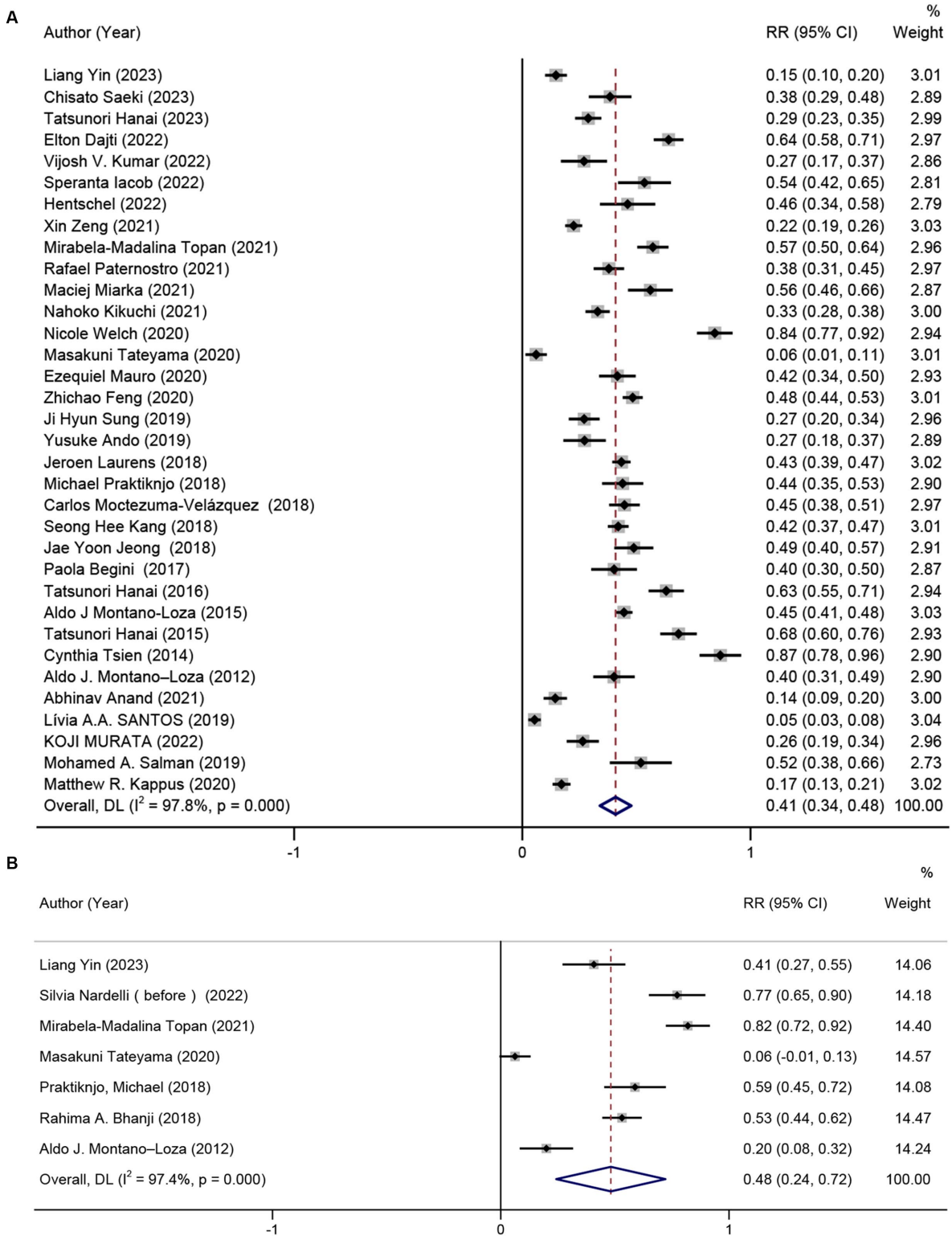

FIGURE 2
Prevalence of sarcopenia in patients with **(A)** cirrhosis, and **(B)** hepatic encephalopathy due to cirrhosis.

From a pathophysiologic point of view, sarcopenia results from an imbalance between protein synthesis and degradation caused by multiple pathways. Chronic sarcopenia is characterized by loss of muscle mass, metabolic and biochemical abnormalities, and disruption of protein homeostasis throughout the body (63). Increased hepatic gluconeogenesis in cirrhotic patients is most

TABLE 2 Subgroup analysis of the correlation between depression and Internet addiction based on gender, definition of sarcopenia, measure muscle mass, HCC and country.

Subgroup	RR	95% CI	*p*	I^2
Gender				
Men	44%	38%–51%	<0.001	95.2%
Women	35%	27%–44%	<0.001	95.0%
Definition of sarcopenia				
EWSOP2	50%	46%–53%	<0.001	67.5%
JSH	24%	21–26%	<0.001	92.6%
Others	37%	35%–38%	<0.001	97.5%
Source of sample				
General patients	41%	33%–49%	<0.001	97.9%
Patients underwent the TIPS	29%	0%–58%	<0.001	96.8%
Listed for LT (before LT)	46%	17%–76%	<0.001	98.5%
Measure muscle mass				
L3 SMA	67%	32%–100%	<0.001	97.2%
L3 SMI	40%	33%–46%	<0.001	96.6%
L3 PMI	36%	18%–55%	<0.001	82.3%
L3 TPMT	26%	4%–49%	<0.001	96.7%
HCC				
Y	38%	26%–51%	<0.001	98.3%
N	39%	31%–47%	<0.001	97.2%
Country				
Asia	34%	25%–43%	<0.001	97.8%
Europe	59%	38%–80%	<0.001	94.4%
North America	45%	36%–54%	<0.001	95.8%

EWGSOP2, 2019 European Working Group on Sarcopenia in Older Adults; JSH, Japan Society of Hepatology; EASL, European Association for the Study of the Liver; Y, There are such patients in the cohort, N, There were no such patients in the cohort; LT, liver transplant; TIPS, Transjugular intrahepatic portosystemic shunt; L3 SMA, skeletal muscle area at the L3 vertebra; SMI, muscle mass index at the L3 vertebra; L3-PMI, the psoas muscle index at the L3 vertebra; L3 TPMT, transversal psoas muscle thickness at the L3 vertebra; N, There were no such patients in the study population; Y, There are such patients in the study population.

likely due to limited hepatic glycogen content and insulin resistance, resulting in insufficient supply of branched-chain amino acids and glucose to muscle cells. In addition, recent clinical trials have found testosterone deficiency in cirrhotic patients, indicating increased muscle cell apoptosis and myogenic protein activity (64). Sarcopenia may be also induced by chronic catabolic conditions, such as cachexia due to cirrhosis, increased energy consumption, and decreased food intake due to loss of appetite. Maintaining muscle mass and avoiding rapid loss of muscle mass and transition to sarcopenia appear to be critical for the prognosis of patients with cirrhosis. Therefore, improving survival and quality of life by monitoring body composition and screening for sarcopenia should be a priority in the clinical management of cirrhosis.

Our analysis suggests that sarcopenia constitutes a risk factor for hepatic encephalopathy. In the context of hepatic encephalopathy, ammonia plays a pivotal role in the development of HE in individuals with liver cirrhosis. Previous studies have also demonstrated that the toxicity of ammonia impacts muscles and other organs. In chronic liver disease, muscles play a critical compensatory role in ammonia clearance. However, as the ammonia clearance rate decreases in cirrhotic patients, the compensatory function of patients with sarcopenia weakens. This results in the influx of ammonia into the blood in substantial quantities, greatly increasing the likelihood of hepatic encephalopathy. During hyperammonemia, the loss of muscle mass and subsequent impairment, induced by mitochondrial dysfunction and reduced adenosine triphosphate, or altered protein modification due to various factors, contribute to the onset of sarcopenia, creating a vicious cycle (65). Diagnosing hepatic encephalopathy is vital for the prognosis of patients with liver cirrhosis. However, due to equipment and personnel constraints, hepatic encephalopathy is not routinely examined in clinical practice (66). Therefore, medical staff should be attentive to such patients and implement more targeted screening and interventions for their benefit. The earlier sarcopenia is detected, the sooner the prevention and treatment programs can be initiated to prevent significant impact on HE.

The limitations of this article can be summarized as follows. First, the patient characteristics of the included studies were inconsistent, such as their demographics (i.e., the severity of and causes of cirrhosis) and methods used to determine sarcopenia. This inconsistency may have contributed to the observed heterogeneity. To address this issue, future studies should standardize diagnostic criteria as much as possible to include more patients with cirrhosis and/or sarcopenia with the same

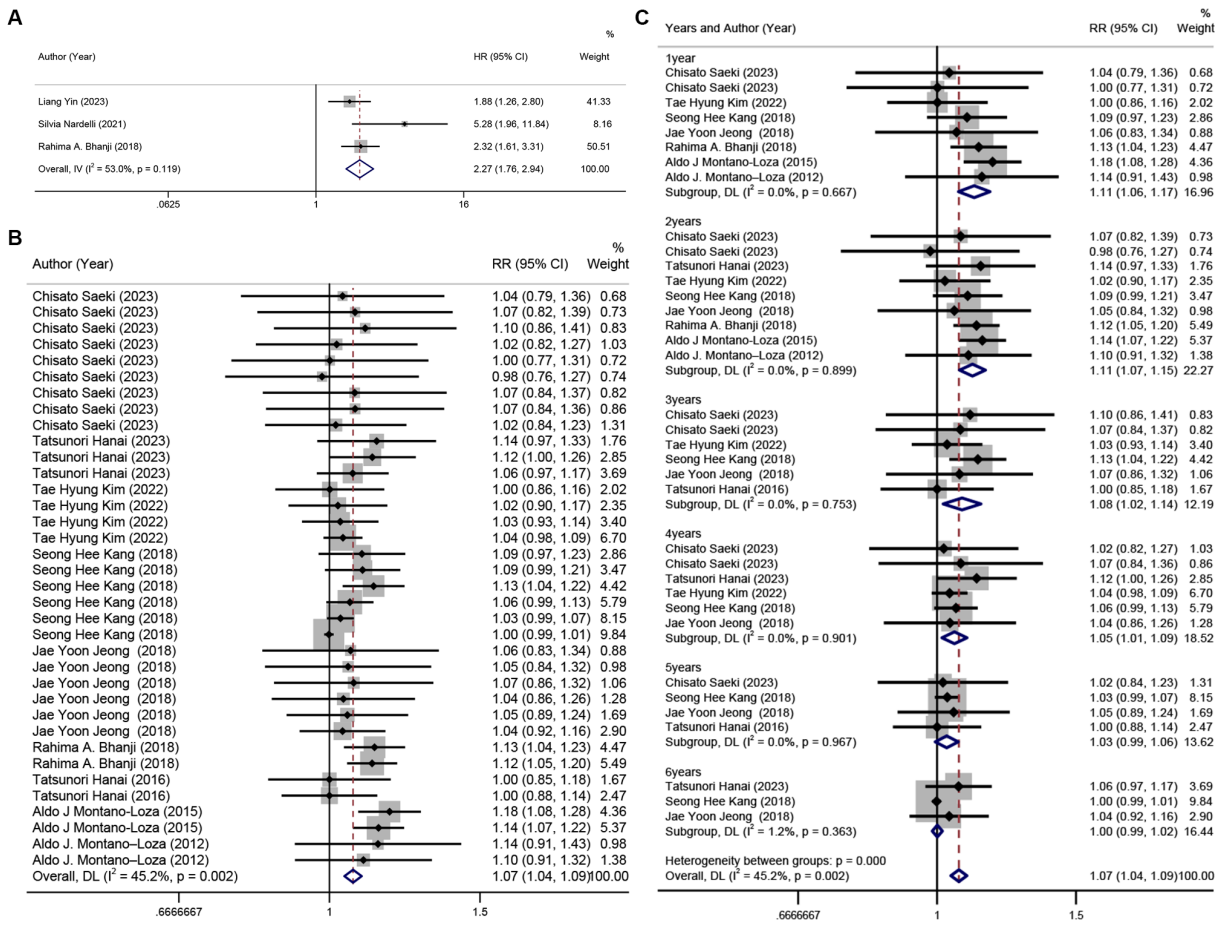

FIGURE 3
Forest plot. **(A)** univariate analysis of the incidence of hepatic encephalopathy in patients with sarcopenia and cirrhosis, **(B)** 6 years cumulative survival rate in patients with or without sarcopenia, **(C)** annual survival rate subgroups in patients with and without sarcopenia.

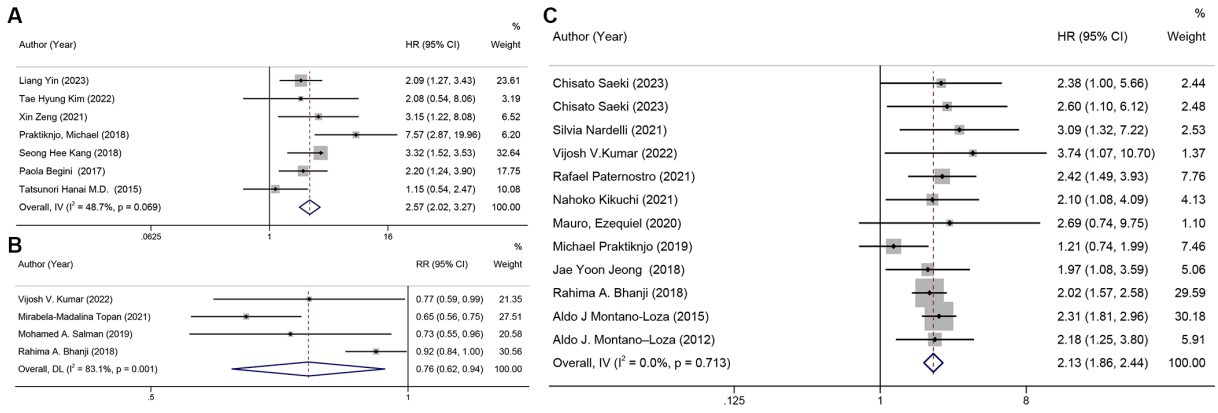

FIGURE 4
Forest plot. **(A)** univariate analysis of sarcopenia and survival rate, **(B)** mortality in patients with sarcopenia and cirrhosis, **(C)** univariate analysis of mortality in patients with sarcopenia and cirrhosis.

characteristics. Second, the majority of the studies included in our analysis were observational cohort studies, potentially introducing bias due to variations in statistical methods, diagnostic criteria, cut-off values for defining sarcopenia, and the distribution of viral liver disease, alcoholic liver disease, and the percentage of cases involving hepatocellular carcinoma (HCC). We attempted to mitigate these limitations through subgroup and sensitivity analyses. Nevertheless, it is imperative to adopt standardized definitions and criteria when conducting meta-analyses of individual sarcopenia data to better elucidate the prevalence of sarcopenia and provide a more comprehensive description of cirrhosis patients with sarcopenia. Finally, we only included studies

published in English and may have excluded relevant studies published in other languages, so there may be biases.

In conclusion, this systematic review and meta-analysis establishes that patients with cirrhosis have a prevalence of sarcopenia of 41%, with up to half of these individuals developing cirrhosis due to either alcoholic liver disease or viral hepatitis. Moreover, sarcopenia is closely associated with the survival and mortality rates in patients with cirrhosis, the study reveals that sarcopenia is linked to a greater than twofold rise in the risk of mortality and a decline in survival rates across most subgroups. Hepatic encephalopathy may interact with sarcopenia in patients with cirrhosis. Based on our comprehensive analysis, sarcopenia should be included as an integral component of the initial assessment for all cirrhosis patients.

Data availability statement

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

Author contributions

YC: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. MZ: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. JG: Conceptualization, Formal analysis, Investigation, Writing – review & editing. JJ: Conceptualization, Formal analysis, Investigation, Writing – review & editing. HW: Conceptualization, Supervision, Writing – review & editing. XW: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1342100/full#supplementary-material

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Natural products and dietary interventions on liver enzymes: an umbrella review and evidence map

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Background: The association between natural products and dietary interventions on liver enzymes is unclear; therefore, this study aimed to examine their effects on liver enzymes in adults.

Methods: PubMed, Embase, and Cochrane Library of Systematic Reviews databases were searched from inception until March 2023. The Assessment of Multiple Systematic Reviews-2 (AMSTAR-2) and Grading of Recommendations Assessment, Development, and Evaluation (GRADE) systems were used to assess the methodological and evidence quality, and the therapeutic effects were summarized in a narrative form.

Results: A total of 40 meta-analyses on natural products (n = 25), dietary supplements (n = 10), and dietary patterns (n = 5) were evaluated, and results were presented in a narrative form. The overall methodological quality of the included studies was relatively poor. The results indicated that positive effects were observed for nigella sativa, garlic, artichoke, curcumin, silymarin, vitamin E, vitamin D, L-carnitine, propolis, and polyunsaturated fatty acids on certain liver enzymes. The dietary patterns, including high-protein, Mediterranean, and calorie-restriction diets and evening snacks, may reduce liver enzymes; however, other supplements and herbs did not reduce liver enzyme levels or have minimal effects. The evidence quality was generally weak given the risk of bias, heterogeneity, and imprecision.

Conclusion: This umbrella review suggests that natural products and dietary interventions have beneficial therapeutic effects on liver enzymes levels. Further clinical trials are necessary to establish the effectiveness of supplements that reduce liver enzymes.

KEYWORDS

natural products, dietary interventions, liver enzymes, umbrella review, evidence map

1 Introduction

The liver is an important metabolic organ and is rich in enzyme systems that play an important role in the metabolism and protein synthesis in the body (1). Specifically, it is applied to degrade toxins, secrete bile, store glycogen, and metabolize drugs (2). These physiological functions can be disrupted by liver disease and/or drug use (3, 4). Several serum liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamine transferase (GGT), and alkaline phosphatase (ALP), are highly sensitive to liver dysfunction and injury (5, 6). When hepatocytes are damaged enzymes within the cytoplasm are released, raising serum enzyme activity, which can reflect various pathological conditions in the liver. Although chronic liver injury patients are usually asymptomatic, the liver enzymes appear generally significantly higher (7). In recent decades, several studies have indicated that liver enzyme disorders are associated with various diseases, such as chronic obstructive pulmonary disease (8), metabolic diseases (9), cardiovascular disease (10), and type 2 diabetes mellitus (T2DM) (11). Meanwhile, liver enzyme disorders are a significant predictive factors for increased patient mortality (12). Hence, there is an urgent clinical need to improve liver dysfunction.

The application of complementary and alternative therapies has increased in recent years, as nearly half of the US population is reported using at least one dietary supplement, and 10% of the participants used at least four dietary supplements (13–15). Similarly, the use of herbal medicines has also increased in several Asian countries (16). Another study reported similar percentages of herbs used as complementary and alternative medicines to treat chronic liver disease (17, 18). The herbal and dietary supplements have been found to promote liver function (19, 20). For example, silymarin acts as a free radical scavenger and has been reported to modulate elevated liver enzymes (21). Similarly, resveratrol reduces diet-induced liver fat accumulation via increased fatty acid oxidation and lipogenesis reduction, exerting hepatoprotective effects in liver injury, and thus, could be used to develop hepatoprotective drugs (20, 22). Natural products also offer a significant supply of antioxidants that can regulate liver enzymes, are affordable, have fewer side effects, and are more easily accessible than their synthetic counterparts (23, 24).

In recent decades, many observational studies and randomized controlled trials (RCTs) focusing on the association between supplement intake and liver enzymes levels have been published (25, 26). Thus, before developing consensus and guideline policies, a comprehensive assessment of the quality of available evidence on the association between natural products and dietary supplements and liver injury outcomes is required. It is imperative to conduct an integrated review of various herbs and dietary interventions since not all are reported to have beneficial effects on liver function (4). Therefore, to summarize the evidence of the effect of natural products and dietary interventions on liver enzymes, a comprehensive review of meta-analyses was conducted to help elucidate their effects in regulating liver enzyme status.

2 Materials and methods

Umbrella reviews are systematic searches, integrating and evaluating available evidence on specific exposure factors and health outcomes in systematic reviews and/or meta-analyses (27). The results of natural products, dietary supplements, and dietary patterns on liver enzymes in the meta-analysis were reviewed, excluding systematic reviews without meta-analysis.

2.1 Literature search

PubMed, Embase and Cochrane Library of Systematic Reviews databases were searched from their inception to March 2023 to identify meta-analyses of natural products, dietary supplements, and dietary patterns on liver enzymes. We used the following search terms: (alanine transaminase or aspartate aminotransferases or gamma-glutamyl transferase or ALS or AST or GGT or ALP or liver enzymes or liver function) and (systematic review or meta-analysis) (see **Supplementary Table 1**). Two reviewers (J.W. and J.J.S.) independently screened titles and/or abstracts, and selected potential articles for full-text review. Then, they independently reviewed the full article for eligibility. In case of any disagreement, a third reviewer (Y.D.W) was engaged to resolve the issue. Moreover, references for eligible articles were manually searched to identify additional studies that fulfilled the inclusion criteria.

2.2 Inclusion and exclusion criteria

All articles that were meta-analyses and conducted using systematic reviews were deemed eligible for the study. The specific criteria for inclusion regarding population, interventions or exposures, comparators, outcomes, and study design were as follows: (1) Participants: adult subjects with or without any health condition; (2) Interventions: oral natural products, dietary supplements as well as dietary patterns; (3) Controls: placebo, no treatment, and conventional treatments; (4) Outcomes: serum liver enzymes, including ATL, AST, GGT, and ALP levels; (5) Study type: meta-analysis based on RCTs. Non-human studies, genetics studies, original studies, and conference abstracts were excluded. Similarly, meta-analyses with fewer than two primary studies and studies on combinations of multiple herbs were also excluded. The most recent, most significant, and updated meta-analyses were preferred if multiple meta-analyses were performed on the same interventions and outcomes (28). When the most recent meta-analysis was not the largest in number, one that included more primary studies was selected.

2.3 Data extraction

The data from eligible articles were extracted by two independent reviewers (J.W. and Y.P.Z.). The extracted data included the first author, publication year, study population, number of RCTs, sample size, supplements (herbs and dietary) and dietary patterns, dosage, frequency of administration, form of intervention, treatment duration, registration information, risk of

bias tool, and outcomes of interest. Any discrepancies in the data extraction were resolved by consensus.

2.4 Assessing the methodological quality and quality of evidence

The methodological quality of the meta-analysis was evaluated by the Assessment of Multiple Systematic Reviews (AMSTAR)-2 checklist, which is a validated and reliable tool for appraising systematic reviews and interventional and observational meta-analyses (29). AMSTAR-2 checklist contains an assessment on search, analysis, and transparency of meta-analysis, and classifies studies as "high," "moderate," "low," and "critically low" quality.

Similarly, the Grades of Recommendations, Assessment, Development, and Evaluations (GRADE) system was used to assess the quality of evidence for outcomes (30), which provides clear criteria for grading the quality of the evidence, including bias risk, imprecision, inconsistency, indirectness, and publication bias. An evidence map was developed to present the plausible benefits of each intervention and the certainty of evidence. Based on the GRADE system (GRADEpro GDT),[1] the certainty of the evidence was categorized into "high," "moderate," "low," or "very low" quality.

2.5 Statistical analysis

The results were generated using a narrative approach (31), including the risk ratio (RR), odds ratio (OR), mean difference (MD), weighted mean difference (WMD), standardized mean difference (SMD), 95% confidence interval (CI), and *p*-value. The heterogeneity of each meta-analysis is presented by I^2 statistics, and the cutoff values for "low," "moderate," and "high" heterogeneity were 25, 50, and 75%, respectively. If the included meta-analyses present subgroup analyses were conducted based on the factors such as sex, age, health status, country, dose, and study duration, they will be reported. In addition, the publication bias was also investigated using funnel plots, Begg's or Egger's regressions. Data were analyzed using Excel 2016 (Microsoft Corporation, WA, USA).

3 Results

3.1 Literature screening results

The preliminary search identified 8,043 potential articles, and after removing duplicates, 6,274 records remained. Subsequently, 6,112 articles were excluded after screening the titles and abstracts. The full texts of the 162 selected records were further evaluated, and the final 40 articles (32–71) were included for analysis, as revealed in **Figure 1**, displaying the study selection flowchart.

1 https://gdt.gradepro.org/app/

3.2 Basic characteristics

The basic characteristics of included meta-analysis are presented in **Table 1**, where all published meta-analyses in peer-reviewed journals between 2013 and 2023 are given. The included meta-analyses were distributed across ten geographic regions, including 20 from Iran, ten from China, two from Greece and Korea, and one from Pakistan, Malaysia, Canada, the United States, the United Kingdom, and Egypt. The number of RCTs included in the meta-analysis ranged from 2 to 26, with subjects between 150 and 3,637, respectively. Twenty-five meta-analyses were found to have used only placebo controls, with the remaining studies using placebo, no treatment, or conventional regimens as controlled treatments. Moreover, seven meta-analyses reported dosing frequencies that ranged from two to three times daily. The treatment period ranged from 2 weeks to 5 years. Sixteen meta-analyses registered their protocols on public platforms, whereas 24 lacked statements. For the risk bias assessment, 35 meta-analyses used the Cochrane risk of bias, three used the Jadad scale, and two were not evaluated in this domain.

3.3 Quality assessment

The results of the overall methodological quality evaluated using the AMSTAR-2 checklist are presented in **Supplementary Table 2**. The analysis revealed that only two studies were of moderate quality, while 12 studies were of low quality, and the remaining 26 were of critically low quality. Methodological quality limitations included item two (57.5% of studies failed to register protocols before conducting the review), item three (95% of studies failed to explain the selection of the study designs in the review), item seven (77.5% of studies did not provide a list of excluded studies and justify the exclusions), and item 10 (95% of studies did not report the source of funding for the individual studies) (**Supplementary Table 2**).

3.4 Effect of natural products and dietary interventions on liver enzymes

3.4.1 Natural herbs

The effects of eight natural herbs, including cinnamon, grape, ginseng, nigella sativa, green coffee beans, garlic, green tea, artichoke, saffron, and sumac, as dietary supplements, were analyzed in the included meta-analysis. Compared with placebo, cinnamon supplementation had no significant effect on serum ALT, AST, and ALP levels (51). However, subgroup analyses found that doses of <1,500 mg/day or duration longer than 12 weeks, as well as T2DM, and elderly patients, demonstrated significantly reduced ALT levels (51). The beneficial effects of cinnamon consumption on serum AST levels have been observed in patients with T2DM and a follow-up period of more than 12 weeks (51). Compared with placebo, grape products failed to reduce the serum concentrations of ALT and AST levels (58). However, the subgroup analysis indicated that grape products effectively reduced ALT and AST levels when consumed for ≥12 weeks (58). There was consistent evidence showing that ginseng failed to reduce ALT, AST, GGT,

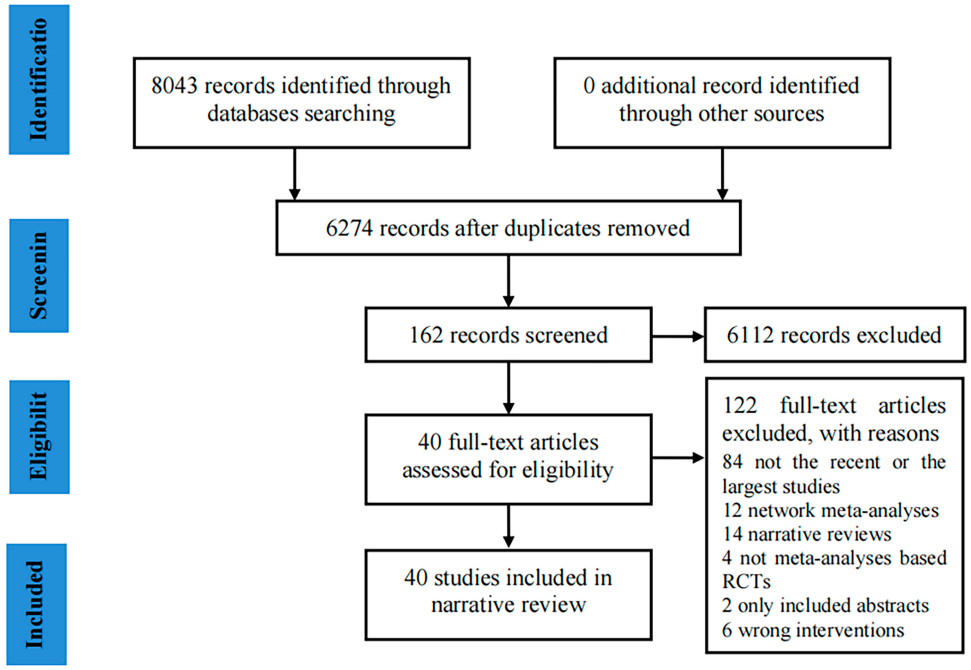

FIGURE 1
Flow chart for the included studies.

or ALP concentrations compared to placebo, while no significant changes in the overall effect of ginseng supplementation were observed during subgroup analysis (40). Similarly, green coffee bean supplementation failed to affect serum ALT, AST, and ALP levels compared to placebo in adults (45). Compared to placebo, saffron supplementation did not appear to improve AST, ALT, or ALP levels in adult (50).

In non-alcoholic fatty liver disease (NAFLD) patients, nigella sativa significantly reduced AST and ALT levels compared to placebo (54) and was also found to reduce ALP levels while not affecting AST and ALT in adults (44). Similarly, the garlic group depicted a reduction in ALT and AST levels in NAFLD patients compared to the placebo group (66). However, compared to placebo, it significantly reduced AST levels but did no significant affect ALT levels in adults (42). Compared to placebo, the overall effect of green tea supplementation on the ALT, AST, and ALP was non-significant (41), while subgroup analysis demonstrated that it reduced the AST and ALT levels in NAFLD patients, but a slightly significant increase in liver enzymes was observed in healthy subjects (41). Compared to controls, artichokes significantly reduced AST and ALT levels in obese liver patients, but did not affect ALP levels (61). In addition, sumac consumption also had non-significant effects on AST and ALT levels in patients with metabolic syndrome and related disorders (47).

3.4.2 Phytonutrients

The efficacy of phytonutrients, including curcumin, berberine, anthocyanin, silymarin, conjugated linoleic acid, and resveratrol, in modulating liver function was assessed. The results demonstrated that curcumin supplementation reduced serum ALT and AST levels in NAFLD patients compared to placebo (64). Subgroup analysis revealed that only curcumin doses less than 500 mg/day reduced serum liver enzymes (64). Similarly, berberine was found to effectively reduce serum AST and ALT levels in NAFLD patients compared to controls (35), but no such effect was observed when berberine was compared to placebo (39). Betaine does not affect liver enzymes, including ALT, AST, and GGT levels (57). Similar results were observed with anthocyanin supplementation, which did not indicate a significant effect on ALT and AST levels compared to placebo (69), which was found to be not associated with dose and treatment duration (69).

Silymarin has been revealed to have hepatoprotective effects on anti-tubercular drug-induced liver enzymes compared to placebo, which could reduce serum ALT, AST, and ALP levels and significantly improve liver function (38). In patients with hepatitis B, it was found to reduce serum AST and ALT levels as effectively as the hepatoprotective drugs, which were significantly lower in the silymarin combined with hepatoprotective drug group than in the hepatoprotective drug group (32). However, oral silymarin on ALT was not different from placebo in patients suffering from chronic hepatitis C (33). The treatment with silymarin was more efficacious than placebo therapy in decreasing ALT and AST levels in patients with NAFLD (49).

Conjugated linoleic acid (CLA) supplementation failed to alter ALT and AST levels compared to placebo (59). However, the effect of CLA on ALT and AST in the unhealthy group was significant in subgroup analysis (59). Resveratrol supplementation did not significantly affect liver biomarkers, including ALT, ALP, AST, and GGT levels, in adult participants (71). However, the subgroup analysis suggested that ALT and GGT levels were significantly lower in patients with liver disease following resveratrol supplementation (71). And high-dose resveratrol supplementation (> 1,000 mg/day) increased ALP and ALT levels in the elderly (≥60 years) (71). In addition, compared to placebo, resveratrol intake did not

TABLE 1 The basic characteristics of included studies.

References	Country	Health status	Interventions/ comparations	Number of primary studies	Sample size	Dose	Frequency	Form	Duration	Registration information	Bias of risk assessment	Reported outcomes	Safety
(51)	Iran	Adults (T2DM = 3, RA = 1, Prediabetes = 1, NAFLD = 1, Impaired glucose tolerance = 1)	Cinnamon/Placebo	7	256	120–10,000 mg/d	Twice = 1, Third = 2 NR = 4	Water soluble extract = 1, capsules = 6	4–13 weeks	OSF (ID: https://doi.org/10.17605/OSF.IO/XSNYW)	Cochrane	AST, ALT, ALP	NR
(71)	Iran	Adults (Healthy individuals = 5, obese patients with metabolic disorders = 20, NAFLD and liver diseases = 8, heart diseases = 2, chronic obstructive pulmonary disease = 1, osteoarthritis = 1)	Resveratrol/Placebo	37	2,114	8–3,000 mg/d	NR	NR	4–48 weeks	PROSPERO (CRD42022326132)	Cochrane	AST, ALT, ALP, GGT	NR
(70)	Canada	Adults (NIDDM = 1, T1DM/T2DM = 2, Healthy = 4, hypertension = 2, hyperlipidemia = 1)	Non-nutritive sweetener/Placebo	10	854	30–1,800 mg/d	NR	NR	4 weeks to 104 weeks	PROSPERO (CRD42021250067)	Cochrane	AST, ALT, GGT	NR
(58)	Pakistan	Adults (Mets = 1, T2DM = 1, hypertension = 1, cardiovascular disease = 2, healthy adults = 1, overweight/obese = 2)	Grape products/Placebo	8	291	200–60 g/d	NR	Powder = 3, extract = 5	3 weeks to 12 months	No	Cochrane	AST, ALT	NR
(64)	Malaysia	NAFLD	Curcumin/Placebo	16	1,028	50–1,500 mg/d	NR	Capsules = 16	8–24 weeks	PROSPERO (CRD42021229160)	Cochrane	ALT, AST	Yes
	Iran	Adults	Almond/Placebo, no intervention	26	1,750	5 and 85 g/d	NR	NR	4 and 20 weeks	No	Cochrane	ALT, AST, GGT	NR
	China	Metabolic disorders (T2DM = 4, NAFLD = 3, dyslipidemia = 1, hyperlipidemia = 3, pre-diabetes or early untreated diabetes = 1)	Anthocyanins/ Placebo	12	893	0.77–600 mg/d	NR	Extract = 7, purified anthocyanins = 3, Juice = 1, blueberry = 1	4–12 weeks	No	Cochrane	AST, ALT	NR

(Continued)

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TABLE 1 (Continued)

References	Country	Health status	Interventions/ comparations	Number of primary studies	Sample size	Dose	Frequency	Form	Duration	Registration information	Bias of risk assessment	Reported outcomes	Safety
(57)	Iran	Adults	Betaine/Control	12	528	1.5–20 g/day	NR	NR	2–52 weeks	No	Cochrane	AST, ALT, GGT	NR
(66)	Iran	NAFLD	Garlic/Placebo	2	186	800 mg to 1.6 g/d	NR	Powder = 2	12–15 weeks	Tabriz University of Medical Sciences (ID 38774)	Cochrane	AST, ALT	No
(62)	China	Individuals with any health condition (Fatty liver disease = 1, T2DM = 1, postmenopausal women = 1, Breast cancer = 1)	Tocotrienols/Placebo	4	437	400 mg to 860 mg/d	Twice = 3, NR = 1	NR	8 weeks to 5 years	No	Cochrane	AST, ALT	NR
(59)	Iran	Adults (Obese or overweight = 13, healthy adults = 4, postmenopausal women with T2DM = 1, NAFLD = 1)	Conjugated linoleic acid/Placebo, control diet	19	848	1.17–10.8 g/d	NR	NR	3–104 weeks	PROSPERO (CRD42022331111)	Cochrane	AST, ALT	NR
(65)	Korea	Chronic Liver Disease (Chronic hepatitis = 6, NASH = 1, NAFLD = 2, NAFLD with T2DM = 2, hepatic Encephalopathy = 3)	L-Carnitine/Placebo, no L-Carnitine	14	1,217	600 mg to 4 g/d	NR	NR	NR	PROSPERO (CRD42022332856)	Cochrane	AST, ALT	NR
(1)	USA	Adults	High-protein diet/standard protein diet, conventional diet	5	558	NR	NR	NR	12 weeks to 12 months	PROSPERO (CRD42019140511)	Cochrane	AST, ALT	Yes

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TABLE 1 (Continued)

References	Country	Health status	Interventions/ comparations	Number of primary studies	Sample size	Dose	Frequency	Form	Duration	Registration information	Bias of risk assessment	Reported outcomes	Safety
(67)	Iran	Adults (NAFLD = 6, obstructive sleep apnea = 1, obese patients with diabetes = 1, subjects with high cardiovascular Risk = 1, CVD = 1)	Mediterranean diet/low-fat diet, energy restriction diet, American Diabetes Association diet and without any dietary treatment	10	705	NR	NR	NR	1.5–72 months	PROSPERO (CRD42021233214)	Cochrane	AST, ALT, GGT	NR
(60)	UK	NAFLD	Mediterranean diet and calorie restriction/Standard care	21	3,037	NR	NR	NR	2–104 weeks	PROSPERO (CRD42019118537)	Cochrane	AST, ALT	NR
(61)	Egypt	Fatty Liver	Artichoke/Control	5	333	150–2,700 mg/d	NR	Tablet = 3; powder extract = 2	4–24 weeks	PROSPERO (CRD42020182502)	Cochrane	AST, ALT, ALP	NR
(45)	Iran	Adults (hypertension = 2, Obese = 1, xerotic skin = 1, NAFLD = 1, hypercholesterolemic = 1, health = 2, Mets = 1)	Green coffee bean extract/Placebo	8	NR	50–1,200 mg/d	NR	NR	8–12 weeks	No	Cochrane	ALT, AST, ALP, GTP	NR
(54)	China	NAFLD	Nigella sativa/Placebo	5	358	75 mg to 2 g/d	Twice = 2, tid = 1, per = 2	Powder = 3; oil = 2	8–24 weeks	No	Cochrane	AST, ALT	Yes
(48)	Iran	NR	Propolis/Placebo	5	313	500 mg to 1,000 mg	NR	NR	8 week to 24 months	No	NR	AST, ALT	NR
(47)	Iran	Mets and related disorders (Hyperlipidemia = 1, NAFLD = 1)	Sumac fruit/Placebo	2	150	1,000 to 2,000 mg/d	NR	NR	6–12 weeks	PROSPERO (CRD42021227425)	Cochrane	AST, ALT	NR
(53)	Iran	NAFLD	Vitamin D/Placebo	16	657	NR	NR	NR	4–48 weeks	No	Cochrane	AST, ALT, ALP, GGT	NR

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TABLE 1 (Continued)

References	Country	Health status	Interventions/ comparations	Number of primary studies	Sample size	Dose	Frequency	Form	Duration	Registration information	Bias of risk assessment	Reported outcomes	Safety
(55)	Greece	Adults (NAFLD = 4, NASH = 3)	Vitamin E/Placebo	7	465	400 to 800 mg/d	Twice = 2, NR = 5	NR	8–96 weeks	OSF (https://osf.io/w4fh3/)	Cochrane	AST, ALT	NR
(49)	Greece	NAFLD	Silymarin/Placebo	8	622	140 to 2,100 mg/d	NR	NR	8–48 months	OSF (https://osf.io/2b54z/)	Cochrane	AST, ALT	NR
(52)	Iran	NAFLD	Resveratrol/Placebo	5	216	300 to 1,500 mg/d	NR	NR	12–24 weeks	No	Cochrane	AST, ALT, ALP, GGT	NR
(50)	Iran	Adults (Schizophrenia = 1, depressive disorder = 1, diabetes = 4, NAFLD = 2, and healthy populations = 2)	Saffron/Placebo	12	608	30 to 100 mg/d	NR	Tablets = 5, capsule = 6, powder = 1	4–12 weeks	No	Cochrane	ALT, AST, ALP	NR
(46)	Iran	T2DM	Chromium/placebo	3	160	400 to 1,000 μg/d	NR	NR	12–25 weeks	No	Cochrane	ALT, AST	NR
(56)	China	NAFLD	Soy diet/control	3	172		NR	NR	8 weeks	No	Jadad Scores	AST, ALT	NR
(40)	Iran	Adults (T2DM = 4, healthy subjects = 4, NAFLD = 1, postmenopausal women = 1, ovarian cancer = 1, Mets = 1, overweight and obese adults = 1, ischemic stroke = 1)	Ginseng/Placebo	14	992	0.75–6 g/day	NR	NR	2–24 weeks	No	Cochrane	ALT, AST, GGT, ALP	NR
(39)	Iran	NR (Allograft renal transplant operation = 1, Low cardiovascular risk = 1, NAFLD = 1, T2DM = 1)	Berberine/Placebo	4	430	600 to 1,500 mg/d	NR	NR	1 to 4 months	No	Cochrane	AST, ALT	NR

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TABLE 1 (Continued)

References	Country	Health status	Interventions/ comparations	Number of primary studies	Sample size	Dose	Frequency	Form	Duration	Registration information	Bias of risk assessment	Reported outcomes	Safety
(44)	Iran	Adults (Stable angina = 1; hypertension = 2; kidney disease = 2; T2DM = 2; Obese = 3; Liver diseases = 2; healthy individuals = 4; osteoporosis = 1; hypercholesterolemia = 1; Menopause women = 1)	Nigella sativa/Placebo	19	1,295	500 mg to 6,000 mg/d	NR	Oil = 12; powder = 7	28 to 294 days	PROSPERO (CRD42018102229)	Cochrane	ALT, AST, ALP	NR
(42)	Iran	NR (Adults with elevated serum Gamma-Glutamyl Transferase = 1, coronary artery diseases = 1, mild hypercholesterolemia = 1, uncontrolled hypertension = 1, peritoneal dialysis patient = 1, arsenical palmer keratosis = 1)	Garlic/Placebo	6	301	10 mg to 2.4 g/d;4 ml/d	NR	Capsule = 1, powder = 1, tablet = 2, extract = 2	8 weeks to 1 year	No	Cochrane	AST, ALT	NR
(41)	Iran	Adults (NASH = 1, NAFLD = 4, T2DM = 3, healthy subjects = 2, obese and overweight = 4, postmenopausal women = 2, hypercholesterolemia = 1)	Green tea/Placebo	17	NR	300 mg to 1,500 mg/d	NR	NR	3 to 48 weeks	No	Cochrane	AST, ALT, ALP	NR

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TABLE 1 (Continued)

References	Country	Health status	Interventions/ comparations	Number of primary studies	Sample size	Dose	Frequency	Form	Duration	Registration information	Bias of risk assessment	Reported outcomes	Safety
(43)	Iran	Adults (Thyroid patients = 2, T2DM with NAFLD = 1, NAFLD = 1, cystic acne = 1, NASH = 4, patients with suspected acute myocardial infarction = 1, cirrhotic = 1, Healthy = 3, hepatocellular carcinoma = 1, hemodialysis = 2, obese = 1)	L-carnitine/Placebo, no treatment	18	1,161	500 to 4,000 mg/d	Twice = 1, NR = 17	NR	2 to 48 weeks	Shahid Sadoughi University of Medical Sciences (IR.SSU.SPH. REC.1398.016).	Cochrane	AST, ALT, ALP, GGT	NR
(38)	China	Antituberculosis Drug-Induced Liver Injury	Silymarin/Placebo	3	747	210 to 420 mg/d	Tid = 1, bid = 2	NR	2 to 8 weeks	No	Cochrane	AST, ALT, ALP	Yes
(36)	South Korea	NAFLD	Low carbohydrate diet/low fat diet group	5	165	NR	NR	NR	2 weeks to 6 months	No	NR	AST, ALT	NR
(37)	China, Taiwan	Liver cirrhosis	Late evening snacks/non-late evening snacks	6	241	200 to 320 kcal.	09:00 PM and 10:00 PM	NR	2 weeks to 12 months	No	Cochrane	AST, ALT	NR
(34)	China	NAFLD	Omega-3 Fatty Acid/Placebo, no treatment	8	530	830 to 9,000 mg/d	NR	Capsule = 8	8 weeks to 18 months	No	Jadad Scores	ALT, AST, GGT	NR
(35)	China	NAFLD	Berberine/lifestyle intervention, other medicines	5	467	0.9–1.5 g/d	Tid = 5	NR	12 to 16 weeks	No	Cochrane	AST, ALT	NR
(33)	China	Chronic hepatitis C virus infection patients	Silymarin/Placebo	2	2,012	600 to 1,260 mg/d	NR	NR	12 to 24 weeks	No	Cochrane	ALT	Yes
(32)	China	Chronic hepatitis B patients	Silymarin and its combination therapy/Placebo	6	603	NR	NR	NR	1 to 3 months	No	Jadad scale	AST, ALT	Yes

T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; RA, rheumatoid arthritis; NASH, non-alcoholic steatohepatitis; NR, not reported.

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significantly change ALT, AST, GGT, or ALP levels in NAFLD patients (52).

3.4.3 Micronutrients

The effects of five micronutrients–tocotrienol, vitamin E, vitamin D, chromium, and l-carnitine–were analyzed in this review. The results demonstrated that tocotrienol intake was not associated with serum ALT and AST levels compared to placebo (62), whereas reduced ALT and AST levels were observed with vitamin E compared to placebo (55). Similarly, vitamin D supplementation decreased ALT levels, but no significant changes in AST, ALP, and GGT levels in NAFLD patients were observed, compared to placebo (53). Chromium did not affect AST and ALT levels in T2DM patients compared to placebo (46), while l-carnitine significantly reduced serum ALT, AST, and GGT levels compared to placebo or no treatment (43). Subgroup analysis revealed that l-carnitine supplementation had beneficial effects in lowering enzymes when administered at higher doses (≥2,000 mg/day), longer treatment duration (>12 weeks), and in patients with liver disease (43). In patients with chronic liver disease, l-carnitine significantly reduced ALT and AST levels compared with the control group (65).

3.4.4 Dietary patterns

The effects of six dietary patterns, including high-protein, low-carbohydrate, Mediterranean, calorie-restricted, and soy diets, and nighttime eating habits, were examined in the included meta-analysis. The high protein diet group revealed a significant reduction in AST levels in adults, while ALT levels remained independent (68). Similarly, the low-carbohydrate diet demonstrated non-significant differences in AST and ALT levels in NAFLD patients compared to the low-fat diet (36). In contrast, the Mediterranean diet significantly reduced AST and GGT levels but did not substantially affect ALT levels in adults (67). In NAFLD patients, the Mediterranean and calorie-restricted diet interventions significantly reduced post-intervention ALT levels, and the Mediterranean component intervention had no significant effect (60). In contrast, the Mediterranean, Mediterranean component, and calorie-restricted diets did not impact AST levels (60). Compared to NAFLD controls, the soy diet had no significant effect on AST and ALT levels (56). It was observed that evening snacks could reduce serum AST and ALT levels in patients with cirrhosis (37).

3.4.5 Other interventions

The effects of propolis, polyunsaturated fatty acids, nuts, and non-sweet nutrients on liver enzymes have also been reported. One study showed that propolis intake significantly reduced ALT and AST levels compared to placebo (48). Another study observed that almond intake did not significantly alter ALT, AST and GGT levels, but subgroup analysis indicated significantly reduced ALT levels in unhealthy subjects (63). Compared to placebo or no treatment, polyunsaturated fatty acid supplementation favored NAFLD treatment and GGT levels but failed to reduce ALT and AST levels (34). Similarly, non-nutritional sweetener consumption was reported to non-significantly alter the AST, GGT, or ALT levels compared to placebo (70). However, according to the subgroup analysis, >24 weeks of stevioside intervention significantly reduced ALT levels (70). Moreover, non-nutritional sweeteners have been found to significantly reduce AST levels in T2DM patients (70).

3.5 Safety

Six meta-analyses reported on adverse events (32, 33, 38, 54, 64, 68). Gastrointestinal symptoms, including nausea/vomiting, abdominal distension/pain, and anorexia, were the most common. Rash/exanthema, headaches, musculoskeletal pain have been observed in other studies.

3.6 Evidence map

The intervention results are summarized in the evidence map displayed in **Figure 2**. The results demonstrated some evidence regarding nigella sativa, garlic, artichoke, curcumin, silymarin, vitamin E, vitamin D, L-carnitine, and high-protein, Mediterranean, and calorie restriction diets, and evening snacks, propolis, and polyunsaturated fatty acids, which may reduce certain liver enzymes. However, the map also showed that nutritional supplements and dietary patterns did not significantly affect liver enzymes, that the quality of evidence for most interventions ranged from very low to low, and that none of them were supported by high-quality evidence. The evidence quality was generally poor due to the risk of bias, heterogeneity, and imprecision (**Supplementary Table 3**).

4 Discussion

This review presents 40 nutritional supplements and dietary interventions evaluating data from meta-analyses of RCTs, where some evidence was observed stating nigella sativa, garlic, artichoke, curcumin, silymarin, or dietary interventions such as vitamin E, vitamin D, L-carnitine, propolis, polyunsaturated fatty acid, and high-protein, Mediterranean, and calorie restriction diets, and evening snacks may reduce liver enzymes. And it had a good safety profile.

The liver is the largest digestive organ in the body, plays an indispensable role in various physiological mechanisms, and is susceptible to damage by multiple factors, such as metabolites, circulating substances, toxins, and microorganisms (72, 73). Oxidative stress systemic and hepatic inflammation are the main pathological factors associated with developing and progressing liver disorders (51). Several potential mechanisms may elucidate the protective effects on the liver offered by natural products, owing to their antioxidant and anti-inflammatory properties, and, therefore, may benefit liver disorders. Meanwhile, natural products positively affect insulin resistance, decrease fat accumulation in hepatocytes and reduce liver enzymes, especially in patients with T2DM and NAFLD. For example, nigella sativa has high anti-oxidant activity and has been revealed to regenerate pancreatic cells, maintain their integrity and increase insulin secretion (54). Nigella sativa could also reduce inflammatory mediators by increasing the production of prodigiosin E-2 and reducing hepatocyte apoptosis (54). Similarly, S-allyl cysteine, an active ingredient in garlic, exerts potent anti-inflammatory effects by upregulating the peroxisome proliferator-activated receptor, which blocks the proliferation of inflammatory factors, thereby mediating liver enzymes (74). Curcumin has been revealed to inhibit oxidative

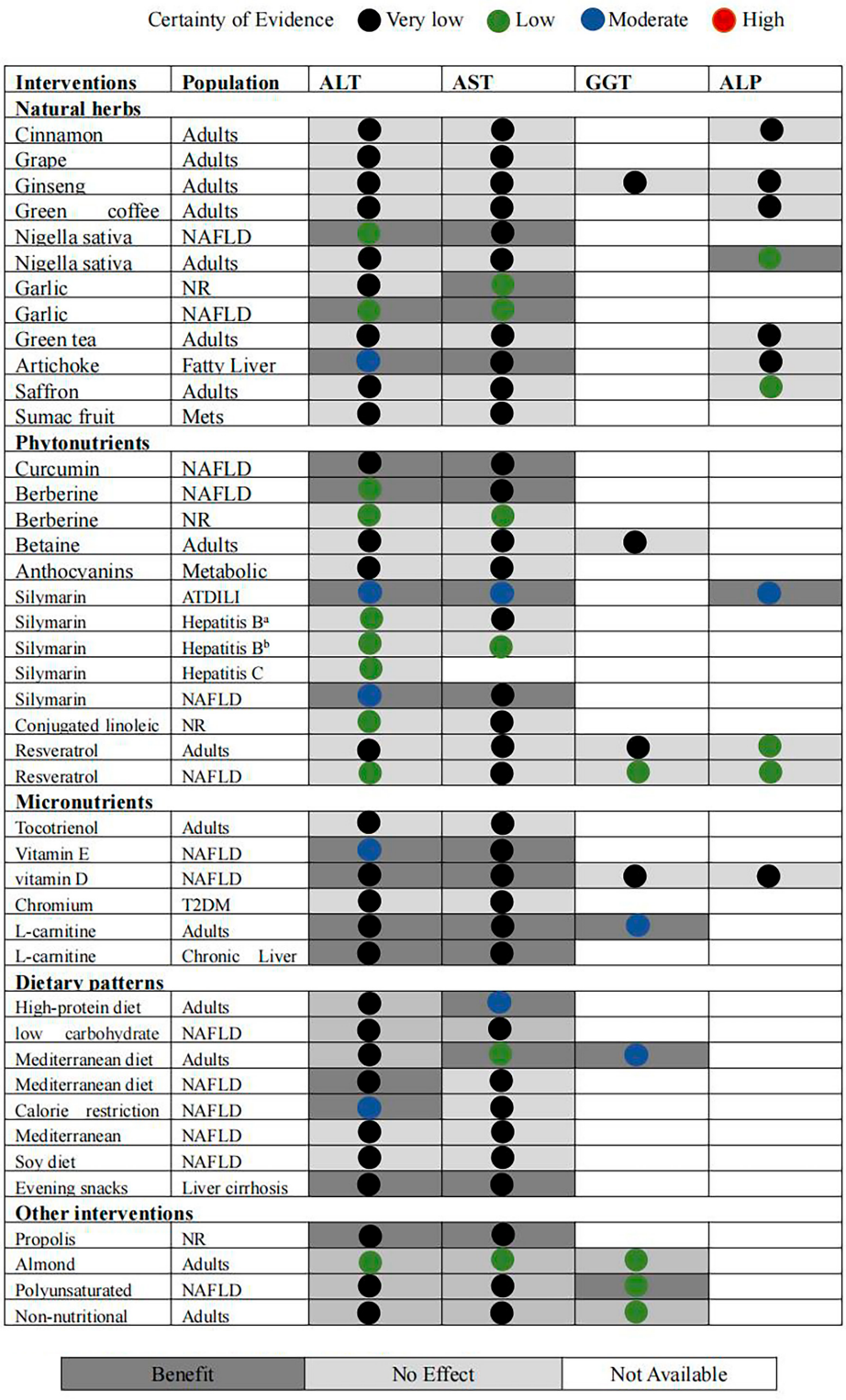

FIGURE 2
Evidence map of availability and appraisal of certainty of the evidence. [a]Silymarin VS protection liver drugs; [b]silymarin plus protection liver drugs/protection liver drugs.

stress-related inflammation via PI3K/AKT and NF-κB related signaling pathways to alleviate liver injury (75), whereas berberine has also been reported to inhibit oxidative stress and hepatic inflammation, preventing NASH progression (76). Silymarin can decrease metabolic stress in liver cells and inhibit inflammation by reducing the infiltration and activation of macrophages and neutrophils to prevent NASH progression (32). These regulatory mechanisms prevent liver damage, stabilize cell membranes, and

reduce cell membrane permeability. The phenolic structure of silymarin allows it to form stable compounds with reactive oxygen species (ROS), which forms the basis of its hepatoprotective and antioxidant properties (77).

The fat-soluble vitamins, including vitamins E and D, are stored and metabolized in the liver. Vitamin E has been clinically proven to improve NAFLD pathology due to its antioxidant effect by reducing steatosis and liver oxidative stress, inflammation, and fibrosis (78). Similarly, vitamin D receptors (VDR) are abundantly expressed in hepatocytes and have been reported to have an exert-inflammatory therapeutic effects on the liver (79). Vitamin D has been found to improve NAFLD and metabolic abnormalities by activating the hepatic VDR, leading to their interaction with HNF4α (80). Our findings reinforced the possible benefits of utilizing vitamins E and D in preventing and treating NAFLD in clinical settings. Moreover, it is well known that disruption of β-oxidation is a significant factor in NAFLD pathogenesis, resulting in fatty acid accumulation within hepatocytes, thereby promoting disease progression. It is possible that the L-carnitine in the transfer of long-chain fatty acids within mitochondria for β-oxidation could potentially contribute to the reduction of ALT and AST levels, particularly in individuals with liver disorders (43).

The development of NAFLD is closely related to diet and lifestyle. It is well supported that different dietary interventions may influence NAFLD pathogenesis and related diseases (81). Many studies have recommended healthy diets and regular physical activities as the primary treatment for NAFLD. Mediterranean diet, as the optimal dietary pattern, has been demonstrated to exert a positive therapeutic effect on liver injury. Our investigation also confirmed a significant reduction in the AST and GGT levels. The Mediterranean diet mainly comprises plant-based foods and fish, with reduced meat and dairy consumption; therefore, it is characterized by a high antioxidant and fiber content, a balanced lipid composition, and a low monosaccharide content in its nutritional composition (82), which is envisaged to reduce hepatic steatosis and metabolic dysfunction in NAFLD patients (83). Other dietary patterns, such as a high-protein diet, can also affect liver enzyme homeostasis. A high-protein diet is associated with satiety, appetite control, and LDL-C and triglyceride levels, suggesting that the diet plays a role in reversing obesity and other chronic diseases. Consequently, the reduction in AST levels we observed in our study may be due to reduced accumulation of triglycerides or alleviated hepatocyte inflammation (84). In addition, our study demonstrated that cirrhotic patients consuming late-night snacks before sleep, providing adequate protein and calories, can reduce protein energy expenditure during early morning hunger (85). The findings of our review are consistent with the evidence that protein intake affects liver function. We also observed that propolis has hepatoprotective effects and reduces AST and ALT levels, mainly mediated by one of its active ingredients, caffeic acid phenethyl ester (86). Similarly, PUFAs have been proven to have a prospective function in NAFLD, which activates the peroxisome proliferator-activated receptor (PPAR), stimulating fatty acid oxidation, and reducing hepatic ROS (87).

There was still considerable room for improvement in the overall methodological quality of the included meta-analyses. First, it has been shown that the availability of registration before a systematic review and meta-analysis affects methodological quality. The systematic review and meta-analysis protocol should be designed and made public before conducting the review to facilitate researchers to reduce implementation and reporting bias. The protocols can be registered on open platforms such as PROSPERO[2] and OSF REGISTRIES[3] or made openly available. Second, the included literature is the cornerstone of systematic reviews and meta-analyses, and a comprehensive search strategy guarantees reliability. To improve the accuracy of systematic review and meta-analysis findings, it is recommended that reviewers employ multiple search methods and databases, trace references, search registration platforms, consult relevant field experts, and search gray literature to prevent missing relevant literature. Third, the rationale and reasons for including a particular study type or studies in systematic reviews and meta-analyses should be explained so that reviewers and readers can understand whether the relevant process makes sense. The authors should list potentially excluded literature and mark reasonable reasons to avoid bias in the outcomes. In addition, commercially funded studies are more likely to reach conclusions favoring the sponsor's product than independently funded studies (88, 89). Therefore, researchers must document the funding source for each study to facilitate judgment of whether financial support gives rise to conflicts of interest that affect the conclusion.

This review has several strengths and limitations. The strengths include meta-analyses based on RCTs while considering dietary interventions and natural products. Because evidence maps were generated from RCTs, this report helps to cover the 'no evidence zone' in this domain. This is the first study to comprehensively review the evidence for natural products, nutrients, dietary supplements, and dietary patterns on liver enzymes, accompanied by a wide-ranging and rigorous study design, including a comprehensive quality assessment of the included literature using the latest version of the AMSTAR-2 checklist. However, it must be acknowledged that the AMSTAR-2 checklist and GRADE system are subjective measures that fail to accurately identify the specific methodological and analytical limitations of the literature. Another limitation of this review was that RCTs without conducted meta-analysis were excluded; therefore, the treatment and safety information may not represent the complete results. The quality of systematic review reports needs to be improved to make more confident recommendations, and the methodological rigor of systematic reviews in nutrition interventions is an important field for future research. Future reviews should be rigorously reported by PRISMA guidelines and incorporate established practice methods such as Cochrane guideline.

5 Conclusion

This umbrella review of the efficacy of nutritional supplements and dietary interventions on liver enzymes revealed that nigella sativa, garlic, artichoke, curcumin, silymarin, vitamin E, vitamin D, l-carnitine, propolis, PUFAs, high-protein, Mediterranean, calorie restriction diets, and evening snacks reduced liver enzymes levels. The results could help professionals make and modify their

2 https://www.crd.york.ac.uk/prospero/

3 https://osf.io/registries?viewonly=

recommendations, provide evidence for clinicians, and guide new research to fill evidence gaps.

Data availability statement

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

Author contributions

YW: Funding acquisition, Supervision, Writing—review and editing. ZL: Conceptualization, Methodology, Writing—original draft. JW: Formal analysis, Investigation, Methodology, Writing—original draft. YZ: Data curation, Formal analysis, Methodology, Writing—review and editing. JS: Formal analysis, Investigation, Methodology, Writing—original draft.

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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.2024.1300860/full#supplementary-material

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Accuracy of prognostic serological biomarkers in predicting liver fibrosis severity in people with metabolic dysfunction-associated steatotic liver disease: a meta-analysis of over 40,000 participants

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Introduction: A prognostic model to predict liver severity in people with metabolic dysfunction-associated steatotic liver disease (MASLD) is very important, but the accuracy of the most commonly used tools is not yet well established.

Objective: The meta-analysis aimed to assess the accuracy of different prognostic serological biomarkers in predicting liver fibrosis severity in people with MASLD.

Methods: Adults ≥18 years of age with MASLD were included, with the following: liver biopsy and aspartate aminotransferase-to-platelet ratio (APRI), fibrosis index-4 (FIB-4), non-alcoholic fatty liver disease fibrosis score (NFS), body mass index, aspartate aminotransferase/alanine aminotransferase ratio, diabetes score (BARD score), FibroMeter, FibroTest, enhanced liver fibrosis (ELF), Forns score, and Hepascore. Meta-analyses were performed using a random effects model based on the DerSimonian and Laird methods. The study's risk of bias was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2.

Results: In total, 138 articles were included, of which 86 studies with 46,514 participants met the criteria for the meta-analysis. The results for the summary area under the receiver operating characteristic (sAUROC) curve, according to the prognostic models, were as follows: APRI: advanced fibrosis (AF): 0.78, any fibrosis (AnF): 0.76, significant fibrosis (SF): 0.76, cirrhosis: 0.72; FIB-4: cirrhosis: 0.83, AF: 0.81, AnF: 0.77, SF: 0.75; NFS: SF: 0.81, AF: 0.81, AnF: 0.71, cirrhosis: 0.69; BARD score: SF: 0.77, AF: 0.73; FibroMeter: SF: 0.88, AF: 0.84; FibroTest: SF: 0.86, AF: 0.78; and ELF: AF: 0.87.

Conclusion: The results of this meta-analysis suggest that, when comparing the scores of serological biomarkers with liver biopsies, the following models showed better diagnostic accuracy in predicting liver fibrosis severity in people with MASLD: FIB-4 for any fibrosis, FibroMeter for significant fibrosis, ELF for advanced fibrosis, and FIB-4 for cirrhosis.

Clinical trial registration: [https://clinicaltrials.gov/], identifier [CRD 42020180525].

KEYWORDS

prognosis, liver biopsy, metabolic dysfunction-associated steatotic liver disease, non-invasive tests, meta-analysis

1 Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is defined as the presence of hepatic steatosis along with at least one of five cardiometabolic risk factors that correspond to the components of metabolic syndrome (MetS) (1). The scenario of MASLD is evolving rapidly; according to the Global Burden of Disease study, MASLD increased considerably in both adolescents and adults between 1990 and 2019 (2, 3). In adolescents, the increase was from 3.73% in 1990 to 4.71% in 2019—an increase of 26.27% (2). In adults, the incidence of MASLD cases increased by 95.4% from 88,177 (95% uncertainty interval (95% UI): 62,304–128,319) in 1990 to 172,330 (95% UI: 125,775–243,640) in 2019. Deaths from MASLD increased by 80.2% from 93,758 (95% UI: 71,657–119,097) per 100,000 population in 1990 to 168,969 (95% UI: 130,575–211,295) per 100,000 population in 2019 (3).

Due to the burden of this disease, early diagnosis of MASLD is an important clinical strategy to prevent its rapid progression to the most severe stages of the disease. According to different international guidelines, liver biopsy is still considered the gold standard for diagnosing liver fibrosis in MASLD (4, 5). However, it is an invasive test that is not free of complications and is not recommended for monitoring disease severity (6). Therefore, the clinical practice guidelines for the management of MASLD recommend the use of non-invasive tests as a resource before the need for liver biopsy in order to stage the disease of fibrosis. These are non-invasive methods that make it feasible to assess disease progression (7).

Different studies have evaluated the diagnostic performance of prognostic models using biomarkers in MASLD (8–10). A meta-analysis of 64 studies published until 2017 compared the diagnostic performance of aspartate aminotransferase-to-platelet ratio index (APRI), fibrosis index-4 (FIB-4), fibrosis score for non-alcoholic fatty liver disease score (NFS), body mass index (BMI), aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AST/ALT ratio), diabetes score (BARD score), FibroScan M probe, FibroScan XL probe, shear wave elastography (SWE), and magnetic resonance elastography (MRE) for staging significant fibrosis (SF), advanced fibrosis (AF), and cirrhosis in MASLD. This study concluded that MRE and SWE may provide better diagnostic accuracy for staging fibrosis in patients with MASLD, with the following results for the area under the receiver operating characteristic (AUROC) curve: SF: MRE: 0.88 and SEW: 0.89;: MRE: 0.93 and SEW: 0.91; and cirrhosis: MRE: 0.92 and SEW: 0.97 (8).

Similarly, a systematic review of 38 studies aimed to evaluate the common non-invasive tests, NFS, enhanced liver fibrosis (ELF), transient elastography, and MRE, in obese patients with SF, AF, and cirrhosis. Evidence showed better accuracy of complex biomarker panels: NFS: summary receiver operator characteristic (SROC): 0.79–0.81 vs. ELF: 0.96; however, the search focused only on studies published until 2016, in English, in four databases, and in individuals with obesity (9). Finally, a recent meta-analysis of 37 studies evaluated the individual diagnostic performance of liver stiffness measurement by vibration-controlled transient elastography (LSM-VCTE), FIB-4, and NFS to derive diagnostic strategies that could reduce the need for liver biopsies. The AUROC results of individual LSM-VCTE, FIB-4, and NFS for AF were 0.85, 0.76, and 0.73, respectively. However, only two invasive tests were included in just one stage of liver fibrosis (10).

Considering the growing body of evidence and lack of consensus on the diagnostic performance of clinical scores, this systematic review and meta-analysis aimed to assess the accuracy prognostic serological biomarkers (APRI, FIB-4, NFS, BARD score, FibroMeter, FibroTest, ELF, Forns score, and Hepascore) in predicting liver fibrosis severity in people with MASLD.

2 Materials and methods

This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy Studies (PRISMA-DTA) guidelines (Supplementary Table S1) (11). The protocol for this meta-analysis was registered in the International Prospective Register of Systematic Reviews database (PROSPERO) under the number CRD42020180525.

2.1 Literature search strategy

This systematic review aimed to answer the following research questions: What is the diagnostic accuracy of the most clinically used serological biomarkers in predicting liver fibrosis severity in people with MASLD? The strategy was based on the participants, index tests, and target condition (PIT) criteria: P: adults ≥18 years with MASLD; I: APRI, FIB-4, NFS, BARD score, FibroMeter, FibroTest, ELF, Forns score, and Hepascore; and T: liver fibrosis. Liver biopsy was used as the reference standard.

We searched the following databases from their inception through December 2021: The Cochrane Hepato-Biliary Group Diagnostic Test Accuracy Studies Register; Medical Literature Analysis and Retrieval System Online (MEDLINE) [via Public/Publisher MEDLINE (PUBMED)]; Excerpt Medical dataBASE (EMBASE); Scientific Electronic Library Online (SciELO); Latin American and Caribbean Health Sciences Literature (LILACS); Cumulative Index to Nursing and Allied Health Literature (CINAHL); and Web of Science (WOS). The reference lists from eligible studies were manually searched to identify additional potentially relevant studies. In addition, we manually searched the abstracts of books from the American Association for the Study of Liver Diseases (AASLD) meetings and European Association for the Study of the Liver (EASL) meetings from the last 10 years. The MEDLINE search strategy was created and adapted for the other databases. There was no language or year of publication restrictions (Supplementary Text S1).

2.2 Eligibility criteria

The eligibility criteria were the PIT criteria described above. Studies were included if they defined liver fibrosis according to the histological classification of the Clinical Research Network (12), included at least 20 adult patients, and provided sensitivity (Sen), specificity (Spe), sample size, or enough information to obtain true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN).

Studies were excluded if participants had viral, autoimmune, or hepatic diseases and chronic hepatitis. Case series, experimental models, replies to letters, editorials, and duplicate publications were also excluded. Studies were considered duplicates if they belonged to the same study group and reported the same inclusion date and individual characteristics. In the case of duplicate studies, the one with the largest sample size was considered.

2.3 Selection of studies

Three review authors (SLT, PBR, and COA) independently selected the articles according to the eligibility criteria in two stages. The first selection stage consisted of screening the titles and abstracts of the articles identified through database searches. In the second stage, full-text articles were assessed using the same methodology. In the case of disagreement between the reviewers, a fourth reviewer (RM) assessed the articles according to the eligibility criteria to resolve any discrepancies.

2.4 Data extraction

Three authors (SLT, PBR, and COA) independently extracted the following data from the selected articles: first author; year of publication; type of paper; study design; study period; country; institution; number of participants; age (years); sex (percentage of males); race (percentages); BMI [kilograms (kg)/meters^2 (m^2)]; hypertension (percentage of participants); diabetes (percentage of participants); dyslipidemia (percentage of participants); MetS (percentage of participants); laboratory tests (AST, ALT, AST/ALT ratio, platelets, glycosylated hemoglobin (HbA1C), glycemia, triglycerides, and cholesterol); and score models (APRI, FIB-4, NFS, BARD score, FibroMeter, FibroTest, ELF, Forns score, and Hepascore). For diagnostic parameters, we considered cutoff values, AUROC, Sen, Spe, TP, FP, TN, and FN. When the authors did not describe TP, FP, TN, or FN, these were calculated based on the Sen and Spe and the number of participants in each study to obtain the values for each model.

2.5 Risk of bias assessment

Three authors (SLT, PBR, and COA) independently assessed the risk of bias in the primary studies using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) (13). QUADAS-2 is a tool for evaluating the quality of primary diagnostic studies by examining quality separately in terms of "risk of bias" and "concerns regarding applicability." Risk of bias assessment items were organized into four domains: patient selection, index test, reference standard, and flow and timing. The applicability of a study was evaluated for the first three key domains and rated as "yes," "no," or "unclear," where "yes" indicated a low risk of bias, "no" indicated a high risk of bias, and "unclear" indicated a lack of sufficient information (13). Disagreements were resolved by consulting a fourth reviewer (RM) to establish a consensus. The methodological quality of individual studies was visualized using the *robvis* web app, which depicts the plots obtained from these analyses (14).

2.6 Data synthesis and analysis

For inclusion in the meta-analysis, the score model should have been used in at least three studies in predicting liver fibrosis severity in people with MASLD. Diagnostic performance statistics were obtained for each study, including Sen, Spe, diagnostic odds ratio (DOR), positive likelihood ratio (LR+), and negative likelihood ratio (LR-), with their respective 95% confidence interval (95% CI). Then, for the DOR, LR+, and LR-, summarized meta-analytical estimates were obtained using a random effects model based on obtaining the variance between studies using the DerSimonian and Laird methods. Heterogeneity was evaluated using Cochran's Q (Q) statistic and I^2 statistic. The Cochran's Q statistic of homogeneity was measured based on the null hypothesis that all eligible studies have the same underlying effect size. The I^2 statistic, which represents the variability between studies, was 0–40%, 40–70%, and 70–100%, indicating low, moderate, and high variance, respectively (15, 16). In addition, summary area under the receiver operating characteristic (sAUROC) curve was obtained using a mixed linear model with known variance estimates according to Reitsma's method. The area under curve (AUC) values were interpreted as follows: <0.5 indicated low accuracy, 0.6 to 0.79 indicated moderate accuracy, 0.8–0.90 showed good accuracy, and > 0.90 represented excellent accuracy (17). A sensitivity analysis was performed to assess whether the results changed when only studies that included the most frequently found scores, FIB-4, APRI, and NFS, and without any fibrosis severity (AF, SF and cirrhosis) were used. All calculations were performed with R version 4.1.3 and Rstudio version 2022.02.1 (Build 461) using the Meta-Analysis of Diagnostic Accuracy (MADA) version 0.5.10 package.[1]

1 https://CRAN.R-project.org/package=mada

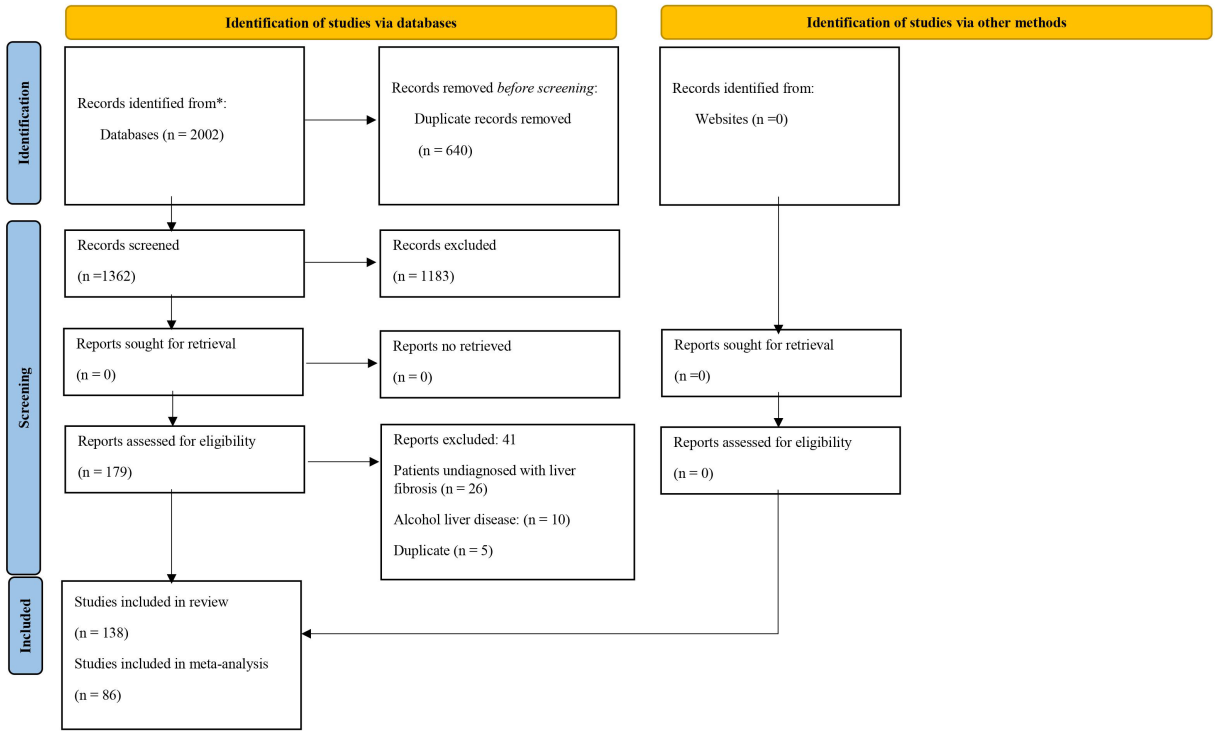

FIGURE 1
PRISMA 2020 flowchart of the study selection process.

The TP, FP, FN, and TN numbers were extracted to construct the 2×2 tables, and the values for each reported test cutoff were calculated. In some studies that did not have the numbers, the prevalence, sensitivity, specificity, and sample size were calculated.[2]

The diagnostic accuracy of the index tests was evaluated in the following dichotomized groups: any fibrosis (AnF) (F0 vs. F1-4), SF (F0-1 vs. F2-4), AF (F0-2 vs. F3-4), cirrhosis (F0-3 vs. F4).

3 Results

3.1 Identification and selection of studies

The search strategy identified 2002 articles. Of these, 640 articles were duplicates, leaving 1,362 for title and abstract assessment. At this stage, 1,183 articles were excluded: 353 on other populations with chronic hepatitis; 130 on patients on autoimmune medication; 74 on animal studies; 198 on alcoholic liver disease; and 428 that did not involve the evaluation or validation of model performance. One hundred and seventy-nine studies were read in full, of which 41 studies were excluded: 26 studies did not include patients diagnosed with hepatic fibrosis; 10 on alcoholic liver disease; and 5 duplicates. Thus, 138 articles were included in this systematic review, of which 86 were included in the meta-analysis and met the eligibility criteria in Figure 1.

2 http://araw.mede.uic.edu/cgi-bin/testcalc.pl

3.2 Characteristics of the included studies

The characteristics of the studies included in the systematic review are described in Table 1. The articles were published between 2004 (123) and 2021 (29, 33, 153). The majority were cross-sectional (68%) (20–22, 27–29, 31–35, 40–44, 46–48, 50–55, 58, 59, 62, 65, 69, 70, 75, 78, 79, 83, 84, 86, 88, 90–93, 95, 97–100, 103–105, 108, 109, 111–114, 116, 117, 119–122, 124, 126–128, 130–136, 138, 140–143, 146, 147, 149–151, 156). Regarding the type of publication, 70.3% of the studies were full-text articles (18, 20, 22, 25, 26, 28–31, 33, 35, 37, 39–42, 44–50, 52, 53, 55, 57, 58, 60, 62, 63, 65, 66, 68, 70, 74, 75, 77–79, 82–88, 90–100, 103, 106, 108, 109, 111–122, 124, 126, 128, 130–135, 138, 140–143, 146, 147, 149–154), and the remaining 29.7% were conference abstracts (19, 21, 23, 24, 32, 34, 36, 38, 43, 51, 54, 56, 60, 61, 64, 67, 69, 71–73, 76, 80, 81, 89, 101, 102, 104, 105, 107, 110, 118, 123, 125, 127, 129, 137, 139, 144, 145, 155). Regarding the geographical origin of the studies, most studies were conducted in Europe (41%) (20, 25, 27, 28, 31, 35–37, 40–44, 47, 51–55, 58, 61, 62, 66, 69, 72, 73, 84–86, 88, 94, 97–100, 102, 103, 105–107, 110, 114, 117, 120, 122, 123, 125, 126, 138–141, 144, 153) and Asia (30%) (22, 29, 46, 59, 67, 70, 71, 74–76, 78, 79, 83, 91, 93, 95, 109, 111, 112, 118, 121, 130–132, 142, 143, 146–152, 154). The total study population consisted of 46,514 participants. The sample size ranged from 29 (46) to 3022 (28) patients. The mean age of the participants ranged from 30 to 67 years old. In 48% of the studies, the majority of participants were male (19, 20, 25, 27, 29–31, 35, 37, 40–42, 44, 46–48, 52–57, 61, 62, 66, 75, 78, 80, 83–86, 91, 93, 95, 97, 98, 103, 106, 107, 112, 114, 120–122, 124, 126, 132, 138, 140–143, 149–151, 153, 154, 156). The mean BMI ranged from 25 (46, 146, 151) to 52.9 (101) kg/m^2.

3.3 Serological biomarkers

The 138 included studies evaluated the nine serological biomarkers (FIB-4; FibroMeter; ELF; NFS; BARD; Hepascore; APRI; FibroTest; Forns score) for liver fibrosis. The most described was the FIB-4, in 89 studies (20–26, 28, 29, 32, 33, 35, 37, 38, 40, 44, 48, 49, 52, 54–56, 58, 66, 67, 71–75, 77–80, 83–86, 89–91, 94, 97–101, 104–107, 109, 110, 112–115, 118, 127–134, 136, 137, 140, 141, 143–146, 148–154), followed by the NFS score in 87 studies (22–27, 30–38, 41, 42, 47–49, 51–56, 58, 61, 66, 67, 71, 73, 74, 76, 77, 80–84, 86, 90, 95, 97–101, 104–107, 110, 111, 113–119, 121, 124, 125, 127, 129, 131, 133–138, 141, 144, 147, 149–154) and the APRI in 80 studies (19–21, 23–26, 29, 30, 32–35, 37, 38, 41, 42, 44, 46, 48, 49, 54, 55, 58, 59, 64, 66, 67, 74, 75, 77, 79–85, 89, 92, 95, 97, 100, 104, 105, 107, 110–113, 118, 121, 125–127, 129–131, 133, 134, 136–138, 141, 144, 145, 148–151, 153, 154). The least used were the ELF in 14 studies (46, 58, 60, 65, 66, 71–73, 105, 106, 120, 142, 143, 147), the Forns score in three studies (58, 100, 151), and the Hepascore in four studies (19, 20, 35, 153). The stage system used to perform the biopsy in most studies was the Kleiner and Brunt system in 55% of the studies (19, 20, 22, 24, 26, 27, 31, 32, 35, 39, 42, 44–50, 52, 53, 56, 58–63, 65, 68, 70, 75, 76, 81–84, 86, 91, 94–100, 106, 107, 113, 114, 117, 119–124, 126, 128, 130–136, 138, 140–144, 149–153). Regarding the severity of fibrosis, AF was the most diagnosed, with 182 studies (20–26, 28, 29, 32, 33, 35, 37, 38, 40, 44, 48, 49, 52, 54–56, 58, 66, 67, 71–75, 77–80, 83–86, 89–91, 94, 97–101, 104–107, 109, 110, 112–115, 118, 127–134, 136, 137, 140, 141, 143–154), followed by SF, with 140 studies (22–25, 30, 35, 36, 38, 41, 42, 47–49, 51, 52, 54, 55, 58, 61, 71, 73, 74, 76, 77, 81–84, 86, 90, 95, 98–101, 105–107, 110, 113, 115, 116, 119, 121, 124, 127, 129, 131, 134–136, 138, 144, 147, 150–152), then by any type of liver fibrosis (107, 112, 114, 120–122, 124, 126, 132, 138, 140–143, 149–151, 153, 154) and cirrhosis (8, 18, 19, 22, 26, 40, 47, 52, 92, 105, 111, 113, 116, 119, 128, 131, 154) in 18 and 16 studies, respectively (Supplementary Text S2 and Supplementary Tables S2, S3). The serological biomarker cutoff values for each severity level have been described in more detail in (Supplementary Table S4).

Table 1. Characteristics of the studies included in the systematic review.

3.4 Analysis of the quality and risk of bias in the included studies

The quality assessment was performed using the QUADAS-2 tool as shown in Figure 2. Studies with patients with MASLD and other morbid conditions were considered a high applicability concern due to the consecutive or random sample of patients enrolled, a case–control design, and inappropriate inclusions such as populations with diabetes, obesity, high levels of transaminases, and selected age.

The risk of bias was unclear in 41% of the studies regarding patient selection (18, 19, 21, 22, 24, 31, 36–38, 51, 56, 59, 61, 72, 73, 76, 83, 85, 88–90, 94, 105, 107, 111, 119, 120, 125, 139, 142, 148, 152, 155, 157). Concerning the reference standard of the studies, several studies did not describe whether all patients received the reference standard and whether all patients were included in the studies, and therefore, 27% of the studies were unclear about the risk of bias (20, 25, 30, 34, 35, 46, 49, 57, 59, 66, 74, 79, 80, 86, 91, 93, 98, 100, 106–109, 115, 116, 118, 123, 129, 130, 138, 142–145, 149, 150, 154, 158). Most of the studies described the pre-specified thresholds (Supplementary Tables S5, S6).

3.5 Meta-analysis results

For inclusion in the meta-analysis, the score model should have been used in at least three studies in predicting liver fibrosis severity in people with MASLD. Only seven scores (APRI, FIB-4, NFS, BARD score, FibroMeter, FibroTest, and ELF) were used in at least three studies to evaluate the four degrees of liver fibrosis severity (AnF, SF, AF, and cirrhosis) and were therefore meta-analyzed (Supplementary Figure S1).

3.6 APRI

The APRI serological biomarker was evaluated for diagnostic accuracy in detecting AnF (>F1) (3 studies), SF (≥F2–F4) (14 studies), AF (≥F3) (33 studies), and cirrhosis (F4) (3 studies) (Supplementary Table S7).

3.6.1 Diagnosis of AnF (F0 vs. F1–F4)

The DOR of the APRI in the diagnosis of AnF was 5.61 (95% CI 4.61–6.82), the LR+ was 2.18 (95% CI 1.63–2.91), the LR- was 0.35 (95% CI 0.22–0.56), and moderate heterogeneity was detected ($Q = 1.04$, $p = 0.59$, $I^2 = 64.35\%$) (Table 2; Supplementary Figures S2, S3). The sAUROC had a moderate diagnostic accuracy of 0.76, Sen of 77% (95% CI 61–88%), and Spe of 64% (95% CI 48–78%) (Figure 3A, Supplementary Table S7, and Supplementary Figure S1).

3.6.2 Diagnosis of SF (F0–F1 vs. F2–F4)

The DOR of the APRI in the diagnosis of SF was 6.29 (95% CI 4.47–8.92), the LR+ was 2.69 (95% CI 2.23–3.23), the LR- was 0.48 (95% CI 0.40–0.58), and low heterogeneity was detected ($Q = 16.13$, $p = 0.24$, $I^2 = 19.40\%$) (Table 2; Supplementary Figures S4, S5). The sAUROC had a moderate diagnostic accuracy of 0.76, Sen of 63% (95% CI 53–72%), and Spe of 79% (95% CI 69–86%) (Figure 3B, Supplementary Table S7, and Supplementary Figure S1).

3.6.3 Diagnosis of AF (F0–F2 vs. F3–F4)

The DOR of the APRI in the diagnosis of AF was 6.45 (95% CI 4.83–8.60), the LR+ was 2.96 (95% CI 2.49–3.52), the LR- was 0.50 (95% CI 0.43–0.57), and low heterogeneity was detected ($Q = 42.78$, $p = 0.009$, $I^2 = 19.40\%$) (Table 2; Supplementary Figures S6, S7). The sAUROC had a moderate diagnostic accuracy of 0.78, Sen of 60% (95% CI 50–69%), and Spe of 82% (95% CI 76–87%) (Figure 3C, Supplementary Table S7, and Supplementary Figure S1).

3.6.4 Diagnosis of cirrhosis (F0–F3 vs. F4)

The DOR of the APRI in the diagnosis of cirrhosis was 6.21 (95% CI 4.34–8.89), the LR+ was 3.11 (95% CI 2.15–4.50), the LR- was 0.53 (95% CI 0.43–0.57), and no heterogeneity was detected ($Q = 1.71$, $p = 0.42$, $I^2 = 0\%$) (Table 2; Supplementary Figures S8, S9). The sAUROC had a moderate diagnostic accuracy of 0.72, Sen of 47% (95% CI 3–84%), and Spe of 87% (95% CI 50–98%) (Figure 3D, Supplementary Table S7, and Supplementary Figure S1).

3.7 FIB-4

The FIB-4 serological biomarker was evaluated for diagnostic accuracy in detecting AnF (>F1) (5 studies), SF (≥F2–F4) (15

TABLE 1 Characteristics of studies included in the systematic review.

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Abe et al. (18)	Japan	Article	289	54.8 ± 14	55	27.6 ± 4.7	Brunt	12.1	39.1	68.1	16.9	49.3	14.8	32.4	17.6	FIB-4, APRI, NFS
Adams et al. (19)	Australia	Abstract	119	48.7 ± 13	54	?	Kleiner and Brunt	41.0	?	?	?	?	?	?	?	APRI, Hepa score, FibroTest
Adams et al. (20)	Australia/Italy	Article	242	46.8 ± 12	60.3	30.2 ± 6	Kleiner and Brunt	35.9	23.9	78.0	18.1	40.1	12.3	22.0	9.5	FIB-4, APRI, Hepa score, FibroTest, BARD score
Ahmed et al. (21)	United States	Abstract	771	?	?	?	Batts Ludwig	?	?	?	?	?	?	?	?	FIB-4, APRI
Aida et al. (22)	Japan	Article	148	61 ± 12	36	26.9 ± 1.25	Kleiner and Brunt	18.9	34.4	71.5	18.2	46.4	16.8	28.2	11.4	FIB-4, APRI
Alkhouri et al. (23)	United States	Abstract	78	30 ± 9	32	?	?	35	42	80	13	23		10		FIB-4, APRI, NFS
Anam et al. (24)	?	Abstract	40	?	?	?	Kleiner and Brunt	40.9	27	80	12.1	32.1	10.7	20	9.3	FIB-4, APRI, NFS, FibroMeter, BARD score
Angelidi et al. (25)	Greece	Article	110	60.1 ± 9.5	52.7	?	?	?	?	?	?	?	?	?	?	FIB-4, APRI, NFS, BARD score
Angulo et al. (26)	United States/ United Kingdom/ Italy/ Australia	Article	1,014	46.9 ± 0.4	58	31.3 ± 0.2	Kleiner and Brunt	34.6	24.7	73.2	13.9	40.5	15.8	26.6	10.8	FIB-4, APRI, NFS, BARD score
Angulo et al. (27)	United States/ United Kingdom/ Italy/ Australia	Article	733	47.7 ± 13.2	52.2	32.3 ± 0.	Kleiner and Brunt	?	26.0	72.9	13.6	40.7	13.0	27.1	14.1	NFS
Anstee et al. (28)	United States/ Europe	Article	3,202	57.5 ± 5.6	47	?	?	26	29	100	45	145	43	100	57	FIB-4, NFS, ELF
Amernia et al. (29)	Iran	Article	205	42.9 ± 10.9	70.2	?	?	?	45.9	78.6	32.7	54.1	14.1	21.4	7.3	FIB-4, APRI

(Continued)

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TABLE 1 (Continued)

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Arora et al. (30)	United States	Article	141	56 ± 4.3	65	?	?	?	?	?	?	?	?	?	?	FIB-4, APRI, NFS, BARD score
Aykut et al. (31)	Turkey	Article	88	46 ± 9	56	30.3 ± 4.6	Kleiner and Brunt	26.0	24.0	69.0	19.0	50.0	21.0	31.0	10.0	NFS, FibroMeter
Balakrishnan et al. (32)	United States	Abstract	122	47 ± 9	20	34 ± 7.5	Kleiner and Brunt	?	?	?	?	?	?	?	?	FIB-4, APRI, NFS, BARD score
Balakrishnan et al.(33)	United States	Article	99	46.8 ± 11.5	26.3	32.4 ± 6.8	Brunt	46.3	38.3	90.7	44.4	63.6	8.1	19.2	11.1	BARD score, FIB-4, APRI, NFS
Barritt et al. (34)	United States	Abstract	859	57 ± 9	38	?	?	?	?	?	?	?	?	?	?	APRI, NFS
Boursier et al. (35)	France	Article	588	55.9 ± 12	57.3	31.7 ± 5.8	Kleiner and Brunt	9	25.9	61.5	26.5	63.3	24.8	38.6	13.8	FIB-4, APRI, NFS, FibroMeter, Hepa score, FibroTest, BARD score
Boursier et al. (36)	France	Abstract	618	?	?	?	?	?	?	?	?	?	?	?	?	NFS, FibroMeter
Boursier et al. (37)	France	Article	938	56.5 ± 12.1	58.5	31.8 ± 5.8	?	9.5	22.8	69.2	26.9	57.7	27.4	30.8	13.4	FIB-4, NFS, FibroTest, FibroMeter, Hepascore
Brandman et al. (38)	United States	Abstract	1,483	50 ± 10	36	?	?	?	?	?	?	?	?	10	?	FIB-4, APRI, NFS, BARD score
Bril et al. (39)	United States	Article	162	57 ± 9	82	34.7 ± 4.6	Kleiner and Brunt	25.1	41.7	83.5	16.5	33.1	12.5	16.5	3.9	FibroTest
Broussier et al. (40)	France	Article	283	56.5 ± 10	53.4	32.9 ± 6.6	?	?	?	?	?	?	?	54.8	?	FIB-4, FibroMeter
Cales et al. (41)	France	Article	235	51.1 ± 11	74.5	28.7 ± 4.9		?	28.9	81.2	8.9	27.7	8.1	18.7	10.6	APRI, NFS, FibroMeter

(Continued)

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TABLE 1 (Continued)

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Cales et al. (42)	France	Article	226	50.9 ± 10.8	75.2	28.7 ± 4.9	Kleiner and Brunt	26.1	29.7	77.5	21.6	44.5	16.2	22.5	6.3	NFS, FibroMeter
Cebreiros et al. (43)	Spain	Abstract	55	43.9 ± 12	24.6	49.9	Metavir	?	?	?	?	?	?	?	?	FibroMeter, ELF
Cengiz et al. (44)	Turkey	Article	123	49 ± 11	56.1	29.5 ± 0.58	Kleiner and Brunt	64.2		86.2	22	35.8	8.9	13.8	4.9	FIB-4, APRI
Chan et al. (45)	Malaysia	Article	147	50.5 ± 11	54.4	29.3 ± 4.5	Kleiner and Brunt	29.3	41.5	79	8.2	29.2	19	21	2	NFS
Chowdhury et al. (46)	India	Article	29	43 ± 4.9	75.8	25.1 ± 2.6	Kleiner and Brunt	41.3	20.6	77.5	10.3	37.9	6.8	27.5	20.6	APRI
Cichoz-Lach et al. (47)	Poland	Article	126	42.7 ± 13	57.9	28.5 ± 2.6	Kleiner and Brunt	26.1	35.7	78.5	16.6	38.0	19.0	21.0	2.3	NFS, BARD score
Cui et al. (48)	United States	Article	102	51.3 ± 14	58.8	31.7 ± 5.5	Kleiner and Brunt	47.1	25.5	81.4	8.8	21.5	12.7	18.6	5.9	FIB-4, APRI, NFS, BARD score
de Carli et al. (49)	Brazil	Article	324	38.7 ± 10.7	34.5	43.8 ± 4.8	Kleiner and Brunt	?	40.8	91.1	4.3	13.2	8.6	8.9	0.3	FIB-4, APRI, NFS, BARD Score
de Cleva et al. (50)	Brazil	Article	131	45.8 ± 11	?	47.8 ± 6.3	Kleiner and Brunt	56.5	29	92.3	6.8	14.4	3.8	7.6	3.8	APRI
Demir et al. (51)	Germany	Abstract	323	?	?	?	?	?	?	?	?	?	?	?	?	NFS, BARD score
Demir et al. (52)	Germany	Article	165	44.8 ± 12	60	28.6 ± 4.3	Kleiner and Brunt	3.6	49.0	87.6	35.1	47.1	9.6	12.0	2.4	FIB-4, NFS, BARD score
Dincses et al. (53)	Turkey	Article	52	45 ± 9	57.6	30.8 ± 5.4	Kleiner and Brunt	?	?	81	?	38	?	19	?	NFS, FibroMeter
Drolz et al. (54)	Germany	Abstract	101	54 ± 10	54	29 ± 1.8	?	?	25.7	45.5	19.8	53.4	13.8	33.6	19.8	FIB-4, APRI, NFS, BARD Score
Dvorak et al. (55)	Czech Republic	Article	56	44.1 ± 15	70	30 ± 3.7	Matteoni	?		51.7	17.8	48.0	16	30.2	14.2	FIB-4, APRI, NFS, ELF, BARD score

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TABLE 1 (Continued)

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Eddowes et al. (56)	?	Abstract	356	53 ± 12	57	34.4 ± 6.5	Kleiner and Brunt	?	?	?	?	?	?	?	?	FIB-4, NFS, FibroMeter
Fagan et al. (57)	Australia	Article	329	45.9 ± 11	64.1	?	Metavir	?	?	?	?	?	?	23.7	?	ELF
Francque et al. (58)	Belgium	Article	542	43.5 ± 12.7	28.6	38.2 ± 6.4	Kleiner and Brunt	64.2	16.3	?	12.1	?	7.0	?	0.2	FIB-4, APRI, NFS, Forns score, BARD score
Fujii et al. (59)	Japan	Article	50	55.8 ± 15.2	26	27.1 ± 3.8	Kleiner and Brunt	?	28.0	56.0	28.0	54.0	26.0	44.0	18.0	APRI
Fujii et al. (60)	Japan	Abstract	122	59 ± 15.3	39	?	Kleiner and Brunt	?	?	55.0	?	?	?	38.0	?	BARD score
Gallego-Duran et al. (61)	Spain	Abstract	49	49 ± 13	61	?	Kleiner and Brunt	?	?	?	?	79.0	?	?	?	NFS, FibroTest
Guha et al. (62)	United Kingdom	Article	192	48.7 ± 12.5	64	32.4 ± 5.7	Kleiner and Brunt	16.1	19.0	77.0	17.0	40.0	13.0	23.0	10.0	ELF
Guillaume et al. (63)	France	Article	417	56.1 ± 1,211	59.2	33.3 ± 6.6	Kleiner and Brunt	29	23.5	67.4	27.3	?	32.4	40.1	7.7	FibroMeter, ELF
Guturu et al. (64)	United States	Abstract	118	?	?	?	Batts Ludwig	?	39.8	75.3	19.4	43.9	8.4	24.5	16.1	APRI, BARD score
Harrison et al. (65)	United States	Article	827	49 ± 5.6	49	33	Kleiner and Brunt	?	24.0	?	80.8	?	?	?	?	BARD score
Hagström et al. (66)	Sweden	Article	646	50 ± 14.8	62	28 ± 3.7	Kleiner	65	40	88	23	35	9	11	3	NFS, BARD score, APRI, FIB-4
Huang et al. (67)	Singapore	Abstract	161	60 ± 14	?	26.8 ± 4.6	?	?	?	?	?	?	?	?	?	FIB-4, APRI, NFS, BARD score
Inadomi et al. (68)	Japan	Article	200	595 ± 17	48	28.1 ± 6.8	Kleiner and Brunt	?	37.5	76	22	58.5	32	36.5	4.5	FIB-4, ELF
Isgro et al. (69)	Italy	Abstract	74	44.3 ± 4.9	?	?	?	8.1	45.8	93.2	39.2	46	5.4	6.8	1.4	ELF
Itoh et al. (70)	Japan	Article	400	56 ± 20	48.7	27.3 ± 9.8	Kleiner and Brunt	16.7	45.7	76.1	13.7	37.5	15.7	23.7	8	ELF

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TABLE 1 (Continued)

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Joo et al. (71)	Korea	Abstract	315	?	?	?	?	?	?	?	?	?	?	?	?	FIB-4, NFS, BARD score
Joo et al. (72)	United Kingdom	Abstract	116	54.3 ± 10.7	?	?	?	?	?	?	?	?	?	?	?	FIB-4
Jouness et al. (73)	Italian	Abstract	254	?	?	?	?	?	?	?	?	?	?	?	?	FIB-4, NFS
Kao et al. (74)	Taiwan	Article	73	35.2 ± 7.7	31.5	41.2 ± 5.6	?	?	?	?	?	22.8	?	11.4	?	FIB-4, APRI, NFS
Kawamur et al. (75)	Japan	Article	90	51.2 ± 5.9	55.5	26, 1	Kleiner and Brunt	?	47.7	61	13.3	52.1	33.3	38.8	5.5	FIB-4, APRI
Kim et al. (76)	Korea	Abstract	481	?	?	?	Metavir	?	?	?	?	?	?	?	?	FIB-4, APRI, NFS, BARD score
Kim et al. (77)	United States	Article	142	52.8 ± 12	26.8	36.3 ± 7.4	Kleiner and Brunt	?	?	?	?	?	?	?	?	FIB-4, APRI, NFS, BARD Score
Kobayashi et al. (78)	Japan	Article	140	56 ± 6.8	54.3	27.1 ± 4	Matteoni	7.1	44.3	74.3	22.9	48.6	21.4	25.7	4.3	FIB-4, APRI
Kolhe et al. (79)	India	Article	100	47 ± 12.3	49	?	Metavir	?	?	73	?	?	?	27	?	FIB-4, APRI
Kosick et al. (80)	Canada	Abstract	541	50.5 ± 13	56.5	32.3 ± 5.5	?	?	?	?	?	?	?	?	45.5	FIB-4, APRI, NFS, BARD score
Kruger et al. (81)	United States	Abstract	111	?	?	?	Kleiner and Brunt	50.0	?	?	?	?	?	?	?	APRI, NFS
Kruger et al. (82)	South Africa	Article	111	52 ± 10	?	?	Kleiner and Brunt	?	?	?	?	?	?	?	17.0	APRI, NFS
Kumar et al. (83)	India	Article	120	39.1 ± 12	75	26.1 ± 3.6	Kleiner and Brunt	26.6	28.3	77.4	22.5	44.8	14.1	22.3	8.3	FIB-4, APRI, NFS, BARD score
Labenz et al. (84)	Germany	Article	261	51 ± 18.5	52.5	30.9 ± 6.9	Kleiner and Brunt	15.5	43.6	84.3	40.9	?	?	?	15.7	FIB-4, APRI, NFS
Lambrecht et al. (85)	Germany	Article	2088	54.5 ± 11.5	64.5	28.6 ± 5.2	?	?	?	?	?	?	?	?	?	FIB-4, APRI

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TABLE 1 (Continued)

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Lang et al. (86)	Germany	Article	96	57 ± 14.6	53	31 ± 6.9	Kleiner and Brunt	?	30.8	?	67.7	130.4	44.4	63.1	18.7	FIB-4, NFS
Lardi et al. (87)	Brazil	Article	73	?	636	?	?	?	?	?	?	?	?	?	?	FibroTest
Lassailly et al. (88)	France	Article	288	41.6 ± 12	33.6	48.6 ± 8	Metavir	59.0	34.0	97.5	4.5	6.9	0.7	2.4	1.7	FibroTest
Le et al. (89)	?	Abstract	254	50.3 ± 10.5	35.4	34.2 ± 6	Metavir	?	?	?	?	44	?	23	?	FIB-4, APRI, BARD Score
Lee et al. (90)	United States	Article	107	48.9 ± 23	38.3	35.9 ± 3.7	?	20.5	18.6	68.0	28.9	48.14	16.8	32.0	14.9	FIB-4, NFS, FibroMeter, BARD Score
Liu et al. (91)	China	Article	349	40.2 ± 12.5	76.5	26.8 ± 3.3	Kleiner and Brunt	?	?	?	?	?	?	?	?	FIB-4
Loaeza-del-Castill et al. (92)	Mexico	Article	30	43 ± 12	43	?	Metavir	26.0	33.0	71.5	40.0	51.5	10.0	10.0	0.0	APRI
Loong et al. (93)	China	Article	215	52 ± 4	55.3	26.8 ± 1.3	?	?	?	?	40.9	27	80	12.1	32.1	FibroMeter
Luger et al. (94)	Austria	Article	46	42 ± 13	20	43.8 ± 4.3	Kleiner and Brunt	?	?	?	?	30	?	13	?	FIB-4, NFS
Mahadeva et al. (95)	Malaysia	Article	131	49.9 ± 12	52.7	?	Kleiner and Brunt	40.8	?	?	35.1	?	35.1	?	6.1	APRI, NFS
Marella et al. (96)	United States	Article	907	46.7 ± 12	32.6	39.9 ± 6 9	Kleiner and Brunt	32.9	36.4	87.2	17.9	30.7	6.9	12.8	5.9	FIB-4, APRI, NFS
McPherson et al. (97)	United Kingdom	Article	145	51 ± 12	61	35 ± 5	Kleiner and Brunt	25.0	43.0	78.0	13.0	29.0	10.0	19.0	9.0	FIB-4, APRI, NFS, BARD score
McPherson et al. (98)	United Kingdom/ Belgium/France	Article	634	49.8	54.8	34 ± 4.5	Kleiner and Brunt	37.4	23.2	?	14.2	?	17	?	8.2	FIB-4, APRI, NFS
McPherso et al. (99)	United Kingdom	Article	305	51 ± 12	60	33.6 ± 4.7	Kleiner and Brunt	?	?	80.5	?	37.5	?	20.5	?	FIB-4, NFS
Meneses et al. (100)	Spain	Article	50	49 ± 8	30	44.3 ± 5	Kleiner and Brunt	60	22	94	12	18	6	6	0	FIB-4, APRI, NFS, Forns score, BARD score

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TABLE 1 (Continued)

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Miao et al. (101)	United States	Abstract	686	?	?	52.9 ± 9.7	?	?	?	?	?	12.3	?	3.1	?	FIB-4, NFS, BARD score
Miele et al. (102)	Italy	Abstract	82	46 ± 12	?	?	?	7.3	39	81.7	35.4	53.7	?	18.3	?	ELF
Miele et al. (103)	Italy	Article	82	46 ± 9	62	28 ± 22–38	?	7.3	39	82.7	35.4	53.7	6.1	18.3	12.2	ELF
Miller et al. (104)	United States	Abstract	354	50 ± 13	42.7	33.9 ± 8.5	?	?	?	?	73.7	?	?		26.3	FIB-4, APRI, NFS
Miller et al. (105)	United Kingdom	Abstract	42	?	?	?	?	?	?	?	?	?	?	?	?	FIB-4, NFS, BARD score
Munteanu et al. (106)	France/Italy/ Brazil/ United Kingdom/ Austria/Greece/ Spain	Article	600	53.2 ± 24	63.3	29.7 ± 0.25	Kleiner and Brunt	20.3	30.8	?	23.3	?	20.2	?	5.5	FIB-4, NFS, FibroTest, BARD score
Nascimben et al. (107)	France	Abstract	884	55 ± 12	61	30 ± 5	Kleiner and Brunt	?	?	?	?	?	?	?	?	FIB-4, APRI, NFS, BARD score
Nassif et al. (108)	Brazil	Article	298	40.1 ± 8	11.1	43.6 ± 10	?	?	?	?	?	?	7.3	?	?	BARD score
Okajima et al. (109)	Japan	Article	163	55.8 ± 14	49.5	27.2 ± 4.3	?	38	34.4	86.5	14.1	26.5	8	12.5	5.5	FIB-4, APRI
Pastor-Ramire et al. (110)	Spain	Abstract	1,256	54.1 ± 14	46	?	?	?	?	?	57.7	?	?	?	?	FIB-4, APRI, NFS, BARD score
Pathik et al. (111)	India	Article	110	42.3 ± 3.2	?	29.1	?	?	?	?	?	?	?	34.5	?	APRI, NFS
Peleg et al. (112)	Israel	Article	153	51.8 ± 17	55.5	29.9 ± 1.6	Metavir	?	?	79.1	?	?	?	20.9	?	FIB-4, APRI
Pérez-Gutiérrez et al. (113)	Mexico/Chile	Article	228	48.6 ± 12	49	?	Kleiner and Brunt	81.6	25.0	88.2	6.6	18.4	7.0	11.8	4.8	FIB-4, APRI, NFS, BARD score
Petta et al. (114)	Italy	Article	321	44.6 ± 12	67.5	29.3	Kleiner and Brunt	?	?	?	?	?	?	22.9	?	FIB-4, NFS
Petta et al. (115)	Italy. Hong Kong. France	Article	741	50.9 ± 12.7	60.2	29.6 ± 4.9	Kleiner and Brunt	?	?	?	?	34.3	?	30.9	?	FIB-4, NFS

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TABLE 1 (Continued)

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Pimentel et al. (116)	Brazil	Article	158	36 ± 10	22.7	41 ± 5	?	?	7.5	30.3	85.9	48.1	61.9	12.0	13.8	NFS
Polyzos et al. (117)	Greece	Article	31	53.3 ± 2.7	25.8	32.2 ± 1.4	Kleiner and Brunt	?	?	?	?	?	?	22.5		APRI, NFS, ELF, FIB-4
Prasad et al. (118)	India	Abstract	240	39.3 ± 10	?	?	?	?	?	?	?	?	?	4	?	FIB-4, APRI, NFS
Qureshi et al. (119)	United States	Article	401	40.5 ± 8.5	17	48.4 ± 7.2	Kleiner and Brunt	43.4	40.0	35.9	86.5	13.8	27.3	11.4	13.5	NFS
Raszeja-Wyszomirska et al. (120)	Poland	Article	104	48 ± 12	65.4	29.6 ± 3	Kleiner and Brunt	?	?	84.6	?	?	?	14.4		BARD score
Rath et al. (121)	India	Article	60	39.7 ± 9.6	85	26.4 ± 3.3	Kleiner and Brunt	31.6	28.3	96.7	36.6	66	3.3	3.3	0	APRI, NFS, BARD score
Ratziu et al. (122)	France	Article	267	50.75 ± 9.4	58	> 27	Kleiner and Brunt	58.2	36.0	79.0	19.0	28.0	5.0	5.0	0	FibroTest
Ratziu et al. (123)	France	Abstract	89	?	?	?	Kleiner and Brunt	36.0	?	?	?	45, 0	?	11, 0	?	FibroTest
Ruffillo et al. (124)	Argentina	Article	138	49 ± 5.6	67	30, 3	Kleiner and Brunt	5.0	6.5	76.9	61.5	88.4	23.1	26.8	3.6	NFS, BARD score
Saez et al. (125)	Spain	Abstract	78	54.2 ± 11	39.7	?	?	?	?	?	?	55, 1	?	?	?	APRI, NFS, BARD score
Sebastiani et al. (126)	France/Italy	Article	190	51.2 ± 13	74.7	28.9 ± 5	Kleiner and Brunt	49.0	36.3	74.7	26.3	51.6	11.6	25.3	13.7	APRI, FibroTest
Seth et al. (127)	United States	Abstract	137	47 ± 11	22	32 ± 6.7	?	?	?	?	?	?	?	40	?	FIB-4, APRI, NFS, BARD score
Shah et al. (128)	United States	Article	541	47.5 ± 12	40	34.7 ± 6.5	Kleiner and Brunt	?	?	76.8	?	?	?	23.1	?	FIB-4
Shaheen et al. (129)	Canada	Abstract	44	51.5 ± 6.6	?	?	?	?	?	?	?	?	?	32	?	FIB-4, APRI, NFS
Shima et al. (130)	Japan	Article	278	57.8 ± 14.8	48.2	27.5 ± 4.7	Kleiner and Brunt	34.1	23.3	72.1	14.7	42.4	23	27.6	4.6	FIB-4, APRI

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TABLE 1 (Continued)

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Shoji et al. (131)	Japan	Article	197	60 ± 14	45.1	27.5 ± 6.2	Kleiner and Brunt	40.6	?	63.9	23.3	59.3	20.8	36	15.2	FIB-4, APRI, NFS, BARD score
Shukla et al. (132)	India	Article	51	50.4 ± 11	53	?	Kleiner and Brunt	?	?	78.4	?	?	?	21.6	?	FIB-4
Siddiqui et al. (133)	United States	Article	145	52.9 ± 11	37.7	35.8 ± 19	Kleiner and Brunt	29	29	64.9	?	?	?	35.2	7.6	FIB-4, APRI, NFS, FibroMeter, BARD score
Siddiqui et al. (134)	United States	Article	1904	50.3 ± 12.2	47	34.4 ± 6.4	Kleiner and Brunt	24	28	72	20	48	20	28	8	FIB-4, APRI, NFS
Simo et al. (135)	United States	Article	225	43.2 ± 9.6	14.7	44.6 ± 5.4	Kleiner and Brunt	?	58.2	21.8	93.4	13.3	19.9	6.2	6.6	NFS
Singh et al. (136)	United States	Article	1,157	51.1 ± 11.5	35.4	35.5 ± 8.1	Kleiner and Brunt	?	?	68.2	?	?	?	38.1	?	FIB-4, APRI, NFS
Singh et al. (137)	?	Abstract	1969	?	?	?	?	?	?	?	?	?	?	7	?	FIB-4, APRI, NFS, BARD score
Sjowall et al. (138)	Sweden	Article	82	59.8 ± 11	67	28.9 ± 4.4	Kleiner and Brunt	?	?	?	?	?	?	17	?	APRI, NFS, BARD score
Stauber et al. (139)	Austria	Abstract	122	?	?	?	?	?	?	?	?	?	?	28	?	ELF
Staufer et al. (140)	Austria	Article	186	52 ± 5.2	57	30.5 ± 2.7	Kleiner and Brunt	?	?	61.8		55		27	?	FIB-4, FibroMeter, ELF
Subasi et al. (141)	Turkey	Article	142	45 ± 9	52.8	30.9 ± 5	Kleiner and Brunt	28.2	35.2	78.9	15.5	36.6	14.1	21.1	7	FIB-4, APRI, NFS, FibroMeter, BARD Score
Sumida et al. (142)	Japan	Article	576	52.3 ± 15	51	27.9 ± 4.9	Kleiner and Brunt	45.6	29.3	?	13.8	24.9	7.8	11.1	3.2	FIB-4, APRI, NFS, BARD score
Takeuchi et al. (143)	Japan	Article	71	50.8 ± 15.7	64.8	29.1 ± 5.1	Kleiner and Brunt	8	17	39	14	46	27	32	5	FIB-4

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TABLE 1 (Continued)

References	Country/ Region	Type of publication	No. patients	Age (SD)	Male %	BMI (SD)	Stage system	Fibrosis 0/1	F1	F0-F1-F2%	F2	F2-F3-F4%	F3	F3-F4%	F4	Serological biomarkers
Tanwar et al. (144)	United Kingdom	Abstract	177	?	?	?	Kleiner and Brunt	59.0	19.2	75.7	17.5	23.8	13.6	23.8	10.2	FIB-4, APRI, NFS, ELF, BARD score
Thanapirom et al. (145)	?	Abstract	92	49.6 ± 13.7	44.9	27.4 ± 5.1	?	97.8	?	100	2, 2	?	?	?	?	FIB-4, APRI
Tomeno et al. (146)	Japan	Article	106	67 ± 7.8	41.5	25.8 ± 3.1	?	?	52.8	10.3	21.6	36.6	11.3	15	3.7	FIB-4
Treeprasertsuk et al. (147)	Thailand	Article	139	40.9 ± 13	47	36.1 ± 14.7	?	?	?	93.5	?	?	?	6.4	?	FIB-4, NFS, BARD score
Uy et al. (148)	Philippines	Abstract	61	46 ± 11	46	29.1 ± 4.3	?	?	?	?	?	?	?	9, 8	?	FIB-4, APRI, BARD Score
Wong et al. (149)	China	Article	246	51 ± 11	54.9	28 ± 4.5	Kleiner and Brunt	28.4	30.4	77.3	18.2	40.9	12.6	22.7	10.1	FIB-4, APRI, NFS, BARD score
Xun et al. (150)	China	Article	152	37.1 ± 9.7	79.6	26.1 ± 3.3	Kleiner and Brunt	31.6	33.5	84.0	19.1	34.9	13.8	15.8	1.9	FIB-4, APRI, NFS, BARD score
Yang et al. (151)	China	Article	453	36.5 ± 16.7	58.9	25.9 ± 3.6	Kleiner and Brunt	?	?	72, 2	?	?	?	27.8	?	FIB-4, APRI, NFS, FibroMeter, Forns score, BARD score
Yoneda et al. (152)	Japan	Article	235	59.9 ± 12	?	26.9 ± 4	Kleiner and Brunt	38.7	27.6	83.8	17.4	33.6	8.9	16.2	7.2	FIB-4, NFS, BARD Score
Younes et al. (153)	Italy, United Kingdom, and Spain	Article	1,173	40 ± 14.1	64.7	29.4 ± 7.5	Kleiner and Brunt									APRI, NFS, FIB-4, BARD score, Hepascore
Zhou et al. (154)	China	Article	207	41.8	73.4	?	?	?	47.8	38.2	96.1	10.1	14	3.9	3.9	FIB-4, APRI, NFS, BARD score
Zou et al. (155)	China	Abstract	107	?	?	?	?	?	?	?	?	?	?	?	28	FIB-4, APRI, NFS, BARD score

APRI, aspartate aminotransferase-to-platelet ratio index; ELF, enhanced liver fibrosis; FIB-4, fibrosis index-4; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NFS, non-alcoholic fatty liver disease score; SD, standard deviation;?, not responded.

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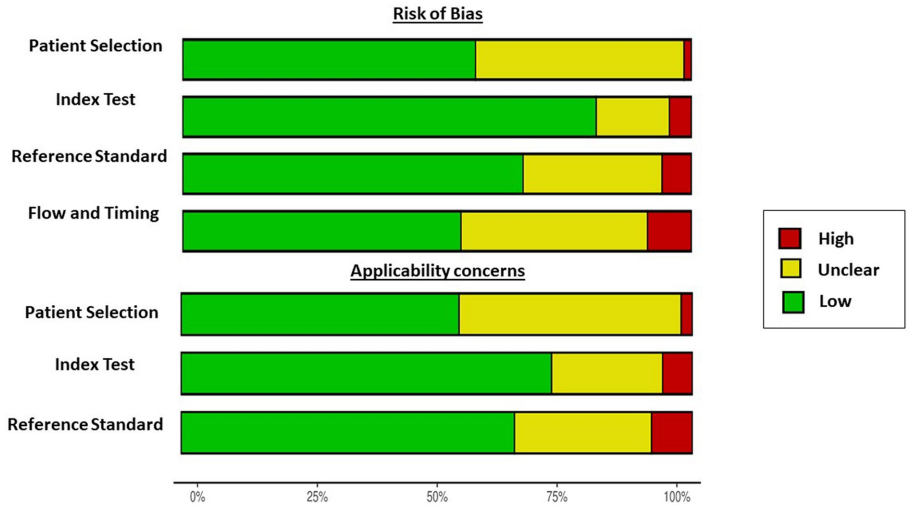

FIGURE 2
Graphical summary of the risk of bias of the included studies using the QUADAS-2 tool.

studies), AF (≥F3) (43 studies), and cirrhosis (F4) (4 studies) (Supplementary Table S7).

3.7.1 Diagnosis of AnF (F0 vs. F1–F4)

The DOR of the FIB-4 in the diagnosis of AnF was 6.57 (95% CI 4.56–9.48), the LR+ was 2.32 (95% CI 1.94–2.77), the LR- was 0.38 (95% CI 0.29–0.49), and low heterogeneity was detected (Q = 5.35, p = 0.25, I^2 = 25.24%) (Table 2; Supplementary Figures S10, S11). The sAUROC had a moderate diagnostic accuracy of 0.77, Sen of 77% (95% CI 61–87%), and Spe of 68% (95% CI 57–78%) (Figure 3A, Supplementary Table S7, and Supplementary Figure S1).

3.7.2 Diagnosis of SF (F0–F1 vs. F2–F4)

The DOR of the FIB-4 in the diagnosis of SF was 5.75 (95% CI 4.11–8.05), the LR+ was 2.51 (95% CI 2.07–3.05), the LR- was 0.50 (95% CI 0.43–0.59), and low heterogeneity was detected (Q = 18.26, p = 0.19, I^2 = 23.33%) (Table 2; Supplementary Figures S12, S13). The sAUROC had a moderate diagnostic accuracy of 0.75, Sen of 64% (95% CI 52–74%), and Spe of 76% (95% CI 66–84%) (Figure 3B, Supplementary Table S7, and Supplementary Figure S1).

3.7.3 Diagnosis of AF (F0–F2 vs. F3–F4)

The DOR of the FIB-4 in the diagnosis of AF was 10.43 (95% CI 7.25–15.02), the LR+ was 4.09 (95% CI 3.33–5.02), the LR- was 0.45 (95% CI 0.39–0.52), and no heterogeneity was detected (Q = 33.1, p = 0.83, I^2 = 0%) (Table 2; Supplementary Figures 14, S15). The sAUROC had a good diagnostic accuracy of 0.81, Sen of 60% (95% CI 52–68%), and Spe of 87% (95% CI 82–91%) (Figure 3C, Supplementary Table S7, and Supplementary Figure S1).

3.7.4 Diagnosis of cirrhosis (F0–F3 vs. F4)

The DOR of the FIB-4 in the diagnosis of cirrhosis was 14.95 (95% CI 9.96–22.44), the LR+ was 4.66 (95% CI 2.41–9.02), the LR- was 0.38 (95% CI 0.19–0.78), and low heterogeneity was detected (Q = 4.16, p = 0.24, I^2 = 27.88%) (Table 2; Supplementary Figures S16, S17). The sAUROC had a good diagnostic accuracy of 0.83, Sen of 69% (95% CI 43–86%), and Spe of 87% (95% CI 57–97%) (Figure 3D, Supplementary Table 7, and Supplementary Figure S1).

3.8 NFS

The NFS serological biomarker was evaluated for diagnostic accuracy in detecting AnF (>F1) (5 studies), SF (≥F2–F4) (14 studies), AF (≥F3) (43 studies), and cirrhosis (F4) (3 studies) (Supplementary Table S7).

3.8.1 Diagnosis of AnF (F0 vs. F1–F4)

The DOR of the NFS in the diagnosis of AnF was 4.85 (95% CI 3.32–7.09), the LR+ was 2.27 (95% CI 1.86–2.78), the LR- was 0.49 (95% CI 0.42–0.57), and moderate heterogeneity was detected (Q = 6.63, p = 0.15, I^2 = 39.66%) (Table 2; Supplementary Figures 18, 19). The sAUROC had a moderate diagnostic accuracy of 0.71, Sen of 66% (95% CI 62–70%), and Spe of 73% (95% CI 64–81%) (Figure 3A and Supplementary Table S7, and Supplementary Figure S1).

3.8.2 Diagnosis of SF (F0–F1 vs. F2–F4)

The DOR of the NFS in the diagnosis of SF was 9.45 (95% CI 5.17–17.5), the LR+ was 3.35 (95% CI 2.42–4.63), the LR- was 0.42 (95% CI 0.33–0.54), and low heterogeneity was detected (Q = 13.53, p = 0.40, I^2 = 3.91%) (Table 2; Supplementary Figures S20, S21). The sAUROC had a good diagnostic accuracy of 0.81, Sen of 69% (95% CI 56–79%), and Spe of 80% (95% CI 71–88%) (Figure 3B, Supplementary Table S7, and Supplementary Figure S1).

3.8.3 Diagnosis of AF (F0–F2 vs. F3–F4)

The DOR of the NFS in the diagnosis of AF was 9.74 (95% CI 6.69–14.17), the LR+ was 3.56 (95% CI 2.93–4.32), the LR- was 0.44 (95% CI 0.38–0.51), and no heterogeneity was detected (Q = 37.99, p = 0.64, I^2 = 0%) (Table 2; Supplementary Figures S22, S23). The sAUROC had a good diagnostic accuracy of 0.81, Sen of 62% (95% CI 53–70%), and Spe of 85% (95% CI 79–90%) (Figure 3C, Supplementary Table S7, and Supplementary Figure S1).

TABLE 2 Comparison of serological biomarkers in predicting liver fibrosis severity in people with MASLD: DOR; LR+, and LR−.

	DOR	(95% CI)	Cochran's Q	*p*	I^2	LR+	(95% CI)	LR-	(95% CI)
APRI									
Any fibrosis	5.61	(4.61–6.82)	1.04	0.59	64.35	2.18	(1.63–2.91)	0.35	(0.22–0.56)
Significant fibrosis	6.29	(4.47–8.92)	16.13	0.24	19.4	2.69	(2.23–3.23)	0.48	(0.40–0.58)
Advanced fibrosis	6.45	(4.83–8.60)	42.78	0.009	25.21	2.96	(2.49–3.52)	0.50	(0.43–0.57)
Cirrhosis	6.21	(4.34–8.89)	1.71	0.42	0	3.11	(2.15–4.50)	0.53	(0.31–0.89)
FIB-4									
Any fibrosis	6.57	(4.56–9.48)	5.35	0.25	25.24	2.32	(1.94–2.77)	0.38	(0.29–0.49)
Significant fibrosis	5.75	(4.11–8.05)	18.26	0.19	23.33	2.51	(2.07–3.05)	0.50	(0.43–0.59)
Advanced fibrosis	10.43	(7.25–15.02)	33.1	0.83	0	4.09	(3.33–5.02)	0.45	(0.39–0.52)
Cirrhosis	14.95	(9.96–22.44)	4.16	0.24	27.88	4.66	(2.41–9.02)	0.38	(0.19–0.78)
NFS									
Any fibrosis	4.85	(3.32–7.09)	6.63	0.15	39.66	2.27	(1.86–2.78)	0.49	(0.42–0.57)
Significant fibrosis	9.45	(5.17–17.5)	13.53	0.40	3.91	3.35	(2.42–4.63)	0.42	(0.33–0.54)
Advanced fibrosis	9.74	(6.69–14.17)	37.99	0.64	0	3.56	(2.93–4.32)	0.44	(0.38–0.51)
Cirrhosis	9.13	(4.25–19.62)	1.72	0.42	0	3.88	(2.35–6.39)	0.43	(0.32–0.58)
BARD score									
Significant fibrosis	5.98	(2.62–13.66)	4.11	0.53	0	2.49	(1.72–3.61)	0.46	(0.30–0.70)
Advanced fibrosis	4.34	(3.40–5.55)	26.11	0.16	23.4	1.88	(1.65–2.14)	0.48	(0.41–0.56)
FibroMeter									
Significant fibrosis	17.82	(4.91–64.7)	2.69	0.44	0	6.00	(2.72–13.23)	0.35	(0.18–0.67)
Advanced fibrosis	13.72	(7.51–25.07)	9.42	0.58	0	4.16	(2.89–5.99)	0.31	(0.24–0.40)
FibroTest									
Significant fibrosis	5.19	(1.77–15.18)	12.21	0.007	75.42	2.10	(1.36–3.25)	0.56	(0.36–0.85)
Advanced fibrosis	7.45	(5.15–10.77)	4.48	0.48	0	3.81	(2.18–6.64)	0.58	(0.43–0.79)
ELF									
Advanced fibrosis	18.82	(9.52–37.18)	7.05	0.21	29.08	4.42	(3.12–6.25)	0.29	(0.23–0.38)

APRI, aspartate aminotransferase-to-platelet ratio index; CI, confidence interval; DOR, diagnostic odds ratio; ELF, enhanced liver fibrosis; FIB-4, fibrosis index-4; I^2, heterogeneity; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NFS, non-alcoholic fatty liver disease fibrosis score; p, statistically significant value, MASLD, metabolic dysfunction-associated steatotic liver disease.

3.8.4 Diagnosis of cirrhosis (F0–F3 vs. F4)

The DOR of the NFS in the diagnosis of cirrhosis was 9.13 (95% CI 4.25–19.62), the LR+ was 3.88 (95% CI 2.35–6.39), the LR- was 0.43 (95% CI 0.32–0.58), and no heterogeneity was detected (Q = 1.72, p = 0.42, I^2 = 0%) (Table 2; Supplementary Figures S24, S25). The sAUROC had a moderate diagnostic accuracy of 0.69, Sen of 63% (95% CI 58–68%), and Spe of 84% (95% CI 73–91%) (Figure 3D, Supplementary Table S7, and Supplementary Figure S1).

3.9 BARD score

The BARD score serological biomarker was evaluated for diagnostic accuracy in detecting SF (≥ F2–F4) (6 studies) and AF (≥ F3) (21 studies) (Supplementary Table S6).

3.9.1 Diagnosis of SF (F0–F1 vs. F2–F4)

The DOR of the BARD score in the diagnosis of SF was 5.98 (95% CI 2.62–13.66), the LR+ was 2.49 (95% CI 1.72–3.61), the LR- was 0.46

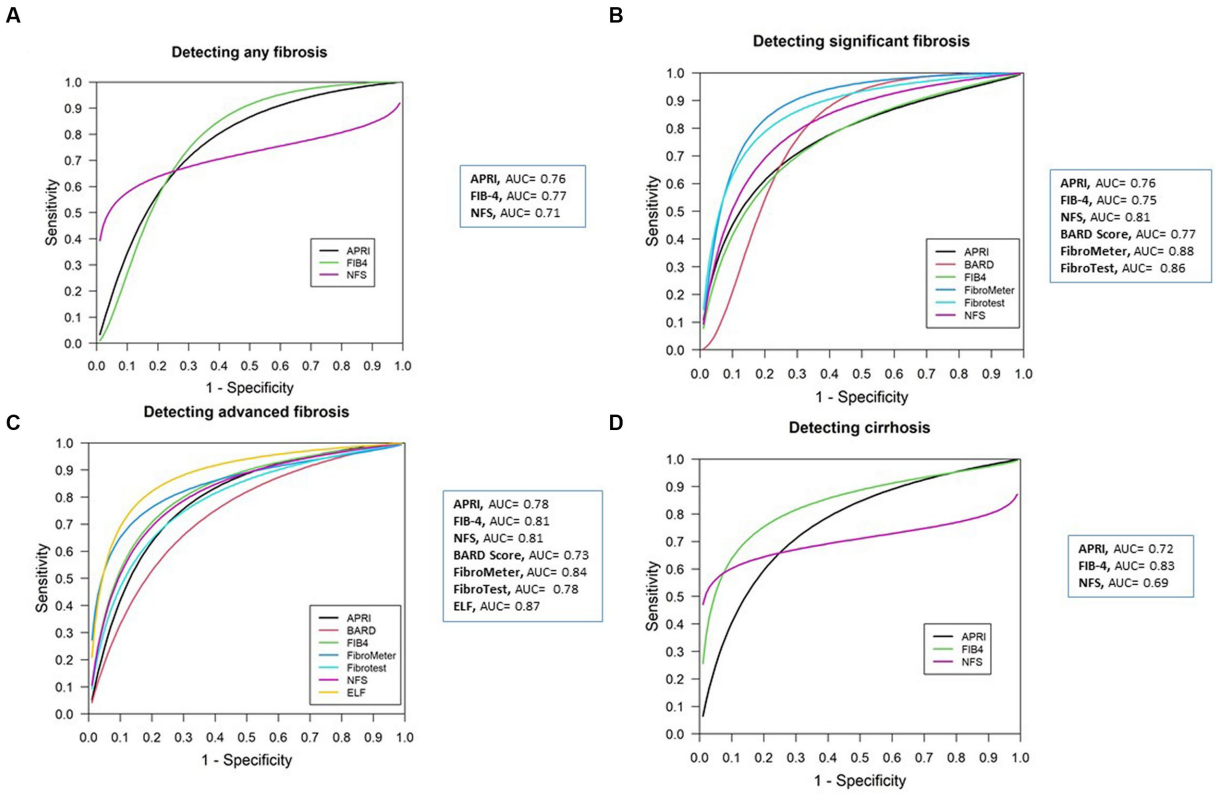

FIGURE 3
Summary AUROC plot of tests. **(A)** APRI, FIB-4, and NFS in detecting any fibrosis. **(B)** APRI, FIB-4, NFS, BARD score, FibroMeter, and FibroTest in detecting significant fibrosis. **(C)** APRI, FIB-4, NFS, BARD score, FibroMeter, FibroTest, and ELF in detecting advanced fibrosis. **(D)** APRI, FIB-4, and NFS in detecting cirrhosis.

(95% CI 0.30–0.70), and no heterogeneity was detected (Q = 4.11, p = 0.53, I^2 = 0%) (Table 2; Supplementary Figures S26, S27). The sAUROC had a moderate diagnostic accuracy of 0.76, Sen of 63% (95% CI 45–82%), and Spe of 79% (95% CI 65–83%) (Figure 3B, Supplementary Table S7, and Supplementary Figure S1).

3.9.2 Diagnosis of AF (F0–F2 vs. F3–F4)

The DOR of the BARD score in the diagnosis of AF was 4.34 (95% CI 3.40–5.55), the LR+ was 1.88 (95% CI 1.65–2.14), the LR- was 0.48 (95% CI 0.41–0.56), and low heterogeneity was detected (Q = 26.11, p = 0.16, I^2 = 23.4%) (Table 2; Supplementary Figures S28, S29). The sAUROC had a moderate diagnostic accuracy of 0.73, Sen of 72% (95% CI 64–79%), and Spe of 63% (95% CI 54–71%) (Figure 3C, Supplementary Table S7, and Supplementary Figure S1).

3.10 FibroMeter

The FibroMeter serological biomarker was evaluated for diagnostic accuracy in detecting SF (≥F2–F4) (4 studies) and AF (≥F3) (12 studies) (Supplementary Table S7).

3.10.1 Diagnosis of SF (F0–F1 vs. F2–F4)

The DOR of the FibroMeter in the diagnosis of SF was 17.82 (95% CI 4.91–64.7), the LR+ was 6.00 (95% CI 2.07–3.05), the LR- was 0.35 (95% CI 0.18–0.67), and no heterogeneity was detected (Q = 2.69, p = 0.44, I^2 = 0%) (Table 2; Supplementary Figures S30, S31). The sAUROC had a good diagnostic accuracy of 0.88, Sen of 68% (95% CI 48–82%), and Spe of 89% (95% CI 80–95%) (Figure 3B, Supplementary Table 7, and Supplementary Figure S1).

3.10.2 Diagnosis of AF (F0–F2 vs. F3–F4)

The DOR of the FibroMeter in the diagnosis of AF was 13.72 (95% CI 7.51–25.07), the LR+ was 4.16 (95% CI 2.89–5.99), the LR- was 0.31 (95% CI 0.24–0.40), and no heterogeneity was detected (Q = 9.42, p = 0.58, I^2 = 0%) (Table 2; Supplementary Figures 32, 33). The sAUROC had a good diagnostic accuracy of 0.84, Sen of 74% (95% CI 68–79%), and Spe of 82% (95% CI 76–87%) (Figure 3C, Supplementary Table S7, and Supplementary Figure S1).

3.11 FibroTest

The FibroTest serological biomarker was evaluated for diagnostic accuracy in detecting SF (≥F2–F4) (4 studies) and AF (≥F3) (6 studies) (Supplementary Table S7).

3.11.1 Diagnosis of SF (F0–F1 vs. F2–F4)

The DOR of the FibroTest in the diagnosis of SF was 5.19 (95% CI 1.77–15.18), the LR+ was 2.10 (95% CI 1.36–3.25), the LR- was 0.56

(95% CI 0.36–0.85), and high heterogeneity was detected (Q = 12.21, $p = 0.007$, $I^2 = 75.42\%$) (Table 2; Supplementary Figures 34, S35). The sAUROC had a good diagnostic accuracy of 0.86, Sen of 72% (95% CI 28–94%), and Spe of 85% (95% CI 45–98%) (Figure 3B, Supplementary Table S7, and Supplementary Figure S1).

3.11.2 Diagnosis of AF (F0–F2 vs. F3–F4)

The DOR of the FibroTest in the diagnosis of AF was 7.45 (95% CI 5.15–10.77), the LR+ was 3.81 (95% CI 2.18–6.64), the LR- was 0.58 (95% CI 0.43–0.79), and no heterogeneity was detected (Q = 4.48, $p = 0.48$, $I^2 = 0\%$) (Table 2; Supplementary Figures S36, S37). The sAUROC had a moderate diagnostic accuracy of 0.78, Sen of 40% (95% CI 15–72%), and Spe of 93% (95% CI 73–99%) (Figure 3C, Supplementary Table S7, and Supplementary Figure S1).

3.12 ELF

The ELF serological biomarker was evaluated for diagnostic accuracy in detecting AF (≥ F3) (6 studies) (Supplementary Table S7).

3.12.1 Diagnosis of AF (F0–F2 vs. F3–F4)

The DOR of the ELF in the diagnosis of AF was 18.82 (95% CI 9.52–37.18), the LR+ was 4.42 (95% CI 3.12–6.25), the LR- was 0.29 (95% CI 0.23–0.38), and low heterogeneity was detected (Q = 7.05, $p = 0.21$, $I^2 = 29.08\%$) (Table 2; Supplementary Figures S38, S39). The sAUROC had a good diagnostic accuracy of 0.87, Sen of 79% (95% CI 68–87%), and Spe of 84% (95% CI 75–90%) (Figure 3C, Supplementary Table S7, and Supplementary Figure S1).

3.13 Sensitivity analysis

The sensitivity analysis showed that there were no changes in the results when only tests with more than 40% of participants (APRI, FIB-4, NFS, and BARD score) and severities (SF, AF, and cirrhosis) were included (Supplementary Figures S40–S58; Supplementary Table S8).

4 Discussion

This systematic review and meta-analysis aimed to assess the accuracy of different prognostic serological biomarkers in predicting liver fibrosis severity in people with MASLD. The serological biomarkers varied according to the different degrees of severity of liver fibrosis. For any type of fibrosis, all the models had moderate precision. For significant fibrosis, the FibroMeter, FibroTest, and NFS models had high precision, and APRI, FIB-4, and BARD score had moderate precision. For advanced fibrosis, the ELF, FibroMeter, FIB-4, and NFS models had high precision, and BARD score, FibroTest, and APRI presented moderate precision. Finally, for cirrhosis, only FIB-4 showed high precision, while APRI and NFS had moderate diagnostic precision in the evaluation of this severity.

The APRI showed moderate diagnostic accuracy across all degrees of liver fibrosis severity, from AnF to cirrhosis, the results that are consistent with previous meta-analyses reporting moderate accuracy in assessing AF with this prognostic model. In addition, different studies have reported inconsistencies in predicting liver fibrosis using this score (8, 96). Therefore, due to conflicting results regarding the effectiveness of the APRI score, the MASLD practice guideline of the AASLD, American College of Gastroenterology, and American Gastroenterological Association recommends using the FIB-4 or NFS score to identify patients with MASLD with stage 3 or 4 fibrosis (6). Our results support this recommendation as FIB-4 and NFS showed good diagnostic accuracy in the assessment of liver fibrosis severity, for SF and AF, and AF and cirrhosis, respectively.

As science has advanced, several serum tests have been developed using either direct biomarkers (reflecting the pathophysiology of hepatic fibrogenesis) or indirect biomarkers (reflecting functional changes in the liver) alone or in combination (57). Complex panels (such as FibroMeter and ELF) have been shown to be more accurate and reproducible for detecting AF than simple panels (159). Our results support these findings, suggesting that both models have good diagnostic accuracy for AF, whereas simple panels such as APRI and BARD score, although cheaper, easier to calculate, and widely available, are not as accurate as complex panels (159).

Different studies have consistently reported that the ELF model provides good results in the assessment of AF, including the 2021 National Institute of Health and Care Excellence guidelines, which established that for the assessment and treatment of people with MASLD, the ELF score is considered "the most cost-effective and appropriate test for AF in adults with MASLD" (160). However, the reality of clinical practice is different as the ELF score is not accessible to frontline health professionals, which may represent a barrier to the detection of liver fibrosis (9, 57).

The FibroTest also showed good diagnostic performance for the assessment of SF in this review. FibroTest and FibroMeter are models that include the analysis of extracellular matrix substances directly involved in the progression of fibrosis and have better Sen and Spe, suggesting that the inclusion of a direct marker of liver fibrosis in a non-invasive test can improve its diagnostic accuracy (8, 9).

Another relevant result was that only three models detected AnF: APRI, FIB-4, and NFS. These models are considered simple scores, that is, none of the complex models analyzed in this review identified this severity. Therefore, there is still a lack of studies evaluating any of these models in the assessment of AnF as most scores have focused on the importance of histological determinants of severe fibrosis and its relevance in the development of future disease. However, the identification of AnF in community settings will allow for the implementation of early lifestyle interventions and consequently inform the decision to refer to secondary care in severe cases (62, 134).

MASLD is also strongly correlated with MetS. Of the 138 included studies, 54.6% reported at least some component of this syndrome. Two recent reviews have suggested that MASLD is both a cause and a consequence of MetS (161, 162). This is because liver fat is presented as a marker of metabolic abnormalities that characterize MetS, and the possibility of MASLD should be considered in all patients diagnosed with MetS with any of the different sets of criteria (161, 162). In the present review, the mean values for both transaminases were above normal, indicating that the studies were conducted in populations with at least some alteration in the serological tests of the liver. In people with

MASLD with normal transaminase levels, 16–24% of them may have AF, with the sAUROC for the BARD score, FIB-4, and NFS ranging from 0.71 to 0.85 (99, 152).

In this review, we found a mean BMI of 32.8 kg/m^2 in the total study population, which is considered grade-I obesity. The findings of a meta-analysis suggest that there is evidence of a high predictive value of abdominal obesity as an indicator of increased risk of metabolic disorders and cardiovascular disease, as well as evidence supporting the cause-and-effect relationship between abdominal obesity and MASLD (163). A recent review showed that there is less evidence when evaluating the tests in populations of patients with obesity, and non-invasive tests tend to be less favorable in these populations due to differences in terms of BMI and alanine aminotransferase levels, which may mean that serum-based scores derived from the liver clinical setting in groups with different hepatic risk profiles do not adequately reflect the accuracy of these tests in the obese population (9).

Conversely, the present results of prognostic models showing moderate diagnostic accuracy may also be related to the fact that this meta-analysis included a larger number of studies, heterogeneous populations and their variables, and all degrees of fibrosis severity compared to previous meta-analyses (9, 10). Although the objective of non-invasive models is not to replace the biopsy, our results highlight the importance of using these models in the evaluation of MALSD patients with suspected liver fibrosis, which determines the prognosis of the disease, as well as the usefulness and feasibility of performing these tests, given the lack of other methods in primary care for these patients (159).

5 Limitations

However, our meta-analysis has limitations. First of all, there was no stratification of the different models by age, race, weight, and morbidities, only by stages of fibrosis, since few studies were conducted in clinical trials to compare homogeneous populations. Another limitation of the present study is the non-inclusion of imaging biomarkers such as MRE. The decision not to include these biomarkers was made to focus on the serological biomarkers recommended by the guidelines to provide a more comprehensive assessment of their performance. However, this is a study with a large sample of participants, with low heterogeneity between the different studies, which aims to contribute to the generalization of results based on possible limitations in health services.

6 Conclusion

The findings of this meta-analysis suggest that when comparing the scores of serological biomarkers with liver biopsies for predicting liver fibrosis severity in people with MASLD, the FIB-4 has good predictive diagnostic accuracy for any fibrosis, the FibroMeter has good predictive diagnostic accuracy for significant fibrosis, the ELF has good predictive diagnostic accuracy for advanced fibrosis, and the FIB-4 has good diagnostic accuracy for cirrhosis. These non-invasive serological biomarkers can thus be considered as an alternative to determine the prognosis of this disease.

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

SL: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. CA: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. PR: Data curation, Writing – original draft. CC: Writing – review & editing, Writing – original draft. MW: Formal analysis, Software, Writing – review & editing, Writing – original draft. AP: Conceptualization, Writing – review & editing, Funding acquisition, Writing – original draft. RM: Conceptualization, Formal analysis, Investigation, Methodology, 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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1284509/full#supplementary-material

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Glossary

95% CI	95% confidence interval
95% UI	95% uncertainty interval
AASLD	American Association for the Study of Liver Diseases
AF	Advanced fibrosis
ALT	Alanine transaminase
AnF	Any fibrosis
APRI	Aspartate aminotransferase-to-platelet ratio
AST	Aspartate aminotransferase
AST/ALT ratio	Aspartate aminotransferase/alanine aminotransferase ratio
AUC	Area under curve
AUROC	Area under the receiver operating characteristic
BARD score	Body mass index, aspartate aminotransferase/alanine aminotransferase ratio, diabetes score
BMI	Body mass index
CINAHL	Cumulative Index to Nursing and Allied Health Literature
DOR	Diagnostic odds ratio
EASL	European Association for the Study of the Liver
ELF	Enhanced liver fibrosis
EMBASE	Excerpt Medical dataBASE
FN	False negatives
FP	False positives
HbA1C	Glycosylated hemoglobin
kg	Kilograms
LILACS	Latin American and Caribbean Health Sciences Literature
LR-	Negative likelihood ratio
LR+	Positive likelihood ratio
LSM-VCTE	Liver stiffness measurement by vibration-controlled transient elastography
m^2	$Meters^2$
MADA	Meta-analysis of diagnostic accuracy
MASLD	Metabolic dysfunction-associated steatotic liver disease
MEDLINE	Medical Literature Analysis and Retrieval System Online
MetS	Metabolic syndrome
MRE	Magnetic resonance elastography
NFS	Non-alcoholic fatty liver disease fibrosis score
PIT	Participants, index tests, and target condition
PRISMA-DTA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy Studies
PROSPERO	International Prospective Register of Systematic Reviews database
PUBMED	Public/Publisher MEDLINE
Q	Cochran's Q
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies-2
sAUROC	Summary area under the receiver operating characteristic
SciELO	Scientific Electronic Library Online
Sen	Sensitivity
SF	Significant fibrosis
Spe	Specificity
SROC	Summary receiver operator characteristic
SWE	Shear wave elastography
TN	True negatives
TP	True positives
WOS	Web of Science

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Exploring the interactions between metabolic dysfunction-associated fatty liver disease and micronutrients: from molecular mechanisms to clinical applications

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Metabolic (dysfunction)-associated fatty liver disease (MAFLD) has emerged as a significant global health concern, representing a major cause of liver disease worldwide. This condition spans a spectrum of histopathologic stages, beginning with simple fatty liver (MAFL), characterized by over 5% fat accumulation, and advancing to metabolic (dysfunction)-associated steatohepatitis, potentially leading to hepatocellular carcinoma. Despite extensive research, there remains a substantial gap in effective therapeutic interventions. This condition's progression is closely tied to micronutrient levels, crucial for biological functions like antioxidant activities and immune efficiency. The levels of these micronutrients exhibit considerable variability among individuals with MAFLD. Moreover, the extent of deficiency in these nutrients can vary significantly throughout the different stages of MAFLD, with disease progression potentially exacerbating these deficiencies. This review focuses on the role of micronutrients, particularly vitamins A, D, E, and minerals like iron, copper, selenium, and zinc, in MAFLD's pathophysiology. It highlights how alterations in the homeostasis of these micronutrients are intricately linked to the pathophysiological processes of MAFLD. Concurrently, this review endeavors to harness the existing evidence to propose novel therapeutic strategies targeting these vitamins and minerals in MAFLD management and offers new insights into disease mechanisms and treatment opportunities in MAFLD.

KEYWORDS

metabolic (dysfunction)-associated fatty liver disease, vitamins, minerals, nutritional assessment, therapeutic strategy

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver disorders, ranging from simple steatosis to more severe conditions like steatohepatitis with fibrosis, and ultimately, cirrhosis. Recognizing its association with hepatic steatosis, obesity, T2DM, and hypertriglyceridemia, NAFLD has been renamed Metabolic (Dysfunction)-Associated Fatty

Liver Disease (MAFLD), highlighting its metabolic underpinnings (1–3).

MAFLD can lead to hepatocellular carcinoma in its more advanced stages, a malignancy known for its high mortality rate. Recent epidemiological studies reveal that MAFLD's global prevalence has reached approximately 30% (4), and this trend shows no signs of abating. Most MAFLD patients initially have a benign condition, MAFL, with over 5% of hepatocytes containing lipid droplets (5). However, 20–30% progress to metabolic (dysfunction)-associated steatohepatitis (6, 7), characterized by significant steatosis, inflammation, and cellular ballooning, primarily in the liver's alveolar zone 3 (8). Alarmingly, up to 38% of MASH patients with fibrosis may develop cirrhosis, and 2.4–12.8% of these individuals are at risk of hepatocellular carcinoma (HCC) (7). Both cirrhosis and hepatocellular carcinoma linked to MAFLD are associated with poor prognoses, highlighting the urgency for timely and effective management strategies in MAFLD patients.

Vitamins and minerals, essential micronutrients predominantly sourced from our diet, play a crucial role in normal body functioning through their antioxidant properties, enzyme activities, and immune system modulation (9). Recent research has brought to light the significant role of certain trace elements, particularly vitamins A, D, E, and minerals like iron, copper, selenium, and zinc. This article delves into their involvement in immune-inflammatory and metabolic processes (10, 11). The destabilization of these micronutrients has been linked to a variety of metabolic diseases (12), including MAFLD (13). Globally, vitamin and mineral deficiencies are widespread (14), and MAFLD patients frequently face similar challenges. These deficiencies are often tied to the dietary choices (15) of the individuals and a reduction in vitamin production due to altered intestinal flora (16). Despite the prevalence of MAFLD, current medical treatments remain inadequate. However, observations of micronutrient imbalances in MAFLD patients and animal models (17), along with the improvements seen in targeted therapies, open up new avenues for treating this condition. The ability of vitamins and trace minerals to positively impact the mechanisms at the core of MAFLD offers promising prospects for its pharmacological treatment (18, 19). This insight, focusing on correcting micronutrient imbalances, could pave the way for innovative strategies in managing and potentially mitigating the progression of MAFLD.

The review aims to provide the latest summary on the pathophysiologic pathways linking micronutrients to the development of MAFLD and to focus on new data from clinical trials exploring the safety and efficacy of vitamin and mineral supplementation on liver outcomes in patients with MAFLD (See Figure 1).

2 Pathogenesis of MAFLD and current therapeutics

2.1 Pathogenesis of MAFLD

The pathogenesis of MAFLD is not well defined, and the "multi-hit theory" is more widely recognized. MAFLD is a complex disease characterized by interactions between the environment and the susceptible polygenic host background that determine the phenotype and progression of the disease, MBOAT7, and other variants in the genes are strongly and consistently associated with MAFLD (20). On this basis, modern high-fat diet and unhealthy lifestyle habits act as triggers for impaired hepatic fat metabolism, hepatocellular fat accumulation producing lipotoxicity (21), endoplasmic reticulum stress (22), increased synthesis of reactive oxygen species, synthesis of adipokines, activation of inflammatory cells and release of inflammatory factors triggering intrahepatic inflammation (23), disruption of hepatic homeostasis, and comorbid insulin resistance (24). Gut microecological changes (25), accelerating the transformation of MAFL to MASH, liver fibrosis and cirrhosis (See Table 1).

2.2 Current therapeutics

Current treatment strategies for Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) are limited in effectiveness. Clinical guidelines primarily advocate for lifestyle interventions (44) and, in cases where obesity has advanced significantly, bariatric surgery (45) to facilitate weight loss. For lean MAFLD patients, the recommended approach is lifestyle modification coupled with a reduction in fructose and sugar-sweetened beverages, aiming for a modest weight loss of 3–5% (46). Other pharmacological treatments, such as metformin, thiazolidinediones, and liraglutide, are generally reserved for patients with concurrent diabetes mellitus. However, their efficacy specifically for MAFLD is not conclusively proven, and they have shown potential side effects or unintended results in animal studies (47).

Research into MAFLD patients' micronutrient levels reveals complex interactions and trends. Vitamins A, E, Zinc, and Copper are often reduced, while Vitamin D varies and Iron increases. These micronutrients interact within MAFLD, complicating disease understanding and progression. This interplay presents challenges yet offers new therapeutic opportunities. Current research focuses on understanding these interactions to develop targeted MAFLD treatments, marking a shift towards more effective management approaches.

3 Vitamins and MAFLD

3.1 Vitamins deficiency status in MAFLD patients

Vitamin deficiency is a global health issue with widespread impact (48). In metabolic diseases related to obesity, most vitamins are found to be deficient (49). Specifically, in MAFLD, the primary vitamins affected are the fat-soluble ones: A, D, and E. MAFLD patients, often consuming diets low in nutrients, rich in high-fat meats/proteins, and high in sodium (50), are prone to lower levels of these vitamins without additional supplementation. Furthermore, alterations in the intestinal microecology significantly influence vitamin absorption, contributing to the vitamin deficiencies observed in MAFLD patients (51, 52). Additionally, vitamin deficiencies play a role in low-intensity inflammation, exacerbated by the release of inflammatory adipokines from adipose tissue, which further aggravates the condition of MAFLD patients. This complex interplay underscores the importance of addressing vitamin deficiencies in managing and improving the health outcomes for those with MAFLD (49).

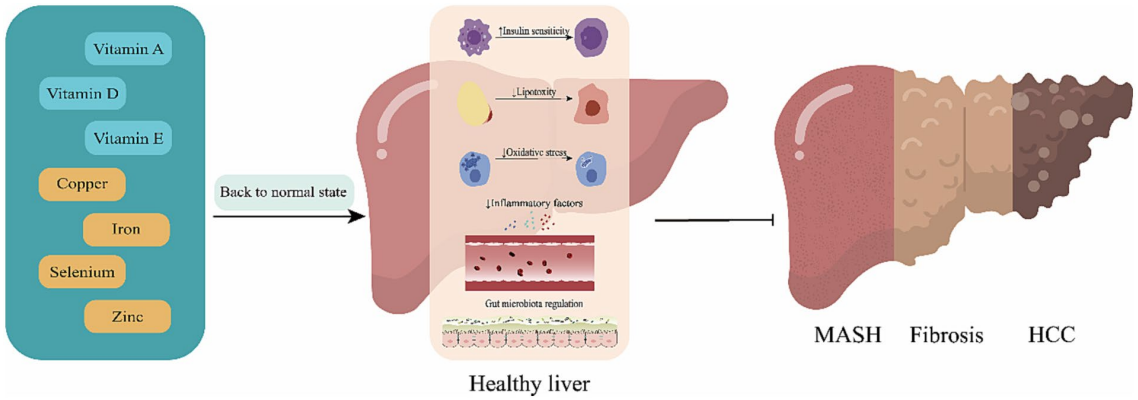

FIGURE 1
Mechanisms of action for the effect of micronutrients. Replenishment of deficient micronutrients plays a pivotal role in reducing the risk and progression of metabolic (dysfunction)-associated fatty liver disease (MAFLD). By restoring these essential nutrients to optimal levels, there is a marked improvement in insulin sensitivity, a reduction in lipotoxicity, a decrease in inflammatory mediators, and a regulation of the intestinal microbiota. These changes collectively contribute to slowing the progression from metabolic (dysfunction)-associated fatty liver (MAFL) to metabolic (dysfunction)-associated steatohepatitis, fibrosis, and potentially hepatocellular carcinoma.

3.2 Role of vitamins in pathogenesis of MAFLD

3.2.1 Vitamin A and MAFLD

Vitamin A, essential for various physiological functions in the human body, relies exclusively on dietary intake. Its primary active form, retinoic acid (RA), plays a pivotal role by binding to retinoic acid receptors to facilitate biological signal transduction (53). Normally, vitamin A, being fat-soluble, is stored in hepatic stellate cells (54, 55). In patients with MAFLD, circulating concentrations of retinoic acid are observed to be lower (56). There is a notable correlation between diminished levels of Vitamin A and the severity of hepatic fibrosis, as well as an increase in liver-related mortality (57). Furthermore, in patients with metabolic (dysfunction)-associated Steatohepatitis (MASH), high expression of hepatic AKR1B10 is linked to reduced hepatic retinoid levels, exacerbating the progression from MASH to Hepatocellular Carcinoma (HCC) (58). This highlights the critical role of Vitamin A in liver health and its potential implications in the progression of liver diseases.

Vitamin A contributes to the management of MAFLD through various mechanisms, including the modulation of lipid metabolism (26, 30, 59), antioxidant effects (28, 60), anti-inflammatory properties (27), and enhancing insulin sensitivity (30, 31). Notably, the retinoic acid receptor β2 agonist AC261066 has been shown to induce changes in the transcriptome and metabolome of hepatocytes (26), reduce the TGF-β1 inflammatory response in Kupffer cells, and alleviate liver fibrosis (27, 61). Dietary supplementation with all-trans retinoic acid (ATRA) notably improved insulin sensitivity in MAFLD model mice (C57BL/6J) (31). Additionally, ATRA acts on the retinoic acid receptor (62) to decrease PPAR-γ2 expression, thereby reducing fat accumulation in the liver (29).

Despite these promising findings, the clinical application of vitamin A in MAFLD treatment is constrained by its narrow therapeutic window and the limited number of clinical trials. This highlights the need for further research to fully understand and harness Vitamin A's potential in MAFLD treatment while ensuring safety and efficacy in human applications.

3.2.2 Vitamin D and MAFLD

Vitamin D, primarily produced in the skin via sunlight exposure, is vital for both skeletal and extra-skeletal health. Clinical guidelines recommend keeping human serum 25(OH)D levels above 50 nmoL/L. Despite this, about 7% of the global population has vitamin D levels below this threshold (63). Studies indicate that vitamin D deficiency is nonlinearly linked to increased MAFLD severity and higher all-cause mortality (64). Lower 25(OH) vitamin D levels are associated with increased MAFLD prevalence and liver fibrosis (65), while higher levels reduce fibrosis risk in MAFLD patients (66).

In a Western diet rat model, Vitamin D deficiency exacerbated MAFLD, potentially via toll-like receptor activation and endotoxin exposure (67). This deficiency also caused insulin resistance, increased hepcidin expression, and heightened inflammation and oxidative stress genes. Key to this severity in vitamin D-deficient MAFLD patients might be the activation of MAPK and NF-κB pathways (68).

In the realm of treating MAFLD with vitamin D, significant strides have been made. Studies across various regions (69, 70) and populations (71, 72) have shown that increased vitamin D levels may help prevent MAFLD. Different dosages of vitamin D exhibit varying degrees of improvement in MAFLD (33, 73).

Vitamin D induces autophagy (18) in mice, primarily by upregulating ATG16L1, thereby inhibiting the p53 pathway to prevent hepatocyte senescence and apoptosis (74). It also reduces inflammation via the enterohepatic axis, underscoring the importance of timely supplementation (75). Phototherapy-enhanced active vitamin D3 in mice mitigates hepatocyte apoptosis, inflammation, fibrosis, and insulin/leptin resistance caused by a CDAA diet (76). Additionally, vitamin D treatment curbs MAFLD induced by a high-fat diet (HFD), involving gut microbiota (77) and metabolic regulation (78), and modulates lipid metabolism through the PPARa signaling pathway (79). Vitamin D-regulated miRNAs are implicated in MAFLD pathogenesis, though more research is needed (80). It also exhibits antifibrotic effects by countering TGF-β signaling in hepatic stellate cells (81).

Contrastingly, some studies have found no correlation between plasma vitamin D levels and insulin resistance, hepatic fat

TABLE 1 Effect of vitamins and minerals in MAFLD.

Author (reference)	Treatment and control	Experimental model	Treatment dosage and administration	Findings
Tang et al. (26)	Treatment: retinoic acid receptor β2 agonist(AC261066) Negative controls:- Positive controls:-	High-fat diet (HFD) induced wild-type (wt) male C57BL/6 mice mouse	3 mg/100 mL drinking water, oral for 2 months	• ↓mRNA increases inPklr, Fasn, Thrsp., and Chchd6 • ↓transcript and protein levels of KHK
Trasino et al. (27)	Treatment: retinoic acid receptor β2 agonist(AC261066) Negative control: RARγ agonist (CD1530) Positive control: no treatment	High-fat diet (HFD) induced Wild type (wt) male C57BL/6 mice	15 IU/vitamin A-acetate/gram, oral for 3 months	• ↓hepatic steatosis and oxidative stress • ↓expression of pro-inflammatory mediators • ↓hepatic stellate cell (HSC) activation • ↓kupffer TGF-β1 expression
Zarei et al. (28)	Treatment: atRA Negative control:- Positive control: -	High-fat diet (HFD) induced male New Zealand rabbits	5 mg/kg/day, oral for 30 days	• ↓liver steatosis • ↓liver oxidative agents • ↑total antioxidant capacity
Kim et al. (29)	Treatment: atRA Negative control: - Positive control: -	WD-fed C57BL/6 mice	Corn oil containing atRA (15 mg/kg/day), oral for 7 days	• ↓adiposity in brown fat
Berry et al. (30)	Treatment: atRA Negative control: - Positive control: -	High-fat/high-sucrose diet C57BL/6Ntac mice	Subcutaneously implanted with an RA pellet or mock pelleted by using a 10-gauge precision trochar	• ↑weight loss • ↑insulin responsiveness
Tsuchiya et al. (31)	Treatment: ATRA Negative control: - Positive control:-	High-fat, high-fructose diet-induced C57BL/6 J mice	50 mg/kg ATRA, oral for 4 weeks	• ↓insulin resistance
Li et al. (18)	Treatment: 1,25 (15)2D3 Negative control: - Positive control: -	High-fat diet (HFD) induced male C57BL/6 mice	2.5 ng/g, three times per week for 4 weeks, i.p.	• ↓liver inflammation • regulated lipid metabolism
Dabbaghmanesh et al. (32)	Treatment: cholecalciferol & calcitriol Negative control: placebo Positive control: no treatment	MAFLD patient	50,000 U vitamin D3 pearl/week for 3 months, oral or0.25 mg calcitriol pearl/day for 3 months, oral	• ↓serumalkaline phosphatase and GGT
Wenclewska et al. (33)	Treatment: cholecalciferol Negative control: no treatment Positive control: no treatment	Metabolic Disorder patients	2000 International Unit (11) cholecalciferol/day oral for three months	• ↓oxidative stress • ↓insulin resistance • ↑metabolic profile
El Amrousy et al. (34)	Treatment: vitamin D Negative control: placebo Positive control: no treatment	100 children with biopsy-proven MAFLD	2000 IU/day orally for 6 months	• ↓hepatic steatosis • ↓lobular inflammation
Mosca et al. (19)	Treatment: vitamin E & hydroxytyrosol Negative control: placebo Positive control: no treatment	Children with MAFLD	3.75 mg of hydroxytyrosol plus 5 mg of Vitamin E/day, oral for 16 weeks	• ↓systemic inflammation • ↓oxidative stress

(Continued)

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TABLE 1 (Continued)

Author (reference)	Treatment and control	Experimental model	Treatment dosage and administration	Findings
Scorletti et al. (35)	Treatment: vitamin E Negative control: no treatment Positive control: no treatment	MAFLD patients	Not for sure	• ↓overall mortality • ↓risk of MAFLD
Vilar-Gomez et al. (36)	Treatment: vitamin E Negative control: no treatment Positive control: no treatment	MASH patients	800 international units/day of vitamin E for ≥2 years	• ↑clinical outcomes
Podszun et al. (37)	Treatment:vitamin E Negative control: no treatment Positive control: no treatment	MAFLD patients	200–800 IU/d, oral for 24 weeks	• ↓oxidative stress
Doboszewskaet al. (38)	Treatment: Zinc Negative controls: Zinc-deficient (ZnD)diet Positive controls: Zinc-adequate (ZnA) diet	Male Sprague-Dawley rats	50 mg Zn/kg or 3 mg Zn/kg, for 4 or 6 weeks	• ↓oxidative damage • ↓pro-inflammatory status
Ma et al. (39)	Treatment: high-iron (HI) diets Negative controls: low-iron (LI) diets Positive controls: -	Male db/db mice	High-iron (HI) diets (1,000 mg/kg chow) or low-iron (LI) diets (12 mg/kg), oral for 9 weeks	• ↑Gluconeogenesis • ↓Lipogenesis • ↑Insulin resistance
Fujiwara et al. (40)	Treatment: high-iron Negative controls: Western diet Positive controls: Western diet + high-iron	Male F344/DuCrlCrlj rats	6% of blending iron citrate (FeC6H5O7 · 5H2O), oral for 26 weeks	• ↑serum triglyceride and cholesterol • ↑hepatic inflammation
Wang et al. (41)	Treatment: Se-enriched spirulina Negative controls: normal diet with Se-enriched spirulina Positive controls: HFD with Se-enriched spirulina	High-fat diet (HFD) induced C57BL/6 mice	Se content 0.45 mg/kg, oral 12 weeks	• ↓hepatic injury and insulin resistance • ↓fat accumulation & expression of lipogenic genes
Xu et al. (42)	Treatment: sodium selenate, Negative controls: - Positive controls: -	Male APP/PS1 transgenic mice	12 μg/mL sodium selenate, oral for 2 months	• ↓insulin resistance
Zhang et al. (43)	Treatment: Se Negative controls: - Positive controls: -	free fatty acid (FFA) induced primary rat hepatocytes	0.1 μM Se, cell cultivation	• ↓oxidative stress • ↓apoptosis
Miyataet al. (6)	Treatment: Selenoneine Negative controls: - Positive controls: -	Fxr-null mice	0.3 mg Se/kg selenoneine-containing diet oral for 4 months	• ↓hepatocellular injury • ↓hepatic steatosis

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accumulation, or MASH severity (82–85). Similarly, randomized trials using vitamin D supplements for MAFLD treatment have not consistently shown benefits (73, 86). Polymorphisms in the Vitamin D receptor gene could explain the varied outcomes observed. While Vitamin D ameliorates liver damage in MAFLD, early expression of its receptor in MAFLD patients' livers and decreased lipid accumulation in mice lacking this receptor gene point to its intricate involvement in MAFLD's development and progression (87).

These findings highlight vitamin D's potential in MAFLD treatment but also reveal its multifaceted and context-dependent nature. The genetics and epigenetics of MAFLD may influence vitamin D's regulatory mechanisms, necessitating further research to elucidate these intricate relationships.

3.2.3 Vitamin E and MAFLD

Vitamin E, currently the only medication recommended by guidelines for treating MASH, is valued for its antioxidant and anti-inflammatory properties (88, 89). It has been observed that patients with MAFLD often have reduced serum levels of both vitamin E and A (90). In a non-randomized, propensity score-adjusted study, a daily intake of 800 IU of vitamin E was associated with significant reductions in total mortality and hepatic decompensation in patients with MASH-induced bridging fibrosis and cirrhosis, both in diabetic and non-diabetic individuals (36). Moreover, vitamin E has been effective in lowering AST and ALT levels in adult patients with MAFLD (91). It inhibits oxidative stress, which reduces *de novo* lipogenesis (DNL) and intrahepatic triglyceride (IHTG) accumulation, thereby disrupting the cycle between oxidative stress and the MAFLD process (92). Histological improvements in MAFLD patients have also been noted with vitamin E treatment, demonstrating its therapeutic potential (92–95).

However, the effectiveness of vitamin E in altering the histological course of MASH in patients with Type 2 Diabetes Mellitus (T2DM) has not been significant (96). Additionally, its use is limited in the treatment of common comorbidities associated with MAFLD (96). While vitamin E shows promise in MAFLD treatment, its role and efficacy may vary depending on specific patient conditions and comorbidities, indicating the need for a nuanced approach in its clinical application.

4 Minerals and MAFLD

4.1 Minerals deficiency status in MAFLD patients

Mineral deficiencies are widely acknowledged as a significant public health issue worldwide, often leading to increased susceptibility to infections. By replenishing these deficient trace minerals to their recommended levels, we can enhance immune function, bolster resistance to infection, and facilitate quicker recovery from such illnesses. While epidemiological data on the connection between trace mineral deficiencies and the onset and advancement of MAFLD are scant, the role of inflammation as a key contributor to MAFLD, coupled with the dietary habits commonly observed in individuals with MAFLD, suggests a potential close link between these mineral deficiencies and the disease's development and progression.

4.2 Role of minerals deficiency in the process of MAFLD progression

4.2.1 Major minerals

Calcium, phosphorus, and magnesium, as major minerals, play important roles in MAFLD. These minerals are key factors in the inflammatory processes related to MAFLD, participating in signaling mechanisms, hepatocyte injury and regeneration, and the regulation of inflammatory factors (62, 97–99). The intricate roles of these major minerals in the human body and their specific associations with MAFLD have been extensively discussed in other reviews (100) and studies (101), and thus fall outside the primary focus of this paper. Instead, this review concentrates on trace minerals, including zinc, iron, copper, and selenium, exploring their relationship with MAFLD and their impact on the progression and management of the disease.

4.2.2 Zinc and MAFLD

Zinc, a crucial trace element, plays vital roles in antioxidant, anti-inflammatory, and anti-apoptotic functions in the human body (102, 103). The risk of zinc deficiency increases with age (104). There is growing evidence linking zinc deficiency to the development of MAFLD (105, 106). In patients with biopsy-proven MAFLD, a J-shaped correlation exists between serum zinc levels and the severity of hepatic necroinflammation (107, 108). Serum zinc deficiency, commonly associated with oxidative stress, endoplasmic reticulum stress, apoptosis, and inflammation, has been noted in MAFLD mouse models (109, 110).

Furthermore, zinc supplementation has been shown to alleviate disorders in lipid and glucose metabolism caused by high-fat diets (111). In diet-induced MAFLD mice, zinc supplementation not only improves liver weight and morphology but also helps prevent hepatic failure (112).

Recent studies reveal zinc's mechanisms in improving MAFLD: in mouse models, PLZF, relying on SIRT1, regulates hepatic lipid and glucose homeostasis (113). The HDAC3/β-catenin pathway promotes lipolysis and inhibits adipogenesis (114). ZHX2 activation of PTEN protects against MASH progression (115). Zinc alpha2 glycoprotein in hepatocytes impacts triglyceride accumulation and key gene expressions (116). The ADA/XO/UA pathway and caspase 3 signaling show potential in liver rescue (116). Zinc oxide nanoparticles in mice reduce hepatic steatosis via the AMPK axis (117), while the Zn2+/MTF-1/PPARa pathway aids in reducing lipid deposition (118). These findings collectively highlight zinc's multifaceted role in addressing various aspects of MAFLD pathogenesis and progression.

Despite that, the specific studies and recommended zinc dosages for clinical treatment of MAFLD still require further exploration and validation.

4.2.3 Iron and MAFLD

The increasingly recognized causal link between iron overload and the progression of MAFLD (119, 120) suggests that dietary iron overload may worsen inflammation and lipid metabolism disorders, akin to human dietary iron overload syndrome (DIOS) (40). Hyperferritinemia the main manifestation of disturbed iron homeostasis often portends more severe metabolic dysfunction and liver injury (121, 122). In the hypoxic intestinal environment, HIF-2alpha plays a crucial role in regulating iron absorption by affecting the DMT1 gene (123). Abnormal iron-induced hepcidin

release, influenced by natural genetic variants may enhance iron absorption (124). Additionally, excess free fatty acids (FFAs) disrupt hepatic iron metabolism, encouraging iron uptake via IRP1 and TfR-1 (125). Iron overload contributes to ferroptosis, initiating inflammation in nonalcoholic steatohepatitis and leading to oxidative DNA damage (126, 127). This condition can be exacerbated by a high-fat diet, which aggravates lipid metabolism disorders, hepatic injury, and oxidative stress (128). Iron-containing extracellular vesicles from hepatocytes induce liver steatosis and fibrosis in mice on a Western diet, causing iron deficiency in hepatocytes and overload in hepatic stellate cells (129). The complex interaction between gut microflora and the host not only impacts MAFLD progression but also influences iron balance (130, 131). This situation results in a detrimental cycle where iron overload increases lipid deposition through oxidative stress-induced mitochondrial dysfunction and activation of the HIF1α-PPARγ pathway (129, 132).

While numerous studies have indicated that bloodletting to address iron overload can improve insulin resistance in patients with MAFLD and hyperferritinemia (133–135), other findings suggest that lowering ferritin through phlebotomy does not necessarily improve liver enzymes, liver fat, or insulin resistance in MAFLD patients (136). This discrepancy highlights the need for more detailed research to unravel these complex interactions and effects.

4.2.4 Copper and MAFLD

Copper, a vital cofactor in numerous physiological redox reactions, has a complex relationship with MAFLD. *In vivo* bioluminescence imaging has shown copper deficiency in a mouse model of MAFLD (137). Concurrently, both hair and hepatic copper concentrations in MAFLD patients are significantly lower and correlate with increased hepatic steatosis, MASH severity, and metabolite alterations (138). Limiting copper intake in mice has been shown to induce hepatic steatosis and insulin resistance, leading to the development of MAFLD (17, 139).

Research exploring the link between copper and lipid metabolism indicates a negative correlation (140). Restoration of intrahepatic copper, achieved by down-regulating copper cyanin, enhances lipolysis through the assembly of copper-loaded SCO1-LKB1-AMPK complexes, showing improvements in MAFLD conditions in mice (141). A case-control study found that high levels of copper significantly improved MAFL in males, highlighting copper's protective role in MAFLD treatment (140, 142).

However, studies also point out the harmful effects of copper overload on lipid metabolism (143, 144) and increased MAFLD risk and severity (145). Moreover, there is a noticeable gap in clinical research exploring the relationship between copper and MAFLD, and the potential toxicology of copper also warrants special attention. Despite these complexities, the intricate link between copper and MAFLD presents a potential avenue for breakthroughs in MAFLD treatment.

4.2.5 Selenium and MAFLD

Selenium, a crucial micronutrient, plays diverse roles in the human body, including antioxidant activities, cancer prevention, and immunomodulation, thanks to its structural and enzymatic functions (146–148). It also has significant implications in metabolic diseases (149). The relationship between selenium and MAFLD is complex and appears to be dose-dependent (150).

Studies have shown that lower blood selenium levels are associated with a higher incidence of advanced liver fibrosis (151). Conversely, higher blood selenium levels (above ~130 μg/L) have been positively correlated with both MAFLD and ghrelin, indicating a dose–response relationship (150, 152). In experimental settings, selenium supplementation in MAFLD mice models has demonstrated beneficial effects, such as mitigating hepatic injury, reducing oxidative stress, lowering insulin resistance, and decreasing inflammation (6, 41, 43, 153).

However, the use of selenium in MAFLD treatment necessitates careful consideration of its delicate balance between therapeutic efficacy and toxicity. Determining the appropriate dosage of selenium is critical and remains a subject of ongoing research and debate in the context of MAFLD treatment. This nuanced understanding of selenium's role underscores the importance of precise dosing in its potential application as a therapeutic agent for MAFLD.

4.3 Relationship between micronutrients in MAFLD

While individual trace elements' roles in MAFLD have been detailed, research on their complex interrelationships is scarce. Zinc and selenium have been linked to reduced cardiovascular risk (109), and Vitamin D and zinc both enhance immune function (154). Additionally, copper and ascorbic acid can interfere with non-heme iron absorption (155). These findings indicate a delicate balance among trace elements, crucial for maintaining overall body homeostasis.

5 Conclusion and outlook

In this review, we examine recent research on the impact of various vitamins and trace minerals on MAFLD. Most studies suggest that deficiencies in vitamins and minerals negatively affect MAFLD. Timely and appropriate supplementation could aid in disease recovery or slow its progression, potentially improving patient prognosis. However, there are also contrasting views or skepticism regarding the causal link between these deficiencies and MAFLD, an aspect this review critically explores.

Currently, there's no unified approach to the pharmacological treatment of MAFLD. Lifestyle interventions and bariatric surgery have shown relative effectiveness, but their success is often limited by patient compliance and eligibility criteria. Hence, their widespread application among MAFLD patients is restricted. The search for effective drugs targeting MAFLD's pathogenesis continues. Vitamins and minerals, crucial in regulating oxidative stress, inflammation, and lipid metabolism, offer promising directions for MAFLD treatment. The need for a drug that can improve the course and prognosis of MAFLD, provided in the necessary amounts for normal body function, is urgent.

Given the complex pathophysiology of MAFLD, the effectiveness of single-agent treatments observed in various studies suggests that individualized combination regimens might be necessary for optimal management of MAFLD. This review seeks to shed light on these multifaceted approaches and the potential of vitamins and minerals in the treatment landscape of MAFLD.

Author contributions

YL: Writing – original draft. XQ: Writing – original draft. TC: Writing – original draft. MC: Writing – original draft. LW: Writing – review & editing. BH: 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

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Nutritional support therapy for liver transplantation in an adult-onset type II citrullinemia patient: a case report

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Liver transplantation is an effective measure to treat adult-onset type II citrullinemia (CTLN2). Active and effective perioperative nutrition support is a very important treatment for the prognosis of such patients. In this paper, we analyzed the process, results, and outcome of nutritional support therapy in a case of CTLN2, and concluded that the perioperative nutritional support program for CTLN2 patients should be followed prior to surgery:1.because of the prevalence of severe malnutrition in CTLN2 patients, Enteral nutrition (EN) combined with Parenteral nutrition (PN) should be the first choice for nutritional support; 2. daily energy intake should be 35 ~ 40 kcal/kg; 3. the nutritional formula should be composed of low-carbohydrates and high medium-chain triglyceride (MCT). Postoperative: initiating EN as soon as possible is recommended to restore intestinal function and adjuvant PN might be taken into consideration in the early stage. The purpose of this case was to provide experience for the development and adjustment of the perioperative nutritional support regimen for CTLN2 patients.

KEYWORDS

CTLN2, liver transplantation, nutritional support therapy, MCT, case report

1 Introduction

Adult-onset type II citrullinemia (CTLN2) is one of the metabolic diseases characterized by hyperammonemia and an elevated level of serum citrulline, which is mainly manifested as wasting and recurring episodes of consciousness disturbance, and shows a dietary preference for protein-and lipid-rich foods (1). It is mostly caused by Citrin deficiency (CD). Citrin is an aspartate-glutamate carrier, which is closely associated with hepatic energy supply. CD is caused by mutations of the *SLC25A13* gene on chromosome 7q21.3 that can cause neonatal intrahepatic cholestasis (NICCD) or CTLN2 (2). Liver transplantation has been considered as the most effective therapy for CTLN2 (3). However, the poor preoperative nutritional status of the patients significantly increases the incidence of post-transplant complications, morbidity and mortality. Therefore, active and efficient nutritional support during the perioperative period is important for the prognosis of patients. Recent studies have indicated that administering arginine and sodium pyruvate alongside a carbohydrate-restricted diet can

serve as an effective therapy for patients with CTLN2. Additionally, it has been suggested that providing intensive nutritional support may enhance patient survival (3). The 2020 European Society of Parenteral and Enteral Nutrition (ESPEN) practical guideline on clinical nutrition in liver disease recommends vegetable protein diets as the preferred nitrogen sources for managing hepatic encephalopathy (HE), while suggesting the avoidance of meat proteins as much as possible (4). Previously, various guidelines have emphasized clinical and nutritional aspects for patients with liver diseases such as HE, liver cirrhosis, and liver transplantation. However, limited information is available regarding CTLN2 (4–8). In this case, the patient's serum ammonia levels were progressively elevated despite receiving the regular enteral nutritional (EN) formulations with vegetable protein preoperatively. The clinical pharmacist analyzed the factors that contributed to the patient's systemic condition and worked with the surgeon to adjust the nutrition support regimen, which allowed the patient's serum ammonia to return to normal and improved his nutritional status. The liver transplantation was subsequently carried out successfully. After liver transplantation, the clinical pharmacist performed regular nutritional assessments of the patient and proceeded to alter the nutritional support protocol based on the patient's systemic condition. The report of this case aimed to provide the clinical treatment experience for the development and adjustment of the perioperative nutritional support regimen for CTLN2 patients with liver transplantation.

2 Case presentation

A 22-year-old male was admitted to the hospital because of an increased level of serum ammonia for 8 months and an altered sleep pattern lasting over 7 months. He developed aberrant behavior with no apparent cause during the previous 8 months, which presented with blurred consciousness, dressing and undressing without purpose, involuntary vocalizations, accompanied by flapping tremors in both hands, lasting four to 5 h, occurring once every five to 6 days; or presented with nighttime sleep disturbances, irritability, and no response to sound, lasting four to 5 h with relief, occurring once every three to 4 days.

The patient was treated symptomatically at a local hospital, but the same kind of symptoms were recurrent. Then he was transferred to Nanjing Brain Hospital. The cranial magnetic resonance imaging (MRI) revealed signals and perfusion abnormalities as follows: 1. Multiple abnormal signals were detected in the bilateral temporal-parietal cortex, bilateral corona radiata and deep within the left temporal lobes. 2. The bilateral temporal-parietal lobes exhibited hypoperfusion, with regional hyperperfusion noted in the left temporal cortex. Abdominal computed tomography (CT) suggested chronic pancreatitis. For further treatment, he was referred to the department of infectious disease of our hospital. On admission, he weighed 32 kg and was 166 cm tall. His body mass index (BMI) was 11.61 kg/m^2. The results of laboratory examinations were shown in Table 1. DNA analysis revealed compound heterozygous mutations in *SLC25A13*, leading to the diagnosis of CTLN2. Although the patient underwent conservative therapy with the administration of arginine and ornithine/aspartate to reduce serum ammonia, lactulose for promoting the excretion of intestinal ammonia, diammonium glycyrrhizinate for hepatocyte protection, and ursodeoxycholic acid for jaundice, he still displayed neuropsychiatric symptoms including recurrent episodes of altered consciousness. Therefore, the patient was advised and opted to undergo liver transplantation. After admission, the patient continued to receive conservative therapy. He was alert with able to eat on his own. Results of nutritional risk screening and nutritional assessment were as follows: ① Nutrition Risk Screening 2002 (NRS 2002) score 4 indicated malnutrition risk. ② Patient-Generated Subjective Global Assessment (PG-SGA) score 10 indicated the nutritional intervention was urgently required. ③ According to body composition analysis (Table 2), the patient was severely malnourished and had abnormally low levels of water, muscle, and fat. ④ Oral intake: patient had normal oral intake where there was no encephalopathy, but it decreased by more than 80% at the onset of encephalopathy.

Considering the patient was Child-Pugh class B, and his poor nutritional condition, elevated serum ammonia levels and triglycerides, the clinical pharmacist advised the patient to take Enteral Nutritional Suspension (TPF-D) (ABBOTT LABORATORIES B.V., Netherlands) with vegetable protein orally (500 mL, once a day) in combination with fat-free parenteral nutrition solution (420Kcal) for initial nutritional support. The next day, the patient's serum ammonia was retested and the results revealed a rise from the earlier level (164 μmol/L → 208 μmol/L). After reviewing the literature, the clinical pharmacist suggested that the Enteral Nutritional Suspension (TPF-D) should be adjusted to Enteral Nutritional Suspension (TP-MCT) (Nutricia, Netherlands) (500 mL, once a day), which is rich in medium-chain triglycerides (MCT), in combination with parenteral nutrition (PN) containing structural fat emulsion (Fresenius Kabi AB, Switzerland) (870 Kcal). Serum ammonia steadily improved.

After receiving 7 days of nutritional support, the patient underwent split liver transplantation (left lobe). 24 h post operatively, patient was started on liquid diet and was treated with Enteral Nutritional Suspension (TP-MCT) (500 mL, once a day) in combination with parenteral nutrition containing structural fat emulsion (870 Kcal). Ammonia returned to normal by post operative day 5 and patient refused to take enteral nutritional supplements. Following nutritional assessment, the clinical pharmacist recommended adding a lipid-restricted and protein-rich diet instead of EN combined with PN and formulated individualized dietary recommendations for the patient (Table 3). On postoperative day 11, his oral intake was almost 900 kcal per day, meeting more than 60% of the daily energy needs. The clinical pharmacist considered adding oral Enteral Nutritional Powder (TP) (ABBOTTLABORATORIES.B.V., Netherlands) at 50 g per day while stopping PN for the patient. On postoperative Day 32, the patient's weight increased by 18.7% to 38 kg, and his albumin level improved by 22% to 39 g/L. Because of his good nutritional recovery, patient was discharged home on post operative day 32. The fluctuation of parameters in relation to liver function and renal function during the patient's hospitalization were shown in Figures 1, 2.

The patient was followed up at 3, 6, and 10 months after liver transplantation, and his body weight and albumin level all gradually increased (Figure 3). The body composition analysis revealed a 40.96% rise in weight, a 37.14% increase in muscle, and a 100% increase in body fat compared to the preoperative period, which was repeated at 10 months post operation (Table 2). Considering the patient's rapid growth in body fat, the clinical pharmacist advised the patient to adjust his diet to increase the proportion of protein intake, and also provided specific recommendations on the amount of food to eat

TABLE 1 The results of laboratory examinations.

Indicators	Admission	Peoperative	Post-1d	Post-5d	Post-11d	Post-1 M	Reference range
WBC (10^9/L)	5.6	4.1	7.7	2.9	7.5	15.7	3.5-9.5
NEUT (%)	27.7	42.5	82.1	86.2	73.5	71.8	40-70
RBC (10^{12}/L)	4.25	2.96	2.96	2.77	2.86	4.26	4.3-5.8
Hb (g/L)	120	93	92	84	90	135	130-175
ALT (U/L)	100.6	22.8	404.1	109.2	22.3	29.0	5-40
AST (U/L)	110.5	31.5	517.7	42.3	15.6	20.2	8-40
ALP (U/L)	219.3	116.1	48.5	47.9	71.5	62.2	47-185
GGT (U/L)	314.5	89.5	68.2	45.9	46.9	48.9	11-50
TBIL (μmol/L)	13.9	3.8	92.1	51.1	90.1	23.8	5-20.5
DBIL (μmol/L)	7.1	2.3	47.0	34.1	57.4	17.2	1.7-6.8
ALB (g/L)	31.9	29.0	33.1	34.6	40.8	40.8	40-55
Triglyceride(mmol/)	2.28	5.17	0.25	0.83	0.59	0.87	0.56-1.7
Cholesterol(mmol/L)	2.87	2.76	1.14	1.74	0.18	3.01	2.9-5.72
AFP (ng/ml)	12.80	6.90	–	–	–	–	0-10
Serum ammonia (μmol/L)	289.0	69.0	28.0	20.0	9.0	9.0	9-30
Uric acid (μmol/L)	433	353	343	304	129	350	90-420
Cre (μmol/L)	45	43	67	60	30	43	44-106
Urea (mmol/L)	2.9	6.3	7.9	10.1	3.2	8.4	2.9-7.5
eGFR	216.5	228.2	136.8	155.4	345.7	228.2	>90

Post-1d, 1 day post-operation; Post-5d, 5 days post-operation; Post-11d, 11 days post-operation; Post-1 M, 1 month post-operation; WBC, White blood cell; NEUT, Neutrophil; RBC, Red blood cell; Hb, Hemoglobin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transferase; TBIL, Total bilirubin; DBIL, Direct bilirubin; ALB, Albumin; AFP, Alpha-fetoprotein; Cre, Creatinine.

TABLE 2 Body composition analysis.

	BW(kg)	BMI (kg/m^2)	Water (e)	skeletal muscle(kg)	Fat(kg)	BMR(kcal)
Admission	33.2	12.3	20.6	14.2	5.2	919 ~ 1041
Post-10 M	46.8	17.4	26.8	19.4	10.4	1143 ~ 1315

BW, Body weight; BMI, Body mass index; BMR, Basal metabolic rate; Post-10 M, 10 months post-operation.

TABLE 3 Individualized suggestions for diet.

Date	Actual intake (kcal/d)	Target intake (kcal/d)	Starch (g)	Meat (g)	Egg (g)	Milk (g)	Vegetables (g)
Post-5d	250	1,274	175	125	50	160	500
Post-11d	990	1,274	175	125	50	160	500
Post-10 M	2400	1,504	225	225	50	320	500

Post-5d, 5 days post-operation; Post-11d, 11 days post-operation; Post-10 M, 10 months post-operation.

(Table 3). During 10 months of follow up after liver transplantation, he remained in good condition without an episode of hepatic encephalopathy, required biliary stent placement for biliary stricture, and his liver function remained normal thereafter. Furthermore, he has also maintained a stable weight of approximately 45 kg.

3 Discussion

CTLN2 is an autosomal recessive disease caused by mutations in the *SLC25A13* gene which encodes Citrin, the liver-specific isoform 2 of the mitochondrial aspartate/glutamate carrier. The prevalence of citrin deficiency (CD) has been reported to be approximately 1 in 7100 persons, and relatively common in East Asia and Southeast Asia. CD patients demonstrate some typical clinical features with age. Neonatal intrahepatic cholestasis (NICCD) caused by citrin deficiency, which manifests as cholestasis, galactosemia, hypoproteinemia, and dysplasia. After recovering from NICCD, many patients are asymptomatic except for their dietary preferences, while others complain of fatigue, hypoglycemia, dyslipidemia and short stature, which are designated as failure to thrive and dyslipidemia caused by CD (FTTDCD). In adulthood, <10 % CD patients develop CTLN2.

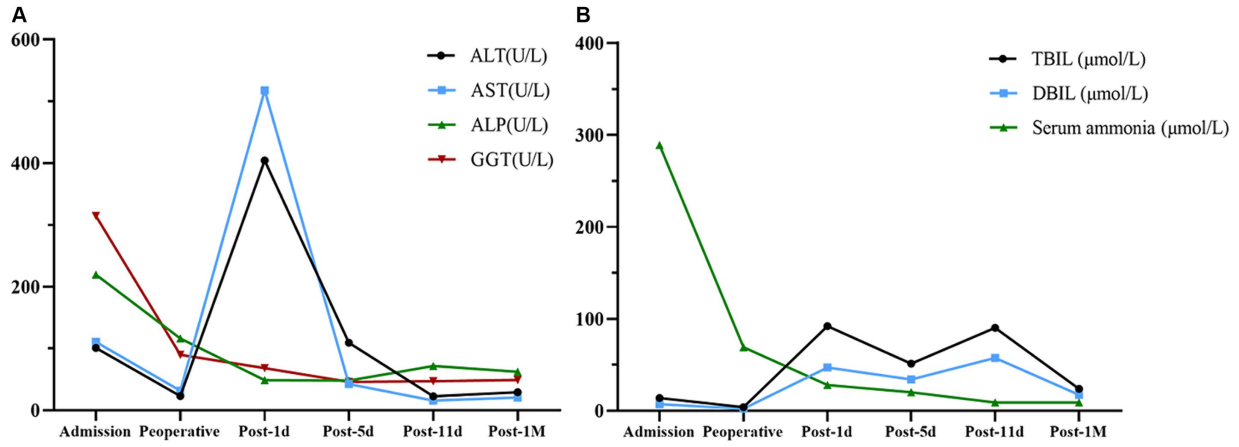

FIGURE 1
Changes in parameters related to liver function. **(A)** Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) fluctuation. **(B)** Total bilirubin (TBIL), direct bilirubin (DBIL), and serum ammonia fluctuation.

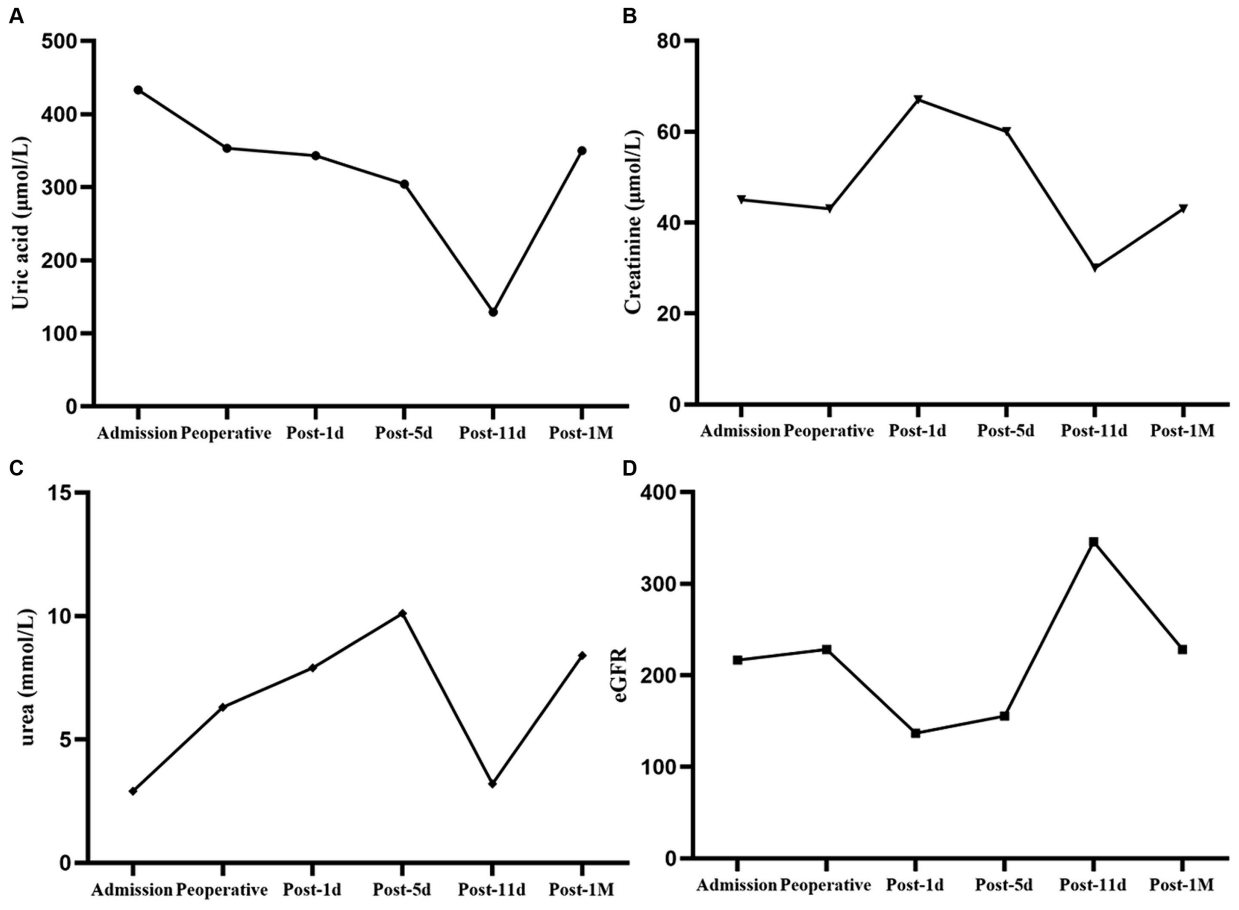

FIGURE 2
Changes in parameters related to renal function. **(A)** Uric acid fluctuation. **(B)** Creatinine fluctuation. **(C)** Urea fluctuation. **(D)** eGFR fluctuation.

CTLN2 shows clinical manifestations with hyperammonemia and citrullinemia, and repeatedly impaired consciousness. It is reported that the prognosis of CTLN2 patients exhibiting hyperammonemia and serious encephalopathy is poor (9). A review of CTLN2 revealed that while conservative therapies, such as arginine and sodium pyruvate administration, high doses of glycerol and fructose infusion for brain edema, hyperalimentation, a low-protein and high-carbohydrate diet, have provided temporary relief from hyperammonemia, they have not improved the long-term prognosis (3). Several studies have demonstrated that the majority of surviving

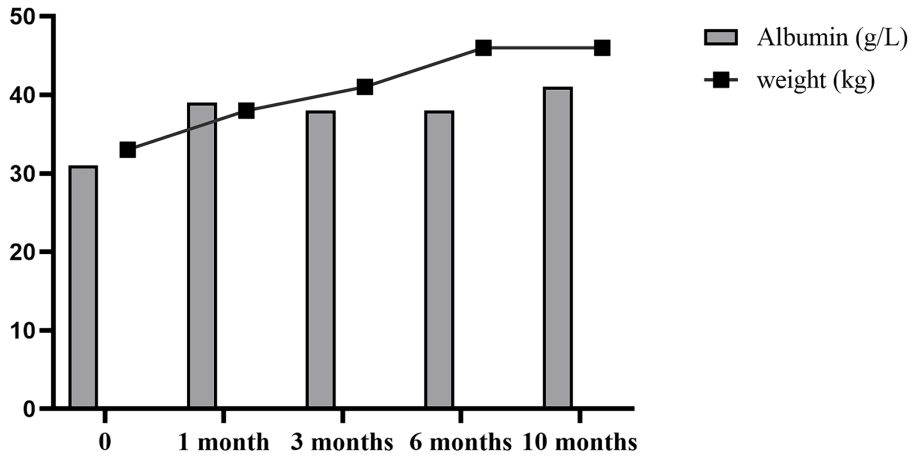

FIGURE 3
Changes in weight and albumin at 1, 3, 6, and 10 months after surgery.

CTLN2 patients undergoing conservative therapies have been managed with the administration of arginine in conjunction with a carbohydrate-restricted diet. Conversely, there is a risk of mortality associated with inadequate treatments, such as solely relying on intravenous infusion of hyperalimentation fluid containing branched-chain amino acids, implementing a low-protein diet, or using glycerol and fructose infusion for brain edema (10–12). Therefore, if therapies aimed at reducing ammonia levels and providing neuroprotection proved ineffective, the decision for liver transplantation should be made. In this case, the patient also underwent an extended period of conservative therapies, including administration of arginine and ornithine/aspartate to reduce serum ammonia, lactulose for promoting the excretion of intestinal ammonia, diammonium glycyrrhizinate for hepatocyte protection, and ursodeoxycholic acid for jaundice. Despite these interventions, the treatment was ineffective, resulting in the development of HE and necessitating a liver transplantation.

2020 European Society of Parenteral and Enteral Nutrition (ESPEN) practical guideline: Clinical nutrition in liver disease recommends that liver cirrhosis patients scheduled for elective surgery or listed for transplantation should be screened and assessed for malnutrition timely to treat malnutrition before surgery and thereby improve body protein status (4). EN should be used when patients cannot meet their caloric requirements through normal food and/or oral nutritional supplement (ONS), and PN shall be commenced immediately in moderately or severely malnourished patients who cannot be nourished sufficiently by oral and/or enteral route (13). For the intake of nitrogen sources in HE, it is advised to consume nitrogen sources rich in plant proteins and to minimize the intake of animal proteins as much as possible (8, 14). In this case, considering the patient's HE and elevated triglyceride (TG) levels, the initial nutritional support provided before liver transplantation consisted of an enteral nutritional suspension containing vegetable proteins along with a fat-free parenteral nutrition solution. Nevertheless, the patient's serum ammonia levels were reassessed after just 1 day on this nutritional support, and surprisingly, the outcome revealed an increase in serum ammonia rather than a decrease. The nutritional recommendations based on the current guidelines were not found to be applicable for practical management of our patient, we had to formulate a more individualized nutritional treatment plan based on the patient's pathophysiological characteristics and thorough review of relevant literature.In light of this, extensive literature review revealed that medium-chain triglycerides (MCT) have shown promise in ameliorating the symptoms of patients with CD. The use of MCT and lactose-restricted formula has been identified as an effective therapy for intrahepatic cholestasis in NICCD (9). Meanwhile, a low-protein, high-sugar diet, a common treatment for hepatic encephalopathy, exacerbates hyperammonemia in CTLN2. NICCD and CTLN2 share a common cause in CD. Citrin is closely linked to hepatic energy metabolism, and its deficiency is anticipated to impair glycolysis and *de novo* lipogenesis, leading to a persistent and cumulative energy deficit in hepatocytes. MCT undergo rapid hydrolysis, traverse the liver via the portal vein, and are metabolized into acetyl-CoA by β-oxidation, thereby enhancing tricarboxylic acid (TCA) cycle activity, augmenting adenosine triphosphate (ATP) production, and facilitating lipogenesis. Moreover, the heightened ATP levels additionally bolster the efficiency of the ammonia-detoxification system (15). Therefore, in this case, the patient's nutritional support was modified to include MCT-rich enteral nutrition suspension combined with parenteral nutrition featuring a structured fat emulsion. Following this nutritional plan, the patient's serum ammonia levels remained stable, and he underwent liver transplantation without experiencing HE. It was documented that intake of low-carbohydrate helps patients with CTLN2 reduce the accumulation of carbohydrates, and a high-MCT diet ameliorates their disorders of energy metabolism disorders, as well as improving their ammonia-detoxification system. The studies by Hayasaka et al. also confirmed this viewpoint (1, 16). At the same time, CTLN2 patients exhibit a dietary preference to a high-fat diet and distaste for carbohydrates due to their unique metabolic patterns. Nutritionists or nutritional pharmacists need to maintain their dietary habits rich in fat and protein to offset their abnormal metabolism (9).

Restoring intestinal function promptly after surgery is a crucial therapeutic objective of postoperative nutritional support for liver transplantation. ESPEN and EASL both recommend initiating EN as soon as possible after liver transplantation to ensure adequate protein and energy supply, reduce infection rates due to altered intestinal flora, and that PN should be used when EN intake falls below 60% of the

target energy and protein requirements. During the perioperative period in liver transplant patients, it is crucial to highlight that the optimal daily energy intake should be 30-35 kcal/(kg d) or 1.3 times the Basal Metabolic Rate (BMR), and protein intake should range between 1.2-1.5 g/(kg d). Research has shown that when EN is administered alone, patients with severe malnutrition have a poor intestinal absorption and tolerance, leading to a high rate of discontinuation EN or unsatisfied intake. In contrast, the combination of EN with PN has been found to be more beneficial for these patients than EN alone (17). In this case, the patient received EN in conjunction with PN 48 h postoperatively to promote early oral feeding. Moreover, we routinely performed nutritional assessments and tailored personalized nutritional support for the patient postoperatively. We also advised dietary interventions to maintain the appropriate protein-fat-carbohydrate ratio. For instance, a recommended ratio of 15-25% protein, 40-50% fat, and 30-40% carbohydrate was suggested (9). Additionally, the patient was required to take long-term immunosuppressants like tacrolimus after surgery to combat immune rejection. Therefore, it is advisable to avoid foods that interact with tacrolimus, such as grapefruit and oranges.

Most liver transplant patients undergo rapid weight gain within 2 years post-surgery attributed to prolonged supplementation, extended period of rest, enhanced liver function, and administration of immunosuppressive medications. Research indicated that up to 26% of liver transplant patients may develop new-onset obesity (18). The increase in weight and obesity can escalate the risk of metabolic syndrome, consequently heightening the chances of cardiovascular morbidity and mortality. The accelerated accumulation of body fat observed in this patient 10 months post-surgery was closely associated with dietary patterns and physical activity levels, potentially escalating the susceptibility to metabolic disorders. Therefore, prolonged dietary management post-liver transplantation should prioritize weight control, particularly focused on curtailing body fat gain, to mitigate the onset of obesity or sarcopenia (19).

4 Conclusion

This paper summarizes the nutritional support treatment for liver transplantation in an adult-onset type II citrullinemia (CTLN2) patient as follows: 1. Preoperative severe malnutrition is prevalent among CTLN2 patients undergoing liver transplantation. It is suggested that adhering to the principles of low-carbohydrate, high-medium-chain triglyceride (MCT) nutritional formulas is beneficial in enhancing the nutritional status of CTLN2 patients. 2. For severely malnourished liver transplant patients, EN combined with supplementary PN can even better achieve the target energy requirement. 3. Long-term nutritional management post-liver transplantation should emphasize weight control to mitigate the onset of metabolic disorders.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Ethics Committee of Nanjing Drum Tower Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YD: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. Y-YF: Conceptualization, Data curation, Writing – original draft, Project administration. YY: Formal analysis, Supervision, Validation, Writing – review & editing. BH: Formal analysis, Supervision, Validation, Writing – review & editing. W-JZ: Supervision, Validation, Writing – review & editing. D-CY: Supervision, Validation, Writing – review & editing, Funding acquisition, Visualization, Resources. X-JB: Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing, Resources.

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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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Probiotic supplementation for 24 weeks in patients with non-alcoholic steatohepatitis: the PROBILIVER randomized clinical trial

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Background and aim: Considering the increasing prevalence of non-alcoholic steatohepatitis (NASH) and treatment gaps, this study aimed to evaluate the effect of probiotic supplementation on liver function markers, nutritional status, and clinical parameters.

Methods: This double-blind, randomized clinical trial (ClinicalTrials.gov ID: NCT0346782) included adult outpatients with biopsy-proven NASH. The intervention consisted of 24 weeks of supplementation with the probiotic mix *Lactobacillus acidophilus* (1×10^9CFU)+*Lactobacillus rhamnosus* (1×10^9CFU)+*Lactobacillus paracasei* (1×10^9CFU)+*Bifidobacterium lactis* (1×10^9CFU), or placebo, twice a day. The following parameters were evaluated: demographic and clinical data, transient elastography (FibroScan), liver enzymes, NAFLD *fibrosis score*, fatty liver index, laboratory assessment, serum concentration of toll-like receptor-4 (sTLR-4) and cytokeratin 18 (CK-18), anthropometric data, dietary intake, and physical activity. Regarding data analysis, the comparison between the groups was based on the delta of the difference of each variable analyzed (value at the end of treatment minus the baseline value) using the *t*-test for independent samples or the Mann–Whitney *U*-test.

Results: Forty-four patients with NASH completed the trial (51.4±11.6years). At baseline, 87% of participants had a mild liver fibrosis degree on biopsy, normal values of liver enzymes, transient elastography values consistent with grade 1 fibrosis in both groups, increased waist circumference (WC), a BMI of 30.97 kg/m^2, and 76% presented with metabolic syndrome (MetS). After the intervention, no differences were observed between the probiotic and placebo groups in terms of MetS, WC, BMI scores, or liver enzyme levels ($p>0.05$ for all). The elastography values remained consistent with grade 1 fibrosis in both groups. Although CK-18 was reduced in both groups, a larger effect size was noted in the probiotic group ($D=1.336$). sTLR-4 was also reduced in both groups, with no difference between groups ($p=0.885$).

Conclusion: Intervention with probiotics in the early stages of NASH demonstrated no significant change in hepatic and clinical parameters.

Clinical trial registration: ClinicalTrials.gov, identifier NCT0346782.

KEYWORDS

non-alcoholic fatty liver disease, probiotics, toll-like receptor-4, cytokeratin 18, metabolic syndrome, microbiota, metabolic dysfunction-associated steatotic liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) and its progressive form of non-alcoholic steatohepatitis (NASH) are considered hepatic manifestations of metabolic syndrome (MetS) (1). Recently, NAFLD was renamed metabolic dysfunction-associated steatotic liver disease (MASLD) (2). The disease affects approximately a quarter of the world's population and is associated with a sedentary lifestyle and a western diet. Thus far, lifestyle changes constitute the first line of treatment, as there is yet no specific pharmacological treatment approved for the disease (1, 2).

It is important to understand the pathogenesis of the disease and the role of the intestinal microbiota in its progression, which may pave the way to discovering new therapies and strategies to control NAFLD. Gut microbiota (GM) has been identified as a potential therapeutic target for patients with NASH (3, 4), as studies have shown changes in the intestinal microbiota of patients with NAFLD and NASH (5–7). A relatively high abundance of genera *Fusobacteria* and a low abundance of genera *Oscillospira* and *Ruminococcus* of the family *Ruminococcaceae* and *Coprococcus* of the family *Lachnospiraceae* were found more in NAFLD patients in parallel with healthy individuals (8). Other bacterial species found in these patients were Proteobacteria, Escherichia, and Enterobacteria (9), and Bacteroides were more common in NASH patients in parallel with healthy individuals (10).

Studies with probiotics have shown benefits in reducing levels of liver enzymes, triglycerides (TGs), low-density lipoprotein cholesterol (LDL-c), steatosis severity, pro-inflammatory cytokines, and even in obesity parameters, such as reduction of visceral and body fat (5, 11, 12). In this context, a recent systematic review followed by a meta-analysis (13), which included 18 RCTs on interventions with probiotic mix (intervention period ranged from 2 to 14 months), demonstrated the effectiveness of these interventions in reducing steatosis (assessed through ultrasound), showing a slight reduction in fibrosis (assessed through elastography), as well as a decrease in levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT). However, the authors state that more studies are necessary due to significant heterogeneity in the probiotic strains used, dosages, and formulations. Another recent systematic review, followed by a meta-analysis (14), evaluated the effect of probiotics on NAFLD parameters and also demonstrated an effect on parameters such as reduction of ALT and AST, serum lipids, glucose, and insulin. In this study, the authors included 21 RCTs, and the intervention time varied from 8 to 56 weeks, with more important effects being observed in studies lasting 12 weeks or longer.

Microbiota dysbiosis is a contributing factor to the disease pathogenesis, related to intestinal barrier dysfunction and increased permeability, exposing the liver to microbial translocation (5). This process activates toll-like receptor 4 (TLR-4), causing an inflammatory response in the liver and initiating the inflammatory cascade (7).

Biopsy is the gold standard for NASH, although it is still considered a risky procedure. Less invasive tests, such as serum cytokeratin 18 (CK-18), have been proposed as promising alternatives to liver biopsy to diagnose NASH and monitor disease progression and response to therapy (15). In addition, scores such as the NAFLD fibrosis score, APRI score, and fat liver index are widely used and have the advantage of not being invasive (16–19). Considering the high prevalence of NASH in the population and the existing gaps in its treatment, this randomized study aimed to evaluate the effect of 24 weeks mix probiotic supplementation [*Lactobacillus acidophilus* (1×10^9 CFU) + *Lactobacillus rhamnosus* (1×10^9 CFU) + *Lactobacillus paracasei* (1×10^9 CFU) + *Bifidobacterium lactis* (1×10^9 CFU)] on liver function markers and clinical parameters in NASH patients.

Methods

This study is a single-center, randomized, double-blind, placebo-controlled clinical trial that included adult subjects at an outpatient clinic of the Gastroenterology and Nutrition and Dietetic Division of Hospital de Clínicas de Porto Alegre, Brazil. The study included only patients with proven liver biopsy NASH (NAS ≥4), defined as the presence of ≥5% of hepatic steatosis and inflammation with hepatocyte injury, with or without fibrosis, and the absence of causes for secondary hepatic fat accumulation, according to the American Association for the Study of Liver Diseases (AASLD) (1). We investigated the history of alcohol use, hemochromatosis, or Wilson disease, and the history of hepatotoxic drug use. Patients who met the inclusion criteria were enrolled after signing written informed consent. Patients with human immunodeficiency virus, hepatitis B or hepatitis C virus infection, cirrhosis, pregnancy, liver transplantation, supplements and foods with probiotics, immunosuppressants, antibiotic use in the last 6 months, and any other chronic inflammatory diseases were excluded.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the ethics and research committee of the *Hospital de Clínicas de Porto Alegre* (Approval Number 16-0438). The protocol was registered at *ClinicalTrials.gov* under the identifier NCT03467282. Written informed consent was obtained from all subjects.

Intervention

Patients were randomized to an intervention or a placebo group in a numerical sequence on the *randomization.com* website. Patients from the intervention group received probiotic supplementation consisting

of a 1 g-sachet containing *Lactobacillus acidophilus* NCFM (1×10^9 CFU) + *Lactobacillus rhamnosus* HN001 (1×10^9 CFU) + *Lactobacillus paracasei* LPC-37 (1×10^9 CFU) + *Bifidobacterium lactis* HN019 (1×10^9 CFU). Patients from the control group received a 1 g sachet with an identical appearance (physical and organoleptic) containing polydextrose/maltodextrin as the placebo. They were instructed to ingest two sachets diluted in water at room temperature daily, before the first meal of the day, for 24 weeks.

During the entire clinical trial period, including the data analysis stage, patients and investigators were blinded to the composition of the sachet content. Only an external researcher had access to this information, and it was only revealed at the end of the study which group each patient belonged to.

All the patients received a spreadsheet to mark the intake of the sachets and write down any symptoms to control their adherence to the treatment. Patients who completed 90% of the recommended treatment were considered in compliance.

In the first visit, patients received general guidance on nutrition, not an individualized plan, and were also instructed to maintain their usual level of physical activity so that this would not be a confounding factor for later interpretation of the results of the intervention. Subsequently, in intermediate visits (days 45, 90, and 135), patients were encouraged to talk about food consumption and daily activities in the last few days in order to identify any changes in the pattern. At these moments, it was again reinforced that the usual standard was maintained.

Figure 1 schematically summarizes the study's logistics, including recruitment, randomization, patients' visiting frequency, and procedures performed at each moment. Full details of the trial protocol and study design have been previously described (20, 21).

Clinical evaluation

The data collection protocol includes demographic and clinical data, medications in use, alcohol intake (with significant intake defined as >20 g/day for men and >10 g/day for women) (22), and smoking status. In addition, details of the disease diagnosis (liver biopsy and transient elastography) were recorded.

The MetS was defined as the presence of ≥3 of the criteria presented according to the *International Diabetes Federation, American Heart Association,* and *National Heart Institute* (IDF/AHA/NHLBI) (23). For waist circumference (WC), we used the database of the ELSA-Brazil study (men ≥92 cm, women ≥86 cm) (24).

Assessment of fibrosis, liver fat, and liver function markers

Before the beginning of the protocol, all patients underwent a liver biopsy to confirm the diagnosis of NASH and determine the degree of fibrosis. We obtained biopsy results for a maximum of 2 years before the start of the intervention. At baseline and the end of the study, participants underwent transient elastography (FibroScan), measurements of liver enzymes, and assessments of serum concentrations of CK-18 and sTLR-4. In addition, the scores were also applied to determine the risk of fibrosis and steatosis.

Transient elastography (FibroScan) was performed in all the patients, and the fibrosis degree was assessed and measured in kilopascals (kPa). The higher the velocity, the greater the stiffness, and the greater the extent of the fibrosis (25, 26). We only considered examinations with ≥10 reliable results, median interquartile range (IQR) values below 30%, and results of transient elastography up to a maximum of 6 months before the intervention. Experienced gastroenterologists performed the imaging examinations (MM and SB).

The immunoenzymatic assay method (ELISA) determined the serum concentration of sTLR-4 (Elabscience, United States) and CK-18 (Elabscience, United States). A spectrophotometer measured the absorbance at a wavelength of 450 nm (Zenyth 200 rt), and the results were expressed in ng/mL and mIU/mL for sTLR-4 and CK-18, respectively. All the processes were performed according to the manufacturer's instructions, and the analyses were duplicated.

Liver function was assessed through laboratory tests. AST, ALT, GGT, bilirubin, and alkaline phosphatase were measured in the HCPA Laboratory routine on the Cobas Mira Plus equipment through a commercial kit.

To assess liver fibrosis risk, we performed the NAFLD fibrosis score, based on age, BMI, presence of diabetes mellitus (DM) or impaired fasting glucose, AST, ALT, platelet, and albumin levels, and the APRI score, which includes AST and platelet count. The steatosis risk was assessed by the fatty liver index (FLI), using BMI, WC, GGT, and TGs levels. The scores were calculated using MDCalc.com.

Lipidic, glycemic, and inflammatory profile assessment

The laboratory assessment included measuring lipid profiles [TGs, total cholesterol (TC), HDL cholesterol (HDL), and LDL cholesterol (LDL)] using the colorimetric enzymatic method; levels of other relevant indices such as C-reactive protein (CRP) were measured by nephelometry using a Bayer® nephelometer; insulin levels were measured by electrochemiluminescence (Elecsys 2010 Equipment); glucose was measured by the colorimetric enzymatic method glucose-peroxidase-Biodiagnostic Kit; homeostasis model assessment of insulin resistance (HOMA-IR) was calculated; albumin and creatinine were measured using a commercial kit in the Cobas Mira Plus equipment. Serum was obtained by centrifugation and stored at −80°C. Quantification of the biomarkers was performed using ELISA according to the manufacturer's instructions.

Anthropometric assessment

The anthropometric assessment included weight and height measurements for calculating the body mass index (BMI). The WC was measured between the last rib and the iliac crest with an inextensible fiberglass tape measure.

Dietary intake assessment

Dietary intake was checked by a 3 days diet record, describing the foods eaten on three non-consecutive days (two weekdays and one weekend day). The patients were oriented on filling it correctly, portion sizes, and details of the consumed foods. The NutriBase® software, 2007, calculated the records.

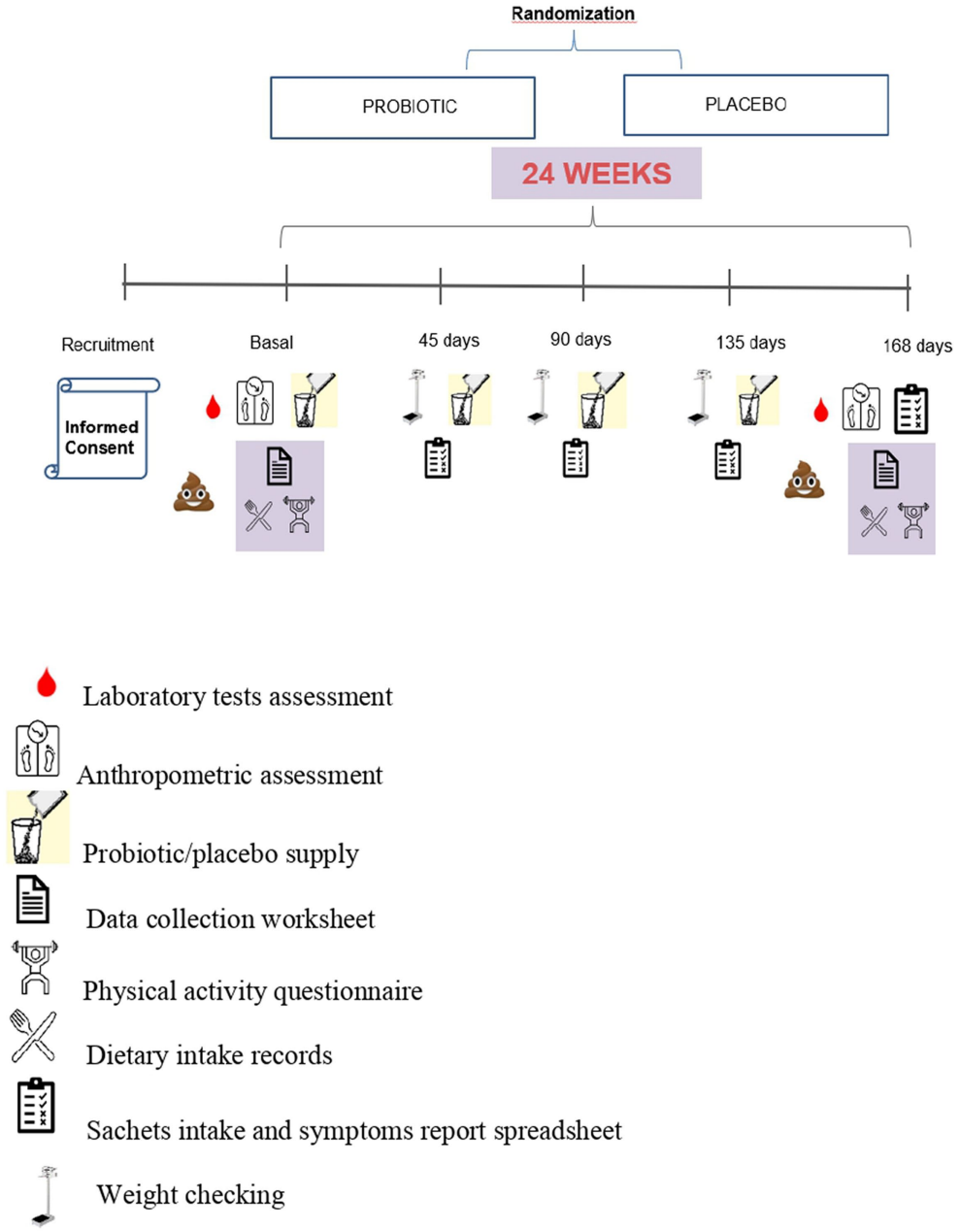

FIGURE 1
Study's logistics—detailing participants' recruitment, randomization, allocation, and methods.

Physical-activity level

The study applied the International Physical Activity Questionnaire—short form (IPAQ) to evaluate the weekly time spent on physical exercise. The values were expressed as metabolic equivalent of tasks (METs) in minutes per week (27).

Statistical analysis

The sample size estimation was carried out in WINPEPI 11.20 (Brixton Health, Israel), based on the data from Eslamparast et al. (28) which found a mean reduction in fibrosis score from 9.36 ± 1.9 to 6.38 ± 1.5 in NAFLD patients taking symbiotic supplementation ($p < 0.001$, compared to placebo).

Regarding data treatment, categorical variables were expressed as absolute frequency (n) and relative frequency (%), and quantitative variables were expressed as the mean ± SD or median and IQRs (25th to 75th percentile). To check the changes that occurred in each group over the 24 weeks, the paired t-test or Wilcoxon test was used. Finally, the comparison between the groups was based on the delta of the difference of each variable analyzed (value at the end of treatment minus the baseline value) using the t-test for independent samples or the Mann–Whitney U-test.

To verify the magnitude of the effect size (D) in the probiotic group and the placebo group, the following reference values were

considered: from 0.2 to 0.4 (small effect), from 0.5 to 0.7 (medium effect), and from 0.8 to ≥1.0 (large effect) (29).

The level of statistical significance was taken as a p-value of <0.05. The data were analyzed using the Statistical Package for Social Sciences version 20.0 (SPSS Inc., United States).

Results

The flowchart in Figure 2 shows 85 eligible patients who underwent ultrasonography, indicating steatosis and had clinical characteristics compatible with NASH, 39 patients left the trial, 38 patients did not have NASH upon biopsy, and 1 patient withdrew from participating in the research protocol. Therefore, 46 patients with NASH were randomized, and a total of 44 subjects completed the clinical trial and were analyzed.

Table 1 presents the patients' demographic and clinical baseline data. As for the number of MetS components, at baseline, 16 patients in the probiotic group and 19 patients in the placebo group had three or more components ($p = 0.428$). Over the 24 weeks, this proportion did not change in any group; specifically, 17 (77.3%) vs. 18 (81.9%), $p = 0.927$ (data not shown in table).

Table 2 shows the clinical characteristics and biochemical parameters before and after 24 weeks. There were no changes in the NAFLD fibrosis score, APRI score, or FLI after the intervention, whereas the biomarkers, CK-18, and sTLR-4 were reduced in both. However, there was no statistical difference between the groups. CK-18 showed a reduction in the probiotic group, and this result was subjected to effect size analysis, finding a large effect ($D = 1.336$) when compared to the placebo group ($D = 0.536$). Regarding the sTLR-4 analysis, the values showed a smaller difference ($D = 0.476$ in the probiotic group and $D = 0.510$ in the placebo group).

Table 3 presents the anthropometric characteristics before and after the intervention. No differences between the groups were observed after treatment.

Table 4 shows the patients' dietary intake characteristics before and after the intervention. There was a significant reduction in energy, carbohydrates, proteins, and lipid intake in the probiotic group and a significant reduction just in cholesterol intake in the placebo group, but when one group was compared to the other, these differences were not significant.

The physical activity was expressed as METs in minutes per week, showing no difference between the groups throughout the study ($p = 0.752$). In the probiotic group, the baseline median was 834 (300–2,118) min, and after the intervention, 773 (596–2,718) min ($p = 0.217$). In the placebo group, the baseline median was 1,060 (299–1,794) min, and after the intervention,1,020 (678–2,810) min ($p = 0.181$).

Compliance and adverse effects

One patient in the probiotic group was considered non-compliant with the protocol; however, they were included in the intention-to-treat analysis. There were no reports of adverse events associated with the probiotic supplementation during the entire trial.

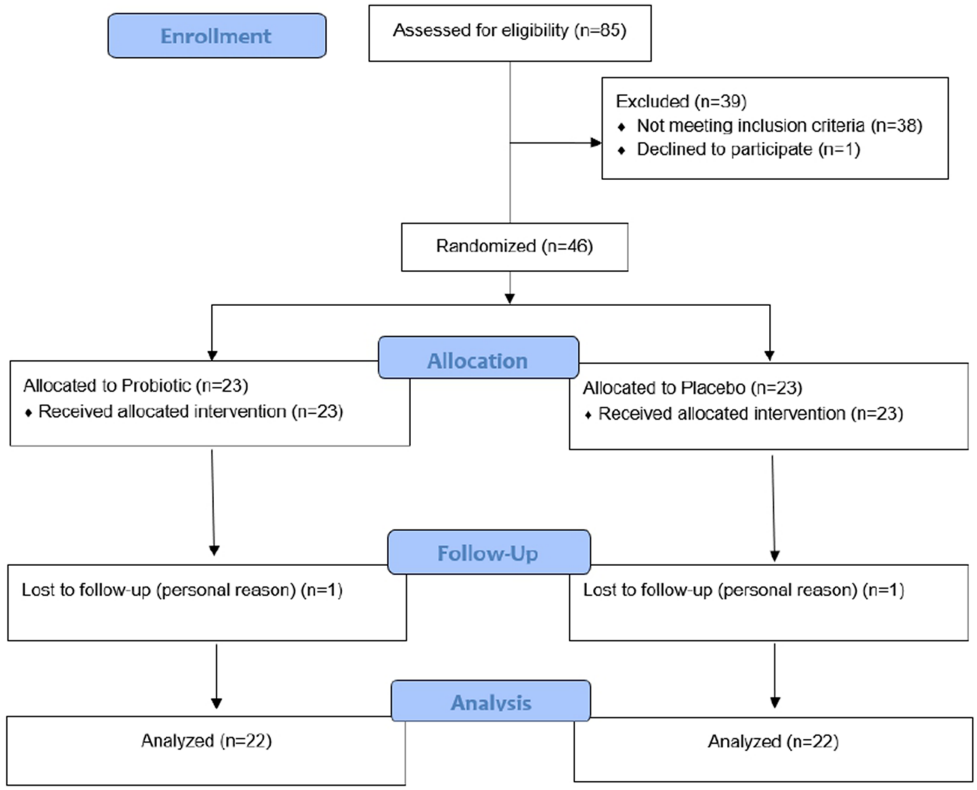

FIGURE 2
CONSORT flowchart detailing participants' recruitment, randomization, and allocation.

TABLE 1 Baseline characteristics of NASH patients.

Variables	All patients (*n* = 46)	Placebo group (*n* = 23)	Probiotic group (*n* = 23)	*p*-value
Female—*n* (%)	27 (58.70%)	12 (52.00%)	15 (65.00%)	0.369
Age (years)	51.35 ± 11.61	51.74 ± 11.94	50.95 ± 11.53	0.983
Waist circumference (cm)	104.70 ± 12.00	104.30 ± 11.00	105.10 ± 13.20	0.807
BMI (kg/m^2)	30.97 (28.36–33.75)	30.80 (28.36–36.96)	31.14 (28.09–33.75)	0.947
Diabetes mellitus	22.00 (47.80%)	13.00 (56.50%)	9.00 (39.10%)	0.376
High blood pressure	30.00 (65.20%)	14.00 (60.90%)	16.00 (69.60%)	0.758
MetS	35 (76.10%)	19 (82.60%)	16 (69.60%)	0.491
Number of MetS components—n (%)				
1	3 (6.50%)	2 (8.70%)	1 (4.30%)	0.428
2	8 (17.40%)	2 (8.70%)	6 (26.10%)	
3	14 (30.40%)	8 (34.80%)	6 (26.10%)	
4	10 (21.70%)	4 (17.40%)	6 (26.10%)	
5	11 (23.90%)	7 (30.40%)	4 (17.40%)	
Degree of fibrosis*				
F0	12 (26.00%)	8 (34.80%)	4 (17.40%)	0.376
F1	28 (61.00%)	12 (15.52%)	16 (69.60%)	
F2	1 (2.00%)	0 (0%)	1 (4.30%)	
F3	5 (11.00%)	3 (13.00%)	2 (8.70%)	
Glucose (mg/dL)	102.00 (88.00–122.00)	108.00 (97.00–144.00)	94.00 (95.00–115.00)	**0.035**
HbA1c %	5.80 (5.40–6.60)	5.80 (5.30–7.20)	5.70 (5.40–6.40)	0.562
Triglycerides (mg/dL)	145.00 (120.00–230.00)	146.00 (102.00–231.00)	143.00 (121.00–230.00)	0.775
Total cholesterol (mg/dL)	176.00 ± 36.00	173.00 ± 41.00	179.00 ± 32.00	0.604
HDL cholesterol (mg/dL)	45.00 (37.00–52.00)	42.00 (37.00–46.00)	48 (35–53)	0.231
LDL cholesterol (mg/dL)	98.30 ± 31.38	97.13 ± 37.45	99.73 ± 22.88	0.788
Insulin (μUI/mL)	15.1 (12–24.4)	14 (12–23.3)	18.3 (12.1–24.4)	0.512
ALT (U/L)	42 (28–63)	44 (35–63)	36 (22–75)	0.258
AST (U/L)	32 (24–46)	32 (24–44)	29 (22–51)	0.545
GGT (U/L)	46 (28–84)	48 (28–81)	43 (28–105)	0.974
Alkaline phosphatase (U/L)	77 (66–90)	71 (63–85)	80 (68–96)	0.368
Total bilirubin (U/L)	0.50 (0.40–0.70)	0.50 (0.40–0.80)	0.40 (0.30–0.60)	0.219
CRP (g/dL)	2 (1–7)	2 (1–7)	2 (1–5)	0.830
CK-18 (mIU/mL)	841.50 ± 320.07	757.95 ± 343.87	925.04 ± 276.95	0.076
sTLR-4 (ng/mL)	9.25 (5.17–16.48)	11.81 (5.17–20.61)	7.75 (4.46–13.35)	0.199

Categorical variables data were expressed as absolute frequency (*n*) and relative frequency (%). Quantitative variables are expressed as the mean ± SD or median and interquartile ranges (25th to 75th percentile). BMI, body mass index; MetS, metabolic syndrome; HbA1 c%, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; CRP, C-reactive protein; CK-18, cytokeratin 18; sTLR-4, soluble toll-like receptor-4; *liver biopsy. Reference values for normality—ALT (♀ < 33 U/L; ♂ < 41 U/L); AST (♀ < 32 U/L; ♂ < 40 U/L); GGT (♀ < 40 U/L; ♂ < 60 U/L); alkaline phosphatase (♀: 35–104 U/L; ♂: 40–129 U/L); total bilirubin <1.2 mg/dL.

Discussion

NASH is currently known as the leading cause of liver transplantation. Its prevalence has increased along with the worldwide increase in obesity (3, 30). Measures are necessary both to prevent the onset of NASH as well as to treat associated complications and prevent its progression to more severe forms (31, 32). The purpose of the present study was to evaluate the effect of 24 weeks probiotic supplementation in NASH patients on liver function markers, nutritional status, and clinical parameters. The 24 weeks intervention with probiotics demonstrated that they do not promote a significant change in liver and clinical parameters.

Despite being young (average age 51 years), NASH patients' baseline demographic and clinical data showed increased WC, obesity, hypertension, and DM. These typical characteristics were present in almost 80% of the patients. There was no difference between the groups, showing the homogeneity of the sample to start the treatment, except for the lower glucose level in the group of probiotics, in which fewer patients were diagnosed with DM.

The patients' routine diet and usual physical activities were assessed before and after treatment to ensure none of these factors interfered with the results. Although the dietary intervention was not our target, especially because we emphasized that patients should not

TABLE 2 Clinical characteristics of NASH patients before and after intervention.

Variables	Baseline	After 24 weeks	*p*-value	Mean change deltas	*p*-value[c]
Transient elastography (kPa)*					
Probiotic group (*n* = 20)	7.80 (6.20–11.20)	7.40 (5.60–11.10)	0.575[a]	−0.40 [(−1.20) to 1.30]	0.577
Placebo group (*n* = 17)	7.70 (5.80–9.50)	6.90 (6.40–10.50)	0.244[b]	−0.50 [(−1.90) to 0.70]	
NAFLD fibrosis score					
Probiotic group (*n* = 21)	−1.81 ± 1.52	−1.57 ± 1.57	0.053[a]	0.23 ± 0.52	0.612
Placebo group (*n* = 22)	−1.30 ± 1.51	−1.17 ± 1.32	0.411[b]	0.13 ± 0.75	
APRI score					
Probiotic group (*n* = 22)	0.39 (0.28–0.74)	0.44 (0.24–0.56)	0.410[a]	0.02 [(−0.10) to 0.08]	0.565
Placebo group (*n* = 22)	0.48 (0.37–0.62)	0.46 (0.30–0.66)	0.386[b]	−0.02 [(−0.08) to 0.13]	
Fat liver index					
Probiotic group (*n* = 21)	85.00 (65.00–90.00)	83.00 (52.00–93.00)	0.600[a]	0.00 [(−11) to 8.00]	0.534
Placebo group (*n* = 19)	89.00 (75.00–95.50)	88.00 (73.50–97.00)	0.655[b]	0.00 [(−5.00) to 1.00]	
CK-18 (mIU/mL)					
Probiotic group (*n* = 22)	913.75 ± 277.10	640.83 ± 246.85	**<0.001**[a]	−272.91 ± 204.29	0.109
Placebo group (*n* = 22)	769.50 ± 323.48	610.10 ± 259.10	**0.020**[b]	−158.53 ± 295.48	0.143
sTLR-4 (ng/mL)					
Probiotic group (*n* = 22)	11.02 ± 7.01	8.99 ± 5.36	0.053[a]	−2.03 ± 4.27	0.885
Placebo group (*n* = 22)	13.28 ± 7.84	11.26 ± 6.94	**0.026**[b]	−2.02 ± 3.10	0.993
ALT (U/L)					
Probiotic group (*n* = 22)	39.00 (23.00–75.00)	32.50 (21–71)	0.831[a]	−2 [(−7) to 4]	0.549
Placebo group (*n* = 22)	45.00 (36.00–63.00)	38.50 (32–48)	0.163[b]	−4 [(−16) to 5]	
AST (U/L)					
Probiotic group (*n* = 22)	32.00 (22.00–51.00)	29.00 (22.00–52.00)	0.864[a]	0.50 [(−9) to 3]	0.473
Placebo group (*n* = 22)	32.00 (25.00–44.00)	28.00 (23.00–37.00)	0.114[b]	−3.50 [(−10) to 2]	
GGT (U/L)					
Probiotic group (*n* = 22)	40.00 (28.00–94.00)	43.50 (24.00–87.00)	0.776[a]	−1 [(−4) to 3]	0.060
Placebo group (*n* = 20)	50.00 (34.50–82.50)	44.00 (30.50–71.50)	0.378[b]	−5 [(−9) to 0.50]	
Alkaline phosphatase (U/L)					
Probiotic group (*n* = 22)	79.00 (68.00–92.00)	73.00 (57.00–91.00)	0.325[a]	−4 [(−8) to 2]	0.481
Placebo group (*n* = 22)	71.00 (63.00–85.00)	72.00 (59.00–77.00)	0.172[b]	−4 [(−10) to 1]	
Total bilirubin (mg/dL)					
Probiotic group (*n* = 22)	0.45 (0.30–0.60)	0.45 (0.30–0.60)	0.684[a]	0 [(−0.10) to 0.10]	0.605
Placebo group (*n* = 21)	0.50 (0.40–0.70)	0.50 (0.40–0.70)	0.256[b]	0 [(−0.10) to 0.10]	
Direct bilirubin (mg/dL)					
Probiotic group (*n* = 22)	0.20 (0.10–0.30)	0.20 (0.20–0.20)	0.414[a]	0 (0–0)	0.818
Placebo group (*n* = 21)	0.20 (0.20–0.30)	0.20 (0.20–0.30)	0.180[b]	0 (0–0)	
Indirect bilirubin (mg/dL)					
Probiotic group (*n* = 22)	0.30 (0.20–0.30)	0.30 (0.20–0.40)	0.666[a]	0 [(−0.10–0.10)]	0.580
Placebo group (*n* = 21)	0.30 (0.20–0.50)	0.30 (0.20–0.40)	0.465[b]	0 [(−0.10–0.10)]	
Insulin (µUI/mL)					
Probiotic group (*n* = 21)	17.80 (12.10–22.10)	14.60 (13.20–20.00)	0.850[a]	0.50 [(−3.40) to 2.40]	0.715
Placebo group (*n* = 20)	14.20 (12.40–25.90)	13.05 (10.05–23.15)	0.836[b]	−0.25 [(−3.90) to 2.30]	
HOMA-IR					

(Continued)

TABLE 2 (Continued)

Variables	Baseline	After 24 weeks	*p*-value	Mean change deltas	*p*-value[c]
Probiotic group (*n* = 22)	74.34 (48.78–104.86)	65.83 (48.32–114.92)	0.332[a]	2.91 [(−10.53) to 18.53]	0.339
Placebo group (*n* = 20)	77.49 (58.98–105.40)	68.86 (53.64–117.99)	0.877[b]	−0.04 [(−34.71) to 14.96]	
Glucose (mg/dL)					
Probiotic group (*n* = 22)	95 (85–115)	98.50 (86–128)	0.850[a]	−1.50 [(−10) to 12]	0.543
Placebo group (*n* = 21)	109 (100–144)	115 (99–134)	0.532[b]	−4 [(−13) to 10]	
Triglycerides (mg/dL)					
Probiotic group (*n* = 22)	142.00 (121.00–207.00)	172.50 (114.00–201.00)	0.287[a]	4.50 [(−30) to 41]	0.366
Placebo group (*n* = 22)	144.00 (102.00–230.00)	135.00 (112.00–184.00)	0.587[b]	−15 [(−44) to 34]	
Total cholesterol (mg/dL)					
Probiotic group (*n* = 22)	179.00 ± 32.00	184.24 ± 42.49	0.194[a]	4.57 ± 21.99	0.392
Placebo group (*n* = 22)	173.00 ± 42.00	172.23 ± 44.17	0.375[b]	−1.50 ± 23.99	
HDL cholesterol (mg/dL)					
Probiotic group (*n* = 22)	48.00 (35.00–53.00)	45.50 (41.00–56.00)	0.897[a]	1 [(−3) to 3]	1.000
Placebo group (*n* = 22)	42.00 (37.00–46.00)	43.50 (38.00–47.00)	0.815[b]	1 [(−5) to 5]	
LDL cholesterol (mg/dL)					
Probiotic group (*n* = 17)	101.19 ± 22.71	105.28 ± 28.11	0.733[a]	−1.36 [(−8.24) to 11.88]	0.794
Placebo group (*n* = 21)	98.42 ± 37.86	97.44 ± 36.74	0.475[b]	0.34 [(−8.70) to 11]	
Creatinine (mg/dL)					
Probiotic group (*n* = 21)	0.83 ± 0.20	0.83 ± 0.18	0.384[a]	0.02 [(−0.02) to 0.04]	0.782
Placebo group (*n* = 21)	0.81 ± 0.16	0.82 ± 0.16	0.520[b]	0.01 [(−0.03) to 0.05]	
Albumin (g/dL)					
Probiotic group (*n* = 19)	4.70 ± 0.40	4.60 ± 0.26	0.228[a]	−0.10 ± 0.26	0.812
Placebo group (*n* = 20)	4.70 ± 0.30	4.64 ± 0.29	0.064[b]	−0.05 ± 0.24	
CRP (g/dL)					
Probiotic group (*n* = 19)	2.00 (1.00–4.00)	3.10 (1.20–4.30)	0.856[a]	−0.10 [(−0.60) to 1.10]	0.339
Placebo group (*n* = 19)	3.00 (2.00–8.00)	4.10 (1.70–7.20)	0.593[b]	0.40 [(−0.70) to 3.95]	
Platelets (μL)					
Probiotic group (*n* = 21)	240.00 ± 58.00	237.29 ± 62.06	0.144[a]	−2.67 ± 19.63	0.258
Placebo group (*n* = 21)	226.00 ± 55.00	214.10 ± 53.45	0.364[b]	−11.90 ± 31.24	

Variables are expressed as the mean ± SD or median and interquartile ranges (25th to 75th percentile). The mean change deltas may not total the subtraction because of the normality of the variable. *kPa, klilopascals—FibroScan; CK-18, cytokeratin 18; sTLR-4, soluble toll-like receptor-4; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein. [a]*p*-value referring to the changes that occurred within the probiotic group over the 24 weeks (paired *t*-test or Wilcoxon test). [b]*p*-value referring to the changes that occurred within the placebo group over the 24 weeks (paired *t*-test or Wilcoxon test). [c]*p*-value mean change deltas between groups (*t*-test for independent samples or Mann–Whitney *U*-test).

change their consumption pattern over the 24 weeks, the fact that they returned on days 45, 90, and 135 for follow-up probably caused "greater attention." The research nutritionists were the ones providing the care, and this may have made them pay more attention to what they were eating. It is an unintentional effect over which we had no control since patients ate their meals in their own homes. Although all patients only received general dietary guidelines at the beginning of the study, they probably began to be more careful with their food choices, which may have affected the results. From a methodological point of view, with appropriate statistical treatment, it was observed that there was a significant reduction in the intake of calories, carbohydrates, proteins, and lipids in the probiotic group, while in the placebo group, only cholesterol intake showed a significant reduction. However, when the groups were compared to each other, these differences were not significant, that is, although there was a reduction in food intake, it was similar in both groups. This unintentional improvement in diet, which occurred in both groups, may have been the reason why it was not possible to observe differences in other parameters evaluated. Concerning physical activity, no difference between the groups was observed.

The RCTs with probiotics are quite heterogeneous, both in terms of intervention time, type of strains, and the amount administered (5, 13, 14, 33–35). These differences between studies do not allow a generalized indication of probiotics as a treatment adjunct (36). There are a lot of different strains, and the effects resulting from their administration can also be quite different (11). Probiotics are expected to reconstitute a healthy microbiome, but the number of bacteria existing in the intestine is far greater than that offered by probiotics in

TABLE 3 Anthropometric characteristics of NASH patients before and after intervention.

Variables	Baseline	After 24 weeks	*p*-value	Mean change deltas	*p*-value[c]
Weight (kg)					
Probiotic group	83.45 ± 16.50	83.64 ± 15.55	0.726[a]	0.50 (0–1)	0.274
Placebo group	87.41 ± 17.59	87.23 ± 18.16	0.702[b]	0 [(−2) to 1]	
BMI (kg/m^2)					
Probiotic group	30.92 (28.09–33.24)	30.70 (28.01–33.75)	0.524[a]	0.18 (0.00–0.43)	0.219
Placebo group	31.16 (29.05–36.92)	30.81 (28.70–35.70)	0.714[b]	0.00 [(−0.70) to 0.43]	
Waist circumference (cm)					
Probiotic group	103.6 ± 11.20	103.52 ± 10.12	0.913[a]	−0.08 ± 3.28	0.875
Placebo group	104.7 ± 11.10	104.76 ± 11.06	0.905[b]	0.05 ± 2.12	

Variables are expressed as the mean ± SD or median and interquartile ranges (25th to 75th percentile). The mean change deltas may not total the subtraction because of the normality of the variable. BMI, body mass index. [a]*p*-value referring to the changes that occurred within the probiotic group over the 24 weeks (paired *t*-test or Wilcoxon test). [b]*p*-value referring to the changes that occurred within the placebo group over the 24 weeks (paired *t*-test or Wilcoxon test). [c]*p*-value mean change deltas between groups (*t*-test for independent samples or Mann–Whitney *U*-test).

TABLE 4 Diet of NASH patients before and after intervention.

Variables	Baseline	After 24 weeks	*p*-value	Mean change	*p*-value[c]
Energy intake (kcal/day)					
Probiotic group (*n* = 17)	2286.71 (2054.96–2516.22)	1758.38 (1366.91–2156.79)	**0.013**[a]	−351 ± 494.03	0.744
Placebo group (*n* = 16)	2286.67 (1708.74–3071.52)	2139.47 (1920.46–2442.13)	0.278[b]	−289.00 ± 716.56	
Carbohydrates (g/day)					
Probiotic group (*n* = 17)	248.50 (221.50–30.9.53)	195.00 (158.38–233.90)	**0.039**[a]	−50.89 ± 94.45	0.892
Placebo group (*n* = 16)	289.40 (208.59–381.27)	255.79 (216.86–307.35)	0.148[b]	−46.28 ± 89.33	
Protein (g/kg/day)					
Probiotic group (*n* = 17)	1.40 ± 0.47	1.06 ± 0.30	**0.014**[a]	−0.33 ± 0.50	0.374
Placebo group (*n* = 16)	1.26 ± 0.44	1.12 ± 0.34	0.258[b]	−0.15 ± 0.50	
Fat (g/day)					
Probiotic group (*n* = 17)	86.88 (62.74–92.96)	85.81 (74.70–131.46)	**0.049**[a]	−13.08[(−53.60) to (0.08)]	0.581
Placebo group (*n* = 16)	100.27 (61.98–120.67)	82.01 (74.12–102.29)	0.278[b]	3.11 [(−53.58) to 21.92]	
Cholesterol (mg/day)					
Probiotic group (*n* = 17)	344.14 ± 157.71	284.99 ± 141.94	0.263[a]	−59.16 ± 210.26	0.857
Placebo group (*n* = 16)	354 ± 163.23	285 ± 117.71	**0.018**[b]	−69.59 ± 105.38	
Total fibers (g/day)					
Probiotic group (*n* = 17)	17.76 (14.13–25.62)	15.30 (14.22–17.46)	0.124[a]	−4.73 ± 11.19	0.265
Placebo group (*n* = 16)	17.58 (15.15–30.20)	19.38 (16.56–26.53)	0.756[b]	−0.12 ± 12.12	

Variables are expressed as the mean ± SD or median and interquartile ranges (25th to 75th percentile). The mean change deltas may not total the subtraction because of the normality of the variable. [a]*p*-value referring to the changes that occurred within the probiotic group over the 24 weeks (paired *t*-test or Wilcoxon test). [b]*p*-value referring to the changes that occurred within the placebo group over the 24 weeks (paired *t*-test or Wilcoxon test). [c]*p*-value mean change deltas between groups (*t*-test for independent samples or Mann–Whitney *U*-test).

the form of supplements (36). It really makes sense to consider that the human intestine is inhabited by approximately 100 trillion bacteria, viruses, and fungi, forming a truly diverse ecosystem (5, 37). Another important key point concerns the use of combinations of bacteria species at the expense of the use of a single strain. In a systematic review with 22 RCTs using probiotics in NAFLD patients (34), 77.8% of interventions were carried out with a mix of probiotics, and only 4 studies were carried out with a single strain. The justification for a preferred prescription of a mixture of probiotics is that different strains have achieved better outcomes in RCTs and can act on different targets, achieving better results in reducing steatosis, fibrosis, AST, ALT, serum lipids, glucose, and insulin (5, 11, 13, 14, 34, 35, 38).

Despite the use of an accessible mixture of probiotic strains and a high supplemented amount, the expected effects were either very modest or not achieved. The biochemical levels of patients with NASH may be altered more according to the severity of the disease (39). However, from a clinical point of view, considering baseline examinations, the patients included in the study were not poorly managed. Additionally, in relation to the degree of fibrosis and hepatic impairment, most patients had low fibrosis or levels close to normal (stage 1). These reasons may explain the absence of significant

findings, as the treatment might have had limited effectiveness in reducing these parameters, including liver enzymes.

Despite being the gold standard for NASH diagnosis, liver biopsy is an invasive method that causes discomfort and may have some risks (1). Thus, non-invasive tests such as scores and CK18 for screening and risk stratification of advanced liver fibrosis have gained increasing visibility (15–18, 40). CK-18 is the major intermediate filament protein comprising the cytoskeletal structure of hepatocytes. It turns out that during hepatocyte apoptosis, the effector caspase cleaves fragments of CK-18 in the bloodstream (41), thus making it possible to evaluate its serum concentration. A recent study (42) evaluated circulating levels of CK-18 in more than 1,000 patients from different centers who had a liver biopsy (153 with NAFL and 855 with NASH). There was an interaction between CK-18 levels and serum AST and BMI. Furthermore, CK-18 showed a positive association with histological NAS, but with unsatisfactory sensitivity and positive predictive value (55 and 59%, respectively), which led the authors to conclude that the isolated measurement of CK-18 would have limited value for the non-invasive diagnosis of NASH. In the present RCT, CK-18 concentration was evaluated and reduced after intervention in both groups. Although the probiotic group had a greater reduction in CK-18 values and a larger effect size, there was no difference between the groups. We believe that intensive monitoring during the protocol meant that the patients were more careful with their diet over the 24 weeks, as both showed a reduction in calorie intake and macronutrients even without statistical differences between them.

We identified that there was a decrease in sTLR-4 after the intervention, but there was no significant difference between the groups. TLR-4 is a key immunological pathway activated by bacterial lipopolysaccharides that produce pro-inflammatory cytokines, inducing metabolic disorders and promoting greater liver damage in patients with NAFLD (43, 44). A recent review addressed the role of TLR-4 in the development of NAFLD through the activation of the immune response of endotoxin-producing strains (*Enterobacter cloacae* B29, *Escherichia coli* PY102, and *Klebsiella pneumoniae* A7) (45).

These receptors can recognize a variety of stimuli and thus initiate an immunological response through the formation of a protein complex. It is important to point out that all parallel TLR pathways compete and thus restrict each other's activation due to overlapping binding sites (46). A limitation of our study was that the evaluation was restricted to the soluble form of TLR-4. This soluble form might even be considered an anti-inflammatory marker since these soluble receptors neutralize ligands and are negative regulators of inflammation (46). Ideally, it would be necessary to identify the multiprotein complex to confirm that an inflammatory response is occurring.

After 24 weeks of the intervention, it was impossible to perceive a decrease in fibrosis or liver fat through the assessed scores. Following our results, symbiotic supplementation for 12 months also had no impact on the NAFLD fibrosis score (47). However, a 2 years lifestyle intervention resulted in a reduction in the NAFLD fibrosis score, weight, WC, and liver enzymes (48).

Similar to our study, but with a much smaller sample size (49), a study using a probiotic mix twice a day for 24 weeks demonstrated a reduction in the intrahepatic fat content assessed by magnetic resonance imaging. However, the included patients had a degree of fibrosis equal to 2 or 3, had higher baseline values of intrahepatic fat, and were instructed to make lifestyle changes (diet and exercise). However, the glycemic and lipid profiles did not change, as in our study.

As for liver enzymes, unlike other authors, we could not demonstrate any significant reduction after treatment. In a brief report (50), 15 NASH patients with altered hepatic enzymes received *Acidophilus* capsules (2 billion viable organisms) 3 times daily for 1 month. They demonstrated a significant reduction in ALT ($p < 0{,}001$) and AST ($p = 0.03$) levels when comparing the intervention group and control group; however, there was no alteration in the imaging examinations. Other authors reported a reduction in liver enzymes after the supplementation of probiotics (51–53), but it is worth noting that the baseline values of all these patients were high.

In general, changes in anthropometric measures are not expected with probiotics, and Wong et al. (49) and Behrouz et al. (53) did not find changes in these parameters. However, Alisi et al. (54) demonstrated a reduction in BMI after the supplementation of a high concentration of probiotic strains for 16 weeks and Famouri et al. (55) in WC and the weight of obese children after supplementing a mixture of probiotics for 12 weeks. It is important to emphasize that in these two interventions, in addition to probiotics, the participants (children and adolescents) were prescribed a low-calorie diet or were encouraged to increase their daily activity, as well as improve their eating habits by increasing their intake of fruits and vegetables and reducing their consumption of fast food, high-fat meals, and sweet snacks.

As we did not find a change in the number of Mets components after supplementation, a more extended intervention would probably be necessary to achieve these effects. In this regard, Duseja et al. (56) also did not succeed in this regard after a similar intervention.

This study has some solid points, including being a single-center, RCT, double-blind, and adhering to methodological guidelines. There was a minor loss of patients during the follow-up period. All patients underwent liver biopsy for the NASH diagnosis before starting the protocol and were followed up every 45 days for reassessment and compliance monitoring. Nevertheless, the study presents a limited fibrosis assessment. No liver biopsy was performed at the end of the study, and not all the patients were suitable candidates for *FibroScan* due to BMI or central obesity.

Conclusion

In conclusion, the 24 weeks intervention with probiotics demonstrated that it does not promote a significant change in liver and clinical parameters for patients in the early stages of NASH. However, its use for disease prevention or in more advanced stages needs to be further evaluated.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Comitê de Ética em Pesquisa—Hospital de Clínicas de Porto Alegre. 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

AS-S: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft. HM: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. SB: Investigation, Methodology, Resources, Writing – original draft. BM: Formal analysis, Investigation, Methodology, Writing – original draft. LL: Formal analysis, Investigation, Methodology, Resources, Writing – original draft. MM: Investigation, Methodology, Writing – original draft. CC: Investigation, Methodology, Writing – original draft. CU-C: Conceptualization, Funding acquisition, Methodology, Resources, Writing – original draft. TS: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Writing – original draft. MÁ-d-S: Investigation, Methodology, Resources, Conceptualization, Writing – original draft. VD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1362694/full#supplementary-material

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Glossary

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
APRI	Aspartate aminotransferase to platelet ratio index
ASCVD	Atherosclerotic cardiovascular disease
BMI	Body mass index
BMR	Basal metabolism rate
CAP	Controlled attenuation parameter
CFU	Colony-forming units
CK-18	Cytokeratin 18
CRP	C-reactive protein
DM	Diabetes mellitus
GGT	Gamma-glutamyltransferase
GM	Gut microbiota
HbA1c	Glycosylated hemoglobin
HCPA	Hospital de Clinicas de Porto Alegre
HDL	High-density lipoprotein
IPAQ	International Physical Activity Questionnaire
LDL	Low-density lipoprotein
LPS	Bacterial lipopolysaccharide
MASLD	Metabolic dysfunction-associated steatotic liver disease
MetS	Metabolic syndrome
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
RCT	Randomized clinical trial
TC	Total cholesterol
TG	Triglycerides
TLR	Toll-like receptor
sTLR-4	Soluble toll-like receptor-4

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Progress and hotspot of diet or exercise therapy in the treatment of non-alcoholic fatty liver disease

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Introduction: The primary treatment for non-alcoholic fatty liver disease (NAFLD) is modifying lifestyle through dietary or exercise interventions. In recent decades, it has received increasing attention. However, the lack of bibliometric analysis has posed a challenge for researchers seeking to understand the overall trends in this field.

Methods: As of February 3rd, 2024, 876 articles on treating NAFLD through diet or exercise therapy from 2013 to 2023 had been retrieved. Two software tools, VOSviewer and CiteSpace, were utilized to analyze the growth of publications, countries, institutions, authors, journals, citations, and keywords. Additionally, the keywords with strong citation burstiness were identified to determine the changes and future trends of research hotspots in this field.

Results: China had the highest number of articles, followed by the United States and South Korea. Yonsei University and *Nutrients* were the institutions and journals with the most significant contributions. Professor Younossi Zobair M, from the United States, is the most prolific author in this field. Through analyzing the keywords, three research hotspots were identified: research on the pathogenesis of NAFLD, research on the treatment modalities of NAFLD, and research on the risk factors and diagnosis methods of NAFLD. In recent years, the research emphasis in this field has changed, suggesting that future research will focus on two frontier keywords: "oxidative stress" and "aerobic capacity."

Conclusion: In the past eleven years, the attention in this field was still rising, and the authors, journals, countries and so on had formed a considerable cooperative relationship. There were also many highly influential and productive researchers in this field. It is speculated that new research will continue around "aerobic exercise" and "oxidative stress" in the future.

KEYWORDS

VOSviewer, CiteSpace, diet therapy, exercise therapy, non-alcoholic fatty liver disease

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is a metabolic stress liver injury disease with more than 5% steatosis and no signs of hepatocyte damage, closely related to insulin resistance and genetic susceptibility (1). NAFLD is a clinicopathological syndrome characterized by abnormal accumulation of hepatic triglycerides and steatosis of hepatic parenchymal cells. The spectrum of diseases includes simple fatty liver disease, non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma, and these diseases can develop continuously in some individuals (2). With the development of the economy, diet structure and lifestyle are also changing constantly, which leads to a substantial increase in the prevalence of obesity, metabolic syndrome, and type 2 diabetes mellitus (T2DM). A recent study found that the global prevalence of nonalcoholic fatty liver disease increased by 50.4% in 2019 compared with 1990, from 25.26 to 38.00%. Among them, the highest prevalence rate is in Latin America (44.37%), followed by the Middle East and North Africa (36.53%), South Asia (33.83%), Southeast Asia (33.0%), North America (31.20%), East Asia (29.71%), Asia-Pacific (28.02%) and Western Europe (25.10%) (3). As a metabolic disease involving many systems, NAFLD has seriously affected the health of people all over the world.

Currently, the primary goal of treating NAFLD is to lose weight and improve insulin resistance (IR). Among them, the essential factors that lead to obesity, including high-calorie diet structure, lifestyle with less exercise and more sitting, and lack of exercise, are also risk factors for NAFLD. Dietary treatment belongs to the category of modern nutrition. It is an important intervention to treat NAFLD by adjusting the daily diet and recipe collocation, improving human function, and regulating diseases. In 1913, Casimir Fink et al. (4) suggested that food contains "life amines." This "biogenic amine" was separated in 1926. By 1950, all the major vitamins had been divided and synthesized to prevent and treat diseases related to nutritional deficiency. At the same time, due to the different levels of development in other countries, the long-term concern for specific nutrients has affected the direction of scientific research and policy intervention. From 1950 to 1970, Keys et al. (5) suggested that improper fat intake was the leading cause of heart disease. Yudkin et al. (6) indicated that excessive sugar intake was the leading cause of coronary heart disease, hypertriglyceridemia, and other diseases. Dietary fat and sugar were the new concerns in this period. From 1970 to 1990, more attention was paid to diet-related chronic diseases, including obesity, T2DM, and other chronic diseases. Since 1990, many experiments have proved that the single nutrition theory is insufficient to explain the influence of diet on diseases (7), which makes the research direction turn to the complex biological effects between food and dietary pattern and dietary structure. Exercise therapy, an essential part of physical therapy, is a method to treat body dysfunction and regulate diseases through various exercises and is a critical intervention method for treating NAFLD. Patients' habits and preferences mostly influence sports, and there is no requirement for a specific form of sports (8).

Bibliometrics first appeared at the beginning of the 20th century and became an independent discipline in 1969, and it was widely used in literature analysis (9). Bibliometric analysis realizes clear and comprehensive graphic results through modern computer technology, which is helpful for data interpretation and provides a quantitative method for analyzing known literature in a specific field (9). It is crucial that the literature dosimetry analysis can reveal the internal relations between much basic information. Therefore, the development of a field can be observed by bibliometric analysis (10). The databases selected in this study were from the Web of Science (WOS) core collection. As a high-quality digital literature resource database, WOS has a high recognition among researchers and is considered the most suitable high-quality database for bibliometric analysis. CiteSpace and VOSviewer were used to draw the knowledge map. Both of the two software are commonly used tools for visualizing literature information. They have advantages in drawing the knowledge map, and their combination can complement each other (11). CiteSpace is software for measuring the similarity of knowledge units based on the data standardization method of set theory. It runs in a JAVA environment and uses the similarity algorithm in the time dimension to observe the process and historical span of knowledge evolution and to identify the changes and future trends of research hotspots in this field (12). VOSviewer is a software based on probability theory for data standardization. It can be used to construct a visual bibliometric network. It is less complicated, and the network categories for mapping knowledge are rich and beautiful (13).

Quite a few scholars are committed to diet or exercise therapy research for NAFLD, and their treatment methods are constantly improving. However, as far as we know, there is no research topic of bibliometric analysis. This study will reveal the field's current development process and frontier trends through bibliometric analysis and provide the latest inspiration and decision-making basis for researchers and clinicians.

2 Materials and methods

2.1 Data collection

In this paper, the core collection of WOS is selected to ensure the comprehensiveness and accuracy of the retrieved data, considering that dietary intervention belongs to the category of modern nutrition; therefore, the retrieval strategy finally determined in this paper is [TS = (nutrition) OR TS = (exercise or "physical activity")] AND TS = ("non-alcoholic fatty liver disease" or "non-alcoholic fatty liver"). The period was from January 2013 to December 2023, and the retrieval deadline was February 3rd, 2024. The only article was selected as the type of article, and there was no restriction on the language. In addition, for the accuracy of the data finally used for analysis, non-article literature was manually excluded. 876 documents were obtained through the search.

2.2 Data analysis

All recorded and referenced documents are exported as plain text files for those retrieved from the WOS core database. The exported record is "download_*.txt" for subsequent analysis. Microsoft Excel is used to analyze the amount of literature published in different years. Using VOSviewer 1.6.18 software, based on the probability theory data standardization method, a visual atlas of publishing countries, institutions, journals, and cited documents is constructed, and the cooperative network is analyzed. The size of a node is related to its degree, frequency of occurrence, and connection strength. The deeper and thicker the connecting lines between nodes, the stronger the connection and cooperation between the two nodes. The color of nodes represents different clusters. Using CiteSpaceV 6.1.R 6 software, a similarity algorithm in the time dimension draws the timeline and

keyword burst diagrams. The timeline graph is a cluster graph, which can reflect the historical development of each cluster containing keywords over a while. Citation explosion is a crucial index to identify emerging trends. Both are used to determine the changes and future directions of research hotspots in this field.

3 Results

3.1 Information analysis of the published quantity

The 876 papers used in this study came from 5,631 authors of 1,058 institutions in 75 countries, published in 97 journals, and cited 13,286 references from 876 journals. This paper analyzes the information from articles published in the last decade. As shown in Figure 1, as of December 31st, 2023, there were 876 related research results on treating NAFLD with diet or exercise. Judging from the number of papers published each year, although there was a slight decline in 2023, the number of articles published in this field is on the rise, indicating that scholars have paid this research field more and more attention in recent years. Especially after 2020, the number of published articles increased significantly, and the number of published articles remained at more than 100 in 2021–2023. It was predicted that research achievements in diet or exercise therapy for NAFLD will continue to increase.

3.2 Information analysis of countries and periodicals

China scholars have had the most fruitful research results in this field, with 214 papers published, accounting for 24.43% of the total number of papers published in this field, Followed by the United States, with a total of 188. These two countries published far more articles than other countries. In addition, South Korea, Italy, Iran, and other countries have also published several articles. From a centripetal point of view, the United States and England were the strongest, with 0.65 and 0.38, respectively, indicating that the two countries have conducted cooperative research with more countries, indicating that the United States and England were not only high-level and had many research results in the field of diet or exercise therapy for NAFLD, but also pay more attention to cooperation between countries than other countries (Table 1). In addition, among different continents, Asia published the most related articles, with a total of 516 articles, followed by Europe, with a total of 394 articles; in different regions, East Asia published the most related articles, with a total of 362 articles, accounting for 41.32% of the total number of articles (Table 2). Different colors represent different node groups that work closely together. The United States was at the center of the whole picture. It cooperated with many countries, which showed that the United States occupied a significant position in national scientific research cooperation and had made outstanding contributions. At the same time, the distribution of countries in this field was uneven, and the top effect was pronounced. Scholars from several countries wrote most papers. Green part: China, the United States, South Korea, Japan, England, Singapore, and India had close cooperation; Red part: Spain, Germany, France, Sweden, Greece, Switzerland, Finland, and Poland had close cooperation; Yellow part: Italy and Israel were closely cooperating; Blue region: Canada, Australia, Iran, Netherlands, and Brazil had close cooperation (Figure 2).

The journals with more than 25 articles were *Nutrients*, *Frontiers in Nutrition*, and *Clinical Nutrition*, with 65 pieces, 27 articles, and 25 articles, respectively. By analyzing the citation of journals, it was found that the journal with the highest average number of sources was *Journal of Hepatology*, with a total of 20 articles, with an average number of citations of 149.05 times and the highest impact factor of 25.70, which showed that the articles published in this journal are of high quality and have attracted a lot of attention in the field of diet or exercise therapy for NAFLD (Table 3).

3.3 Information analysis of authors and institutions

The organizations of the top 10 high-productivity authors were mainly from Spain. Younossi Zobair M. from the Global NASH Committee of the United States published 19 articles from 2013 to 2023 and obtained 905 citations, each being cited 47.63 times. The second place was Tur, Josep A. from the University of the Balearic Islands in Spain, who published 17 papers and was awarded 132 times, with each article cited 7.76 times. Younossi Zobair M. was also the most frequently

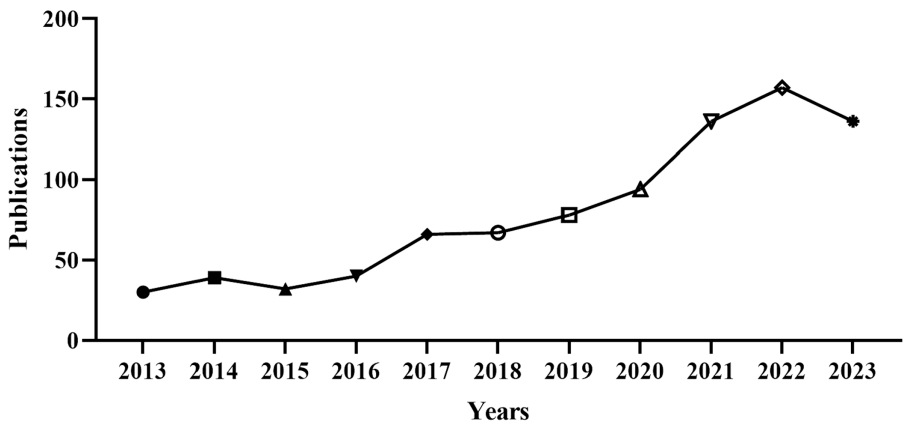

FIGURE 1
The annual distribution of publications. Annual distribution of diet or exercise therapy publications for non-alcoholic fatty liver disease from 2013 to 2023.

TABLE 1 Top 10 countries in terms of publications and centrality.

Rank	Documents	Centrality	Country	Rank	Centrality	Documents	Country
1	241	0.11	China	1	0.65	188	United States
2	188	0.65	United States	2	0.38	66	England
3	103	0.05	South Korea	3	0.18	4	South Korea
4	77	0.06	Iran	4	0.11	214	China
5	73	0.09	Italy	5	0.10	14	Poland
6	66	0.38	England	6	0.10	6	Belgium
7	47	0.03	Spain	7	0.10	2	Ghana
8	46	0.08	Germany	8	0.09	73	Italy
9	45	0.00	Japan	9	0.08	46	Germany
10	30	0.07	Australia	10	0.07	16	France

TABLE 2 Ranking of the number of articles published in different continents/regions.

Rank	Continents	Documents	Rank	Regions	Documents
1	Asia	516	1	East Asia	362
2	Europe	394	2	North America	213
3	North America	213	3	South Europe	152
4	South America	34	4	West Asia	116
5	Oceania	33	5	West Europe	116
6	Africa	27	6	Central Europe	91

cited author, which shows that this author had a high influence in this field (Table 4). Different colors represent different clusters that work closely together. Overall, there was large-scale cooperation among researchers. Through research, we can find that authors in the same country cooperated more closely. Orange part: All authors were from Inova Health System in the United States; Green part: The authors were all from Spain; Dark blue part: The authors are all from Japan; Red part: The authors were all from universities in China (Figure 3).

According to the analysis in Table 5, the number of articles published by Yonsei University in South Korea was the largest, with 20 pieces, followed by Sungkyunkwan University in South Korea, with 19 articles, and the Public University of Navarre in Spain, with 18 reports. On the whole, most of the higher-ranked institutions were from South Korea, Spain, and Iran. These results showed that the institutions were leading in this field. From the perspective of inter-agency cooperation, Yonsei University, Iran University of Medical Sciences, and Sun Yat-sen University were highly concentrated, which showed that they pay more attention to cooperative research with other institutions than other institutions. As shown in Figure 4, the analysis diagram shows the distribution relationship of more than 10 mechanisms, and different colors indicate different node clusters, which are closely matched. At the same time, it can be seen that the blue part was the thickest part between nodes, indicating that these institutions have a very close cooperative relationship.

3.4 Information analysis of cited documents

By presenting the top 10 cited articles in this field, this paper analyzes the highly cited articles of great significance. There were 31 articles cited more than 100 times, most of which were cross-sectional studies and randomized controlled studies, which increased the credibility of the research results (Table 6). The most frequently cited and core article was "Modeling NAFLD Disease Burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, And United States for 2016–2030," published in 2018 by Estes C. This paper used the Markov model to predict the disease burden of NAFLD using the currently available data. It concluded that the burden would continue to increase, showing the necessity and urgency of research on NAFLD's treatment methods. This also means that as the primary treatment of NAFLD, diet and exercise therapy need to be further studied (Figure 5).

3.5 Keywords and hot spot analysis

Keywords reflect the core content of each article. By observing its historical development process and discovering unknown words, we can identify the changes and future trends of research hotspots in this field. By presenting the top 10 keywords in this field, this paper analyzes the overview of keywords in this field. By comparing the frequency and centrality of keywords, it is found that "non-alcoholic fatty liver," "insulin resistance," "metabolic syndrome," "risk," and "prevalence" have a higher frequency and occupy the central position of The relationship network. The high centrality of keywords such as "liver disease," "population," "weight lose," and "cardiovascular disease" means that these keywords are closely related to other keywords. According to the frequency and centrality of keywords, we can judge the hot spots and frontiers in this field (Table 7).

The keyword is the author's refining and summarizing of the article's content, which can reflect the core content of the article.

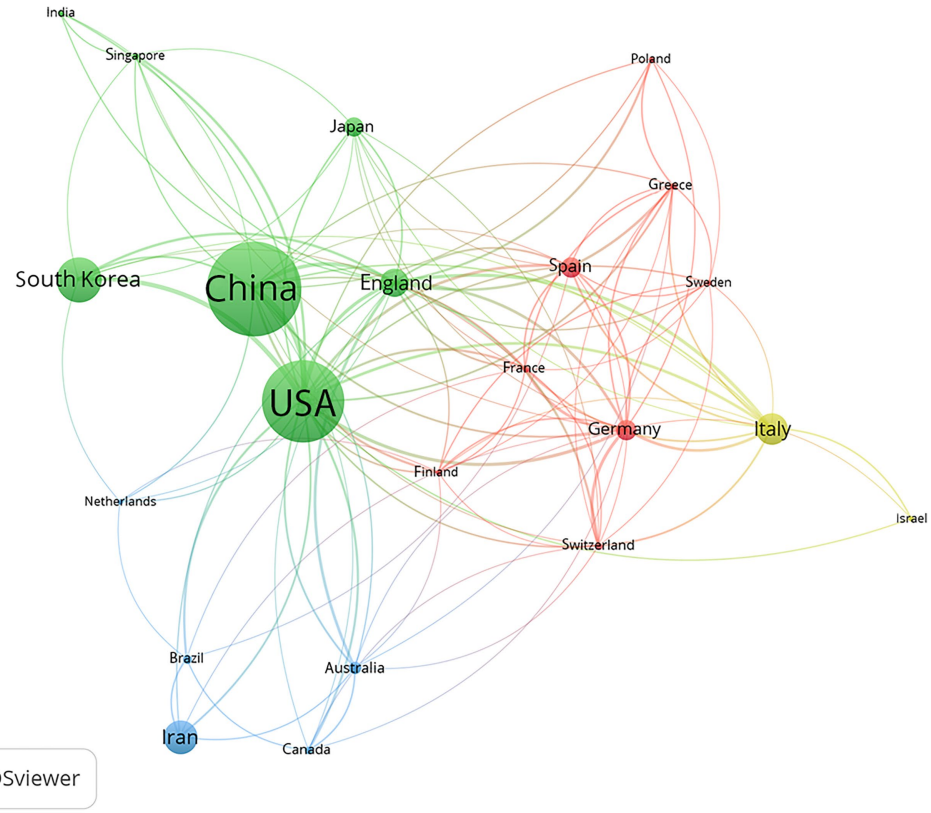

FIGURE 2
Analysis of national cooperation based on the VOSviewer visualization map. The node size is positively correlated with the frequency of node content, and the thickness of the connection line is positively correlated with the co-occurrence times of node content at both ends. Different color clustering reflects the cooperative relationship between countries.

TABLE 3 Top 10 journals in terms of publications and centrality.

Rank	Source	Documents	Citations	Average citation/publication	IF(2023)
1	Nutrients	65	926	14.25	5.90
2	Frontiers in Nutrition	27	113	4.19	5.00
3	Clinical Nutrition	25	821	32.84	6.30
4	Liver International	22	635	28.86	6.70
5	Nutrition Metabolism and Cardiovascular Diseases	22	218	9.91	3.90
6	Scientific Reports	21	261	12.43	4.60
7	Journal of Hepatology	20	2,981	149.05	25.70
8	Journal of Clinical Medicine	18	117	6.50	3.90
9	BMJ Open	17	213	12.53	2.90
10	Frontiers in Endocrinology	16	183	11.44	5.20

In this paper, 55 times or more are set as high-frequency keywords, and VOSviewer is used to present the co-occurrence network of high-frequency keywords. The thickness of the node connection is positively related to the co-occurrence frequency of the nodes at both ends of the link. As shown in Figure 6 keywords mainly form three clusters, representing three main research directions in this field. The red part is primarily composed of "insulin resistance", "obesity", "inflammation", "steatohepatitis", "exercise", "diet", and so on, and is mainly involved in the pathogenesis and treatment of NAFLD. The green part is primarily composed of "risk", "cardiovascular", "diabetes mellitus", "metabolic syndrome", and, so on which are mainly related to the risk factors of NAFLD. The blue part is primarily composed of "steatosis", "diagnosis", "management", and so on; this section mainly introduces the diagnosis and management methods in the development of NAFLD

TABLE 4 Top 10 the most productive authors distributed by publications and citations.

Rank	Author	Documents	Citations	Average citation/ publication	Rank	Cited author	Citations
1	Younossi, Zobair M.	19	905	47.63	1	Younossi, Zobair M	557
2	Tur, Josep A.	17	132	7.76	2	Chalasani, N	243
3	Abete, Itziar	13	187	14.38	3	Targher, G	194
4	Casares, Miguel	12	87	7.25	4	Angulo, P	184
5	Mascaro, Catalina M.	12	87	7.25	5	Marchesini, G	161
6	Montemayor, Sofia	12	87	7.25	6	Zelber-sagi, S	153
7	Fukui, Michiaki	10	239	23.90	7	Bedogni, Giorgio	151
8	Golabi, Pegah	10	616	61.60	8	Musso, GAdams, La	128
9	Hekmatdoost, Azita	10	153	15.30	9	Lonardo, A.	115
10	Ryu, Seungho	10	546	54.60	10	Wong, Vws	105

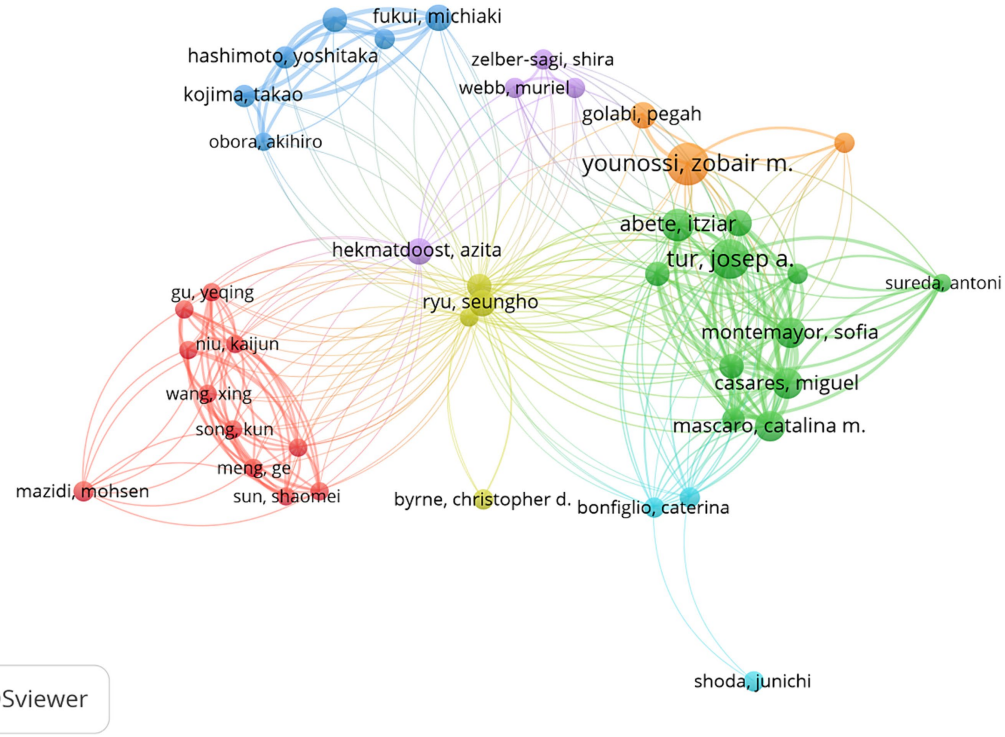

FIGURE 3
Analysis of author cooperation based on the VOSviewer visual map. The node size is positively correlated with the frequency of node content, and the thickness of the connection line is positively correlated with the co-occurrence times of node content at both ends; different color clustering reflects the cooperative relationship between authors.

CiteSpace is a timeline graph used to draw keywords. The timeline of keywords is essentially a clustering diagram, which can observe the historical development of keywords in multiple clusters in 10 years. Wherein each period corresponds to a vertical time axis, and the keywords on the time axis represent the first appearance of the keywords in the period. The node's size indicates the keyword's total frequency in recent years. The annual ring of node content, the color of each layer suggests the year when the keyword reappears, and the thickness is the frequency of this year. Connecting means co-occurrence. We can get nine groups of high-frequency words representing nine main research directions in this field (Figure 7). The greater the number of clusters, the fewer keywords are included. They are "weight loss," "ampk" (activated protein kinase), "mortality," "body composition," "physical activity," "cardiovascular risk," "dietary intervention," "vitamin C," and "fatty liver index." Among them, "weight loss" and "ampk" were the rich categories of articles, and they were the most concerned research directions in this field, which not only have a large number of articles but also have new research content every year from 2013 to 2023. The last new research keywords, "dietary

TABLE 5 Top 10 institutions with the most publications.

Rank	Documents	Centrality	Organization	Country
1	20	0.04	Yonsei University	South Korea
2	19	0.01	Sungkyunkwan University	South Korea
3	18	0.01	Public University of Navarre	Spain
4	18	0.01	Shahid Beheshti University of Medical Sciences	Iran
5	16	0.01	Inova Fairfax Hospit	United States
6	15	0.01	Tehran University of Medical Sciences	Iran
7	15	0.03	Iran University of Medical Sciences	Iran
8	13	0.00	Instituto de Salud Carlos III	Spain
9	13	0.01	University of the Balearic Islands	Spain
10	13	0.03	Sun Yat-sen University	China

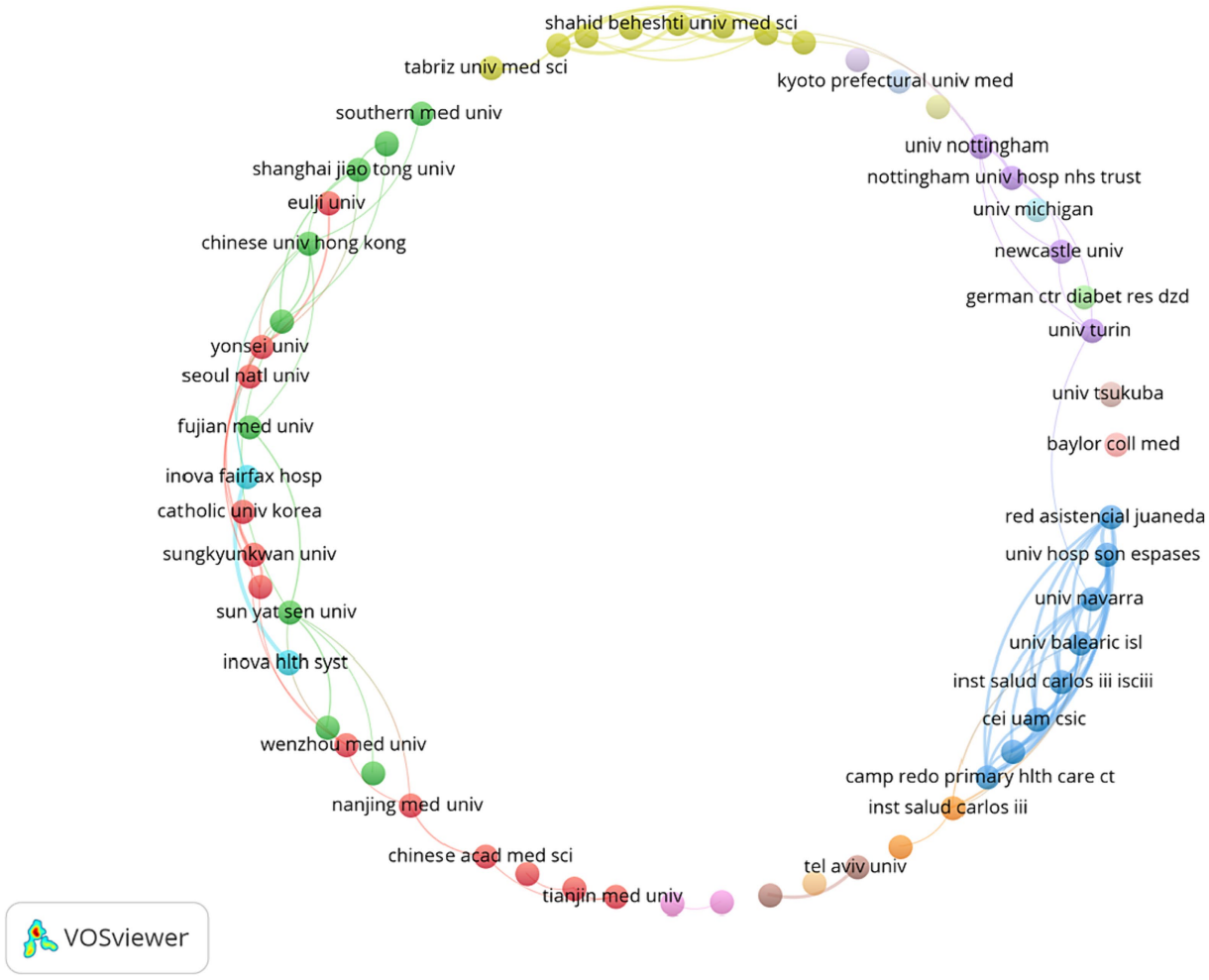

FIGURE 4
Analysis of institutional cooperation based on the VOSviewer visualization map. The node size is positively correlated with the frequency of node content, and the thickness of the connection line is positively correlated with the co-occurrence times of node content at both ends; different color clustering reflects the cooperative relationship between institutions.

intervention" and "fatty liver index" appeared in 2022, and all the other clusters still have new research keywords.

Keyword burst detection can identify the changes and future trends of research hotspots in this field. In the diagram, the blue line represents the timeline, and the red line represents the outbreak period. You can see the first appearance year, outbreak intensity, outbreak starts the year, and outbreak end year of many keywords in the picture. Through keyword burst detection, 17

TABLE 6 Top 10 the most cited literature.

Rank	Count	Title	Author	Journal	Year	Volume	Issue	Page	DOI
1	963	Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016–2030.	Estes, C	Journal of Hepatology	2018	69	4	896	10.1016/j.jhep.2018.05.036
2	267	Epidemiology of chronic liver diseases in the USA in the past three decades.	Younossi, ZM	Gut	2020	69	3	564	10.1136/gutjnl-2019-318813
3	234	Sarcopaenia is associated with NAFLD independently of obesity and insulin resistance: Nationwide surveys (KNHANES 2008–2011).	Lee, YH	Journal of Hepatology	2015	63	2	486	10.1016/j.jhep.2015.02.051
4	233	Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: A randomized controlled trial.	Chen, SH	Digestive and Liver Disease	2015	47	3	226	10.1016/j.dld.2014.11.015
5	216	Community-based lifestyle modification programme for non-alcoholic fatty liver disease: A randomized controlled trial.	Wong, VWS	Journal of Hepatology	2013	59	3	536	10.1016/j.jhep.2013.04.013
6	184	Irisin is inversely associated with intrahepatic triglyceride contents in obese adults.	Zhang, HJ	Journal of Hepatology	2013	59	3	557	10.1016/j.jhep.2013.04.030
7	179	Metabolic dysfunction-associated fatty liver disease is associated with increased all-cause mortality in the United States.	Kim, D	JOURNAL OF HEPATOLOGY	2021	75	6	1,284	10.1016/j.jhep.2021.07.035
8	167	Cadmium Exposure and Liver Disease among US Adults.	Hyder, O	Journal of Gastrointestinal Surgery	2013	17	7	1,265	10.1007/s11605-013-2210-9
9	165	High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance.	Zelber-Sagi, S	Journal of HepatOLOGY	2018	68	6	1,239	10.1016/j.jhep.2018.01.015
10	164	Beneficial effects of lifestyle intervention in non-obese patients with non-alcoholic fatty liver disease	Wong, VWS	Journal of Hepatology	2018	69	6	1,349	10.1016/j.jhep.2018.08.011

10.3389/fnut.2024.1326092

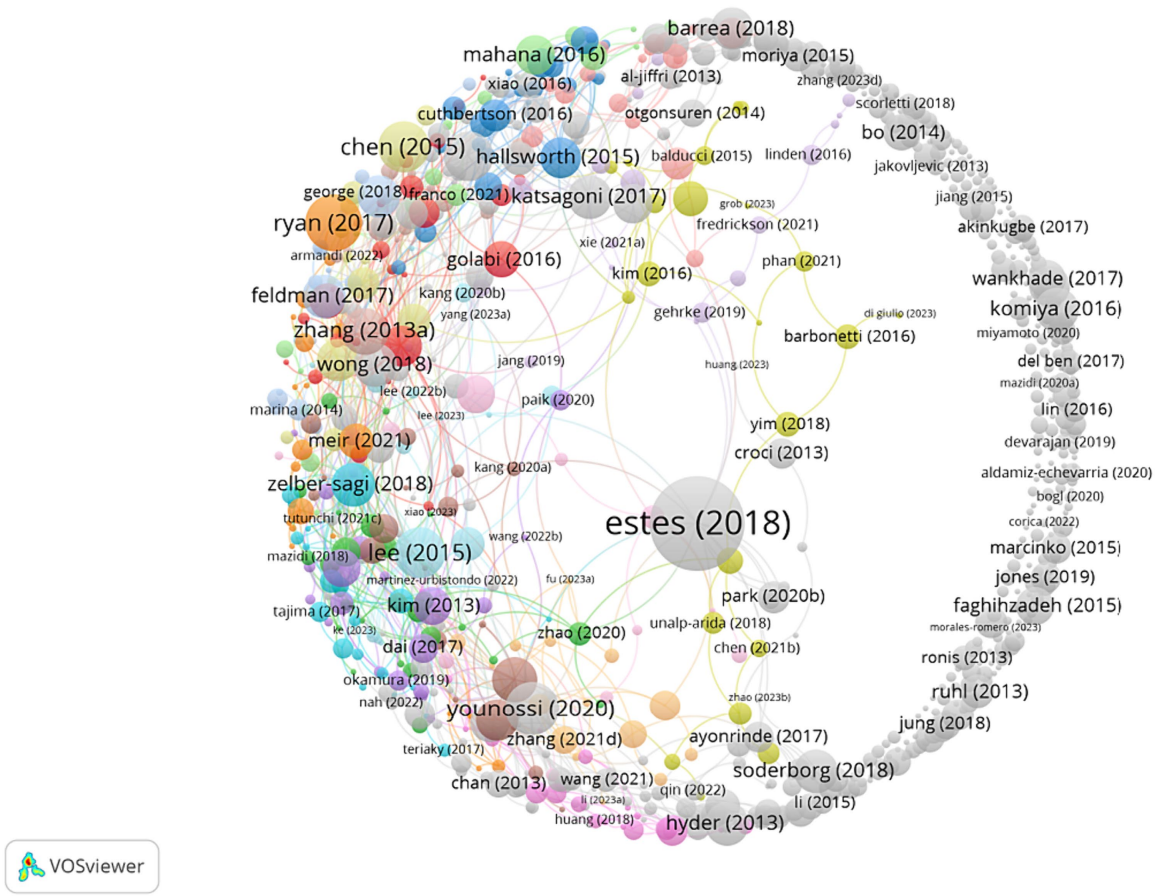

FIGURE 5
Analysis of co-cited literature based on the VOSviewer visualization map In network visualization, the size of nodes depends on the frequency of co-citation. The greater the citation frequency, the greater the contribution of the articles at both ends of the node to the future development of this field.

TABLE 7 Top 10 keywords in terms of publications and centrality.

Rank	Count	Centrality	Keyword	Rank	Centrality	Count	Keyword
1	446	0.01	Non-alcoholic fatty liver	1	0.09	34	Liver disease
3	243	0.02	Risk	3	0.07	62	Weight lose
4	203	0.02	Metabolic syndrome	4	0.06	63	Cardiovascular disease
5	202	0.02	Prevalence	5	0.06	21	Skeletal muscle
6	187	0.02	Fatty liver	6	0.05	158	Obesity
7	176	0.04	Physical activity	7	0.05	125	Association
8	158	0.05	Obesity	8	0.05	112	Disease
9	142	0.02	Hepatic steatosis	9	0.05	87	Exercise
10	133	0.03	Steatohepatiti	10	0.05	66	Nash

outbreak keywords with the strongest references displayed in order of outbreak start year are obtained. From 2013 to 2019, more attention was paid to "metabolic syndrome," "population," "steatohepatitis," "alanine aminotransferase level," "skeletal muscle," "cardiovascular risk," "coronary heart disease," "activated protein kinase," "nutrition examination survey" and "follow up" (Figure 8). From 2019 to 2023, "mice," "intensity," "fructose," and "diabetes mellitus" received more attention. From 2023 to 2024, "cross-sectional study," "oxidative stress," and "aerobic capacity" received more attention. We can infer this field's research focus and frontier (Figure 8).

4 Discussion

In this study, VOSviewer 1.6.18 and CiteSpaceV 6.1.R 6 were used to analyze 876 articles on treating NAFLD by exercise or diet published in the core database of WOS from 2013 to 2023. The following main

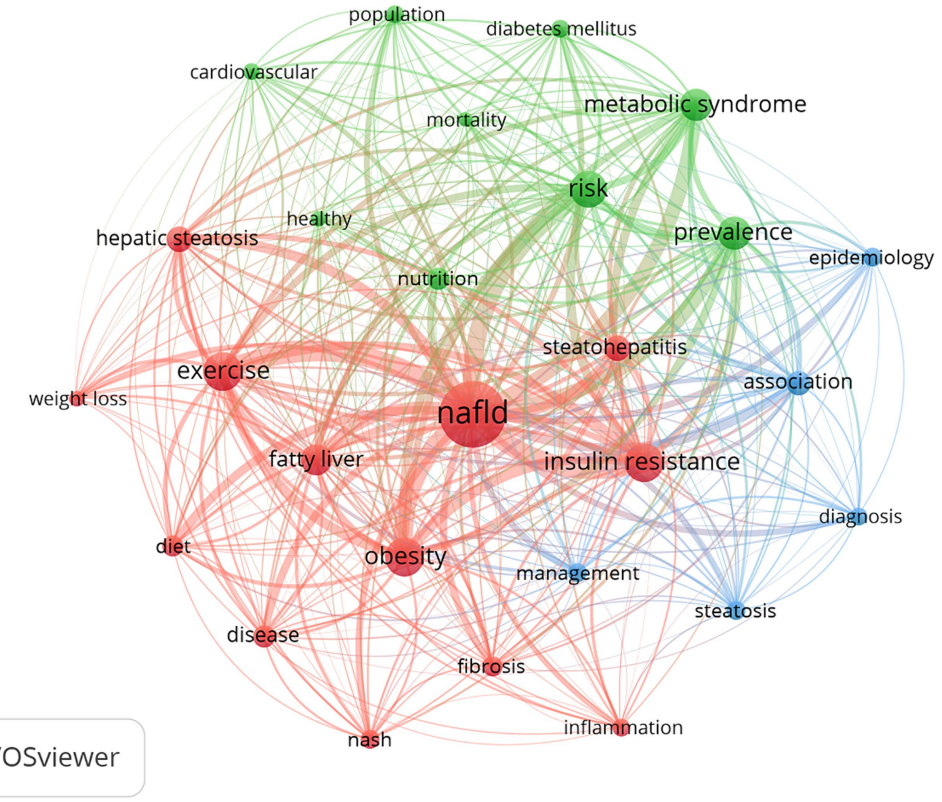

FIGURE 6
Analysis of keywords based on the VOSviewer visual map. The node size is positively correlated with the frequency of node content, and the thickness of the connection line is positively correlated with the co-occurrence times of node content at both ends; different color clustering reflects the cooperative relationship between keywords.

conclusions were drawn from time distribution, space–time distribution, the contribution of authors and articles, hot spots, and frontiers.

4.1 General description

The number of published articles increased from 2013 to 2023, especially after 2020. The number of published articles remained at more than 100 during 2021–2023, indicating a good development trend in this field, which may be attributed to the high prevalence rate of NAFLD caused by various factors, which attracted the attention of many scholars.

Researchers from China have made the most significant contribution to this field, accounting for 24.43% of the total number of papers published in this field, which may be attributed to the fact that the incidence of NAFLD in China has now exceeded the global incidence. At the same time, the United States and England have the highest centrality, which shows that researchers in these two countries paid more attention to academic cooperation. Among them, China ranked second in the number of papers published but fourth in the center, which may be why China started late. The number of papers published in East Asia was the largest, accounting for 41.32% of the total. In addition, the number of published articles was not directly related to the incidence of NAFLD in this area.

Among the top 10 organizations with the highest productivity, three organizations had a large number of publications from Iran. At the same time, the centrality of Yonsei University and Iran University of Medical Sciences was high, which showed that they have high collaboration ability and academic influence.

Younossi Zobair M., from the United States, was the author who has published the most articles in this field and was a member of the global Nash Committee. H index was as high as 103, which shows that he greatly influenced the research field. From the analysis of published papers, Younossi Zobair M. pays more attention to the diagnosis, treatment, pathological causes, and so on of NAFLD, among which he thinks that strenuous exercise is beneficial to adult patients with NAFLD, especially to adult NAFLD patients with metabolic abnormalities (14). The most frequently cited article was by Chris Estes from the US Centers for Disease Control and Prevention. His articles included epidemiological research and disease prediction model research of various liver diseases, which pointed out the direction for clinicians and essential research work.

The impact factor is a standard method to evaluate the quality of journals. According to our results, among the top 10 journals in terms of productivity, "Journal of Hepatology" had the highest impact factor of 25.70, and its 20 published articles have been cited 2,981 times, which indicated that the published articles were of high quality and have been recognized by peers in this field. "Journal of Hepatology" has become the top journal in this field. By analyzing the published literature, it was found that this journal mainly contained empirical

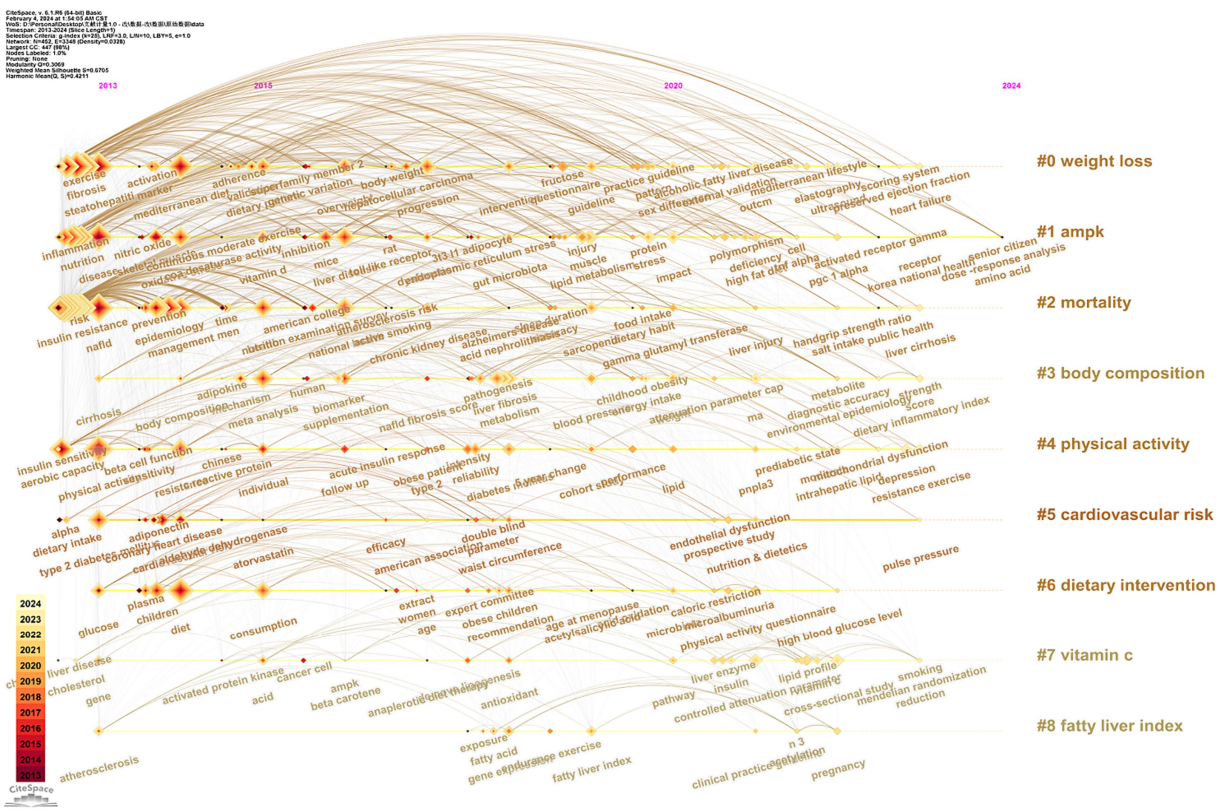

FIGURE 7
A timeline view of keywords. Each period corresponds to a longitudinal time axis, and the keywords on the time axis represent the first appearance of the keywords in this period; The node size represents the total frequency of this keyword for several years. The annual ring of node content, the color of each layer represents the year when the keyword reappears, and the thickness is the frequency of this year; Connecting means co-occurrence.

research papers covering the whole area of medicine-gastrointestinal hepatology. Whether the influencing factors were high or low, journals were influential in promoting diet or exercise therapy to treat NAFLD.

4.2 Hot spots and frontiers

The keyword is the authors' refining and summarizing of the content of the article, which can reflect the core content of the article. Based on keyword analysis, the main research direction of exercise or diet therapy in the field of NAFLD in the last 10 years was observed, that is, the research hotspot, including (1) Studies on the pathogenesis of NAFLD. IR caused by obesity and other factors is not only the strongest predictor of NAFLD (15), but also the critical factor leading to steatosis and abnormal accumulation of hepatocyte fat (16). At this time, abnormal liver fat accumulation will cause oxidative stress and lipid peroxidation, leading to mitochondrial dysfunction and inflammatory mediators and finally resulting in inflammatory necrosis and fibrosis of the liver (17). (2) Studies on the treatment of NAFLD. Many pieces of evidence showed that calorie restriction (18, 19), fat restriction (20, 21), carbohydrate reduction (22, 23), and appropriate vitamin supplementation (24, 25) in diet therapy, aerobic exercise (26, 27) resistance exercise (28, 29) and high-intensity interval training (30) in exercise therapy had good therapeutic effects. Moreover, clinical experiments have proved that combining diet and exercise may achieve better therapeutic efficacy than a single one (31, 32). (3) Studies on risk factors and diagnosis methods of NAFLD. In addition to obesity, dyslipidemia, T2DM, hypertension, and IR, genetic factors are also important risk factors for NAFLD (33–39), such as *PNPLA3* (40) and transmembrane 6 superfamily member 2 (*TM6SF2*) (41, 42) genes. In addition, intestinal flora disorder and the change of metabolites are important risk factors for NAFLD. Intestinal flora can lead to NAFLD by reducing intestinal barrier permeability and participating in immune inflammatory reactions. Targeted regulation of intestinal flora and its metabolites by changing diet and exercise has become a new treatment for NAFLD (43). To diagnose and distinguish the development stage of NAFLD and prevent or reverse the further progression of NAFLD in time, a large number of researchers have devoted themselves to developing non-invasive diagnostic methods (44), such as the serum inflammatory markers, apolipoprotein A-I (APAI1) (45).

Keyword burst detection can identify the change in research hotspots and future development trends in this field. Oxidative stress refers to a physiological state in which the imbalance of the redox system leads to excessive accumulation of free radicals, which plays a vital role in the pathogenesis of NAFLD. Oxidative stress can improve membrane permeability by causing membrane phospholipid peroxidation, thus promoting the release of inflammatory factors, which act on Kupper cells, hepatic stellate cells and hepatocytes, and finally induce the progress of NAFLD (46). Studies have confirmed that some foods, dietary patterns and nutrients can effectively reduce the level of oxidative stress in the body to achieve the effect of treating

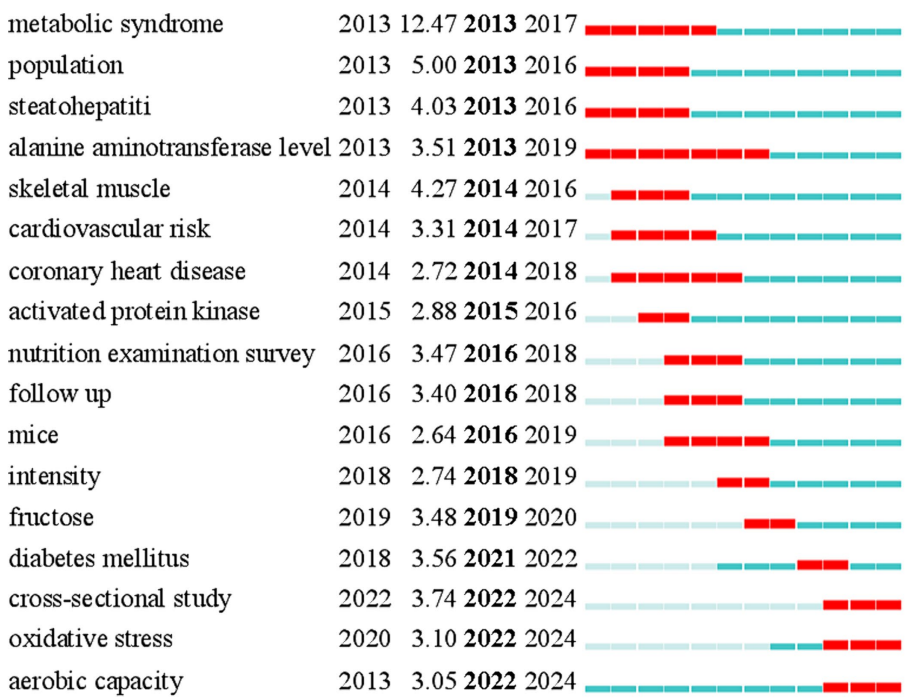

FIGURE 8
Top 17 keywords with the strongest citation bursts. The table shows the outbreak keywords in the field of diet or exercise therapy for non-alcoholic fatty liver disease in recent 10 years and ranks them according to the years when they appeared.

NAFLD, such as fish, lean meat (47), nuts (48) and other foods; such as the Mediterranean diet (49–51), DASH (Dietary Approaches to Stop Hypertension) diet (52), RCR (Calorie-restricted regimen) diet (53) and other dietary patterns; such as carotenoids (54, 55), vitamin C (56), vitamin E (57), polyunsaturated fatty acids (58), methionine (59), isoflavones (60) and resveratrol (61). It is worth mentioning that Marinello et al. (62) found that organic acids in the diet can effectively treat NAFLD. Still, the combination of organic acids and ethanol will aggravate oxidative stress, which means that it is not feasible to treat alcoholic fatty liver with organic acids. Proper exercise can improve the level of antioxidant enzymes, enhance the ability of free radical scavenging, and play a role in preventing more serious oxidative stress damage. Csader et al. (63) proposed that 12 weeks of high-intensity intermittent exercise could change the expression of fat genes without changing the level of oxygen phospholipids. Cook et al. (64) found that restoring redox imbalance mediated by resistance exercise (the utilization of the pentose phosphate pathway) is very important for developing NAFLD. Fernandes et al. (65) found that aerobic exercise can protect against oxidative damage by improving non-enzymatic antioxidant defense but cannot change enzymatic defense. In addition, Barsalani (66) found that the effect of adding isoflavones in exercise training Is more evident than that provided by individual exercise.

Aerobic ability is the ability of the human body to obtain energy through aerobic metabolism when engaged in long-term and moderate-intensity exercise. Aerobic exercise can increase the oxidation function in the liver, reduce free fatty acids and reduce the synthesis of fat in the liver by improving aerobic endurance, thus treating NAFLD. Zhou (67) was included in the randomized controlled trial published in 2010–2021. The results showed that the best intervention effect on blood lipid and liver enzymes in NAFLD patients was aerobic training combined with resistance training followed by high-intensity interval training, resistance training, and aerobic training. Katsagon (68) was included in 20 randomized controlled trials published in 2005–2016. The results showed that resistance exercise was better than aerobic exercise in treating NAFLD. In addition, moderate to high-intensity and moderate-intensity continuous training is more beneficial than low-intensity continuous training and high-intensity interval training. Sabag (69) found that high-intensity interval training and moderate-intensity continuous training have the same effect on improving NAFLD. It can be seen that the impact of various aerobic exercise interventions on NAFLD is still uncertain, so further research is needed

5 Restrictions

There are some limitations in this research. First of all, all the data used in the analysis are only from the WOS core database, not all the databases of WOS. Secondly, the types of articles are limited to papers, and other influential articles published in other forms may also be ignored. In addition, WOS is constantly being updated dynamically; we may miss some new research progress. This has a particular influence on our results.

6 Conclusion

To sum up, this study makes a bibliometric analysis of the field of diet or exercise therapy for NAFLD from 2013 to 2023 for the first time, which helps us to deepen our understanding of this field and

provides a valuable summary for discussing development trends and hot topics. Chinese scholars have contributed the most to this field, followed by scholars from the United States. The latest research results in this field can be found in *Nutrients*, *Frontiers in Nutrition*, and *Clinical Nutrition*. Professor Younossi Zobair M. and Professor Tur Josep A. were highly esteemed academic leaders in this field. In recent years, the research focus in this field has changed from "metabolic syndrome," "population," "steatohepatitis," "alanine aminotransferase level," "skeletal muscle," "cardiovascular risk," "coronary heart disease," and "activated protein kinase" to "cross-sectional study," "oxidative stress" and "aerobic capacity."

Author contributions

XS: Writing – original draft. YX: Writing – original draft. HS: Writing – original draft. FR: Writing – original draft. NT: Writing – original draft. LZhu: Writing – original draft. SL: Writing – original draft. JW: Writing – review & editing. LZha: Writing – review & editing. SY: Writing – review & editing. GJ: Writing – review & editing. BL: Writing – review & editing. NW: 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

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Exploring the link: magnesium intake and hepatic steatosis in Americans

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Purpose: The connection between magnesium and hepatic steatosis has not been well-studied. This study aimed to explore the link between magnesium intake and hepatic steatosis, utilizing data from the National Health and Nutrition Examination Survey (NHANES) 2017–2020.

Materials and methods: The analysis included 5,935 participants, excluding individuals with hepatitis infection or substantial alcohol consumption. Magnesium intake assessment was based on 24-h dietary recalls. Hepatic steatosis evaluation employed the controlled attenuation parameter (CAP), measured via transient elastography. Multivariate regression and subgroup analyses were conducted to scrutinize the relationship between magnesium intake and CAP values.

Results: A higher magnesium intake was associated with lower CAP values, after adjusting for potential confounders. Subgroup analyses indicated an inverted U-shaped correlation between magnesium intake and CAP in women, White people, and non-hypertensive individuals, with respective inflection points at 126, 124.5, and 125 mg/day, respectively. Below these thresholds, a higher magnesium intake correlated with increased CAP values, while above these points, it was associated with decreased CAP.

Conclusion: This extensive population-based study indicates an inverse relationship between magnesium intake and hepatic steatosis in Americans. This relationship displays an inverted U-curve, varying before and after specified inflection points in women, White people, and non-hypertensive individuals. These findings offer insights into tailored magnesium supplementation strategies for preventing and treating liver steatosis, based on gender and ethnicity.

KEYWORDS

magnesium, hepatic steatosis, fatty liver, controlled attenuation parameter, NHANES

Introduction

Hepatic steatosis occurs when fat droplets accumulate within liver cells (1). Fatty liver is diagnosed when the liver's fat content exceeds 5% of its weight (2). The severity of fatty liver varies, ranging from simple fatty liver, which shows no evident inflammation or damage, to non-alcoholic steatohepatitis (NASH), characterized by significant inflammation and liver cell damage (3). The global prevalence of fatty liver is rising in parallel with the obesity epidemic, affecting an estimated 25% of the global population (4). While simple fatty liver can often be managed with lifestyle and dietary changes, about 30% of cases may progress to NASH (5). Without treatment, it can progress to liver cirrhosis, liver cancer, or liver

failure (6). Therefore, early diagnosis and prevention of liver steatosis are crucial (7).

Magnesium, a vital mineral, is involved in more than 300 enzymatic reactions in the human body, such as energy production, carbohydrate metabolism, protein synthesis, and regulating blood pressure (8). Serving as a cofactor for numerous enzymes in carbohydrate and lipid metabolism, magnesium plays a crucial role in metabolic processes (9). Preliminary research links low magnesium intake with a heightened risk of metabolic diseases like type 2 diabetes and cardiovascular disorders (10, 11).

The link between magnesium and fatty liver disease is currently under active investigation. Limited studies have explored the role of serum or dietary magnesium in metabolic dysfunction-associated steatotic liver disease (MASLD) (12–14). Nevertheless, hepatic steatosis in the general population is not well-studied in these investigations. Additionally, previous studies have predominantly diagnosed MASLD using abdominal ultrasonography, a method significantly influenced by the physician's subjective judgment and technical expertise.

The Controlled Attenuation Parameter (CAP), assessed semi-automatically via liver elastography, offers a reliable, non-invasive approach to quantitatively evaluate hepatic steatosis (15). Many studies, corroborated by liver biopsy, demonstrate a significant correlation between CAP values and liver fat levels (16–18). Between 2017 and 2020, the National Health and Nutrition Examination Survey (NHANES) introduced transient elastography to measure one hepatic steatosis, yielding the largest dataset of CAP observations in the United States. Herein, our study aims to examine the relationship between magnesium intake and hepatic steatosis by analyzing extensive data on magnesium intake and CAP values from the 2017–2020 NHANES. We will particularly focus on whether there are unique associations across different demographic subgroups.

Materials and methods

Statement of ethics

This study received approval from the National Center for Health Statistics Research Ethics Review Board, with each participant providing consent.

Study population

To ensure nationwide representation, NHANES, an extensive, continuous cross-sectional survey in the US, employs stratified, multistage, clustered random sampling to gather diet and health data from the entire population (19). Of the 15,560 participants in the 2017–2020 NHANES cycle, 9,698 had available CAP data. We excluded 2,451 participants who tested positive for hepatitis B antigen, hepatitis C antibody, or hepatitis C RNA, 799 with significant alcohol consumption (four or more drinks daily), and 513 lacking magnesium intake data. Ultimately, the study included 5,935 participants. Figure 1 presents the flowchart of sample selection.

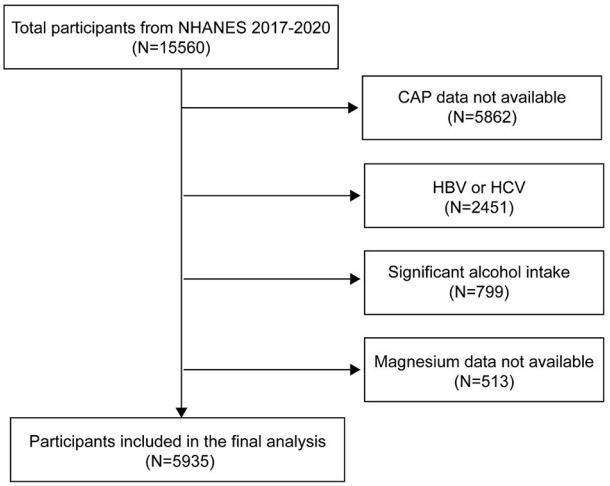

FIGURE 1
Flowchart of participant selection. NHANES, National Health and Nutrition Examination Survey; CAP, controlled attenuation parameter.

Variables

This study focused on magnesium intake as the exposure variable. Daily dietary intake data were gathered via 24-h recall interviews and a 30-day dietary supplement questionnaire. For each NHANES 2017–2018 participant, two 24-h recalls were conducted. The first dietary recall was performed in person at the NHANES Mobile Examination Centers (MEC), and the second via telephone by trained interviewers 3–10 days post-MEC interview. The United States Department of Agriculture's Food and Nutrient Database for Dietary Studies was the source of information on nutrient intakes, including dietary fiber (20). The total amount of magnesium consumed per day from food and dietary supplements was determined. The outcome variable, CAP, was measured using the FibroScan® 502 V2 Touch, equipped with liver ultrasonography transient elastography. This device measures CAP by recording ultrasonic attenuation, indicative of hepatic steatosis and liver fat content.

Categorical variables such as gender, race/ethnicity, education level, marital status, smoking habit, diabetes, hypertension, and cholesterol levels were included in our study. Age, body mass index (BMI), γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum albumin, serum creatinine, and uric acid were among the continuous factors in our study. You may find detailed information about CAP, magnesium intake, and other variables at http://www.cdc.gov/nchs/nhanes/.

Statistical analysis

We utilized a weighted variance estimation technique to tackle notable fluctuations in our dataset. For categorical data, the weighted chi-square test was utilized to evaluate group differences, and for continuous variables, the weighted linear

TABLE 1 Weighted characteristics of the study population based on magnesium intake tertiles.

Magnesium intake (mg/day)	Total ($N = 5,935$)	Low (10.0–185.5, $N = 1977$)	Middle (185.6–284.0,p20mm $N = 1,977$)	High (284.1–1,097.0, $N = 1,981$)	*P*-value
Age (years)	44.83 ± 20.16	40.80 ± 21.08	45.01 ± 20.76	47.75 ± 18.30	<0.0001
Gender (%)					<0.0001
Men	47.51	38.07	43.39	58.40	
Women	52.49	61.93	56.61	41.60	
Race/ethnicity (%)					<0.0001
Mexican American	9.76	9.51	9.52	10.17	
Other Hispanic	7.38	7.76	7.91	6.63	
Non-Hispanic White	62.77	59.67	61.73	66.06	
Non-Hispanic Black	11.22	14.71	11.66	8.16	
Other race	8.86	8.35	9.17	8.99	
Education level (%)					<0.0001
Less than high school	10.41	14.42	9.09	8.93	
High school	28.02	35.70	29.54	21.88	
More than high school	61.57	49.89	61.37	69.19	
Marital status (%)					<0.0001
Married/living with partner	64.06	56.60	62.17	70.35	
Widowed/divorced/separated	18.91	21.58	21.08	15.45	
Never married	17.03	21.83	16.75	14.19	
Income to poverty ratio	3.14 ± 1.63	2.78 ± 1.65	3.13 ± 1.61	3.43 ± 1.59	<0.0001
BMI (kg/m^2)	29.30 ± 7.49	29.15 ± 7.83	29.55 ± 7.72	29.20 ± 6.98	0.2007
Smoked at least 100 cigarettes in life (%)					0.0544
Yes	38.28	40.03	39.23	36.33	
No	61.72	59.97	60.77	63.67	
Diabetes (%)					0.8733
Yes	10.78	11.14	10.67	10.59	
No	86.82	86.56	86.67	87.16	
Borderline	2.40	2.30	2.66	2.25	
Hypertension (%)					0.4496
Yes	31.80	31.40	32.91	31.13	
No	68.20	68.60	67.09	68.87	
High cholesterol level (%)					<0.0001
Yes	34.12	30.76	32.99	37.42	
No	65.88	69.24	67.01	62.58	
AST (IU/L)	21.06 ± 10.32	20.43 ± 12.14	20.54 ± 8.46	21.96 ± 10.26	<0.0001
ALT (IU/L)	21.62 ± 14.75	20.18 ± 15.34	21.03 ± 14.31	23.19 ± 14.52	<0.0001
GGT (IU/L)	26.36 ± 30.09	25.01 ± 32.21	27.81 ± 32.76	26.11 ± 25.70	0.0263
Serum albumin (g/L)	41.22 ± 3.23	41.02 ± 3.34	41.19 ± 3.30	41.40 ± 3.08	0.0022
Serum creatinine (mg/dL)	0.85 ± 0.30	0.83 ± 0.26	0.84 ± 0.29	0.88 ± 0.32	<0.0001
Uric acid (mg/dL)	5.29 ± 1.38	5.18 ± 1.42	5.25 ± 1.38	5.40 ± 1.33	<0.0001
CAP (dB/m)	261.47 ± 63.66	255.96 ± 63.55	265.24 ± 65.89	262.36 ± 61.41	<0.0001

Mean ± SD for continuous variables: the *P*-value was calculated by the weighted linear regression model. (%) for categorical variables: the *P*-value was calculated by the weighted chi-square test.

TABLE 2 The association between magnesium intake (mg/day) and controlled attenuation parameter (dB/m).

	Model	Model 2	Model 3
	β (95% CI) *P*-value	β (95% CI) *P*-value	β (95% CI) *P*-value
Magnesium intake (mg/day)	0.01 (−0.00, 0.02) 0.1402	−0.02 (−0.04, −0.01) 0.0003	−0.01 (−0.03, −0.00) 0.0477
Magnesium intake tertile			
Low (10.0–185.5 mg/day)	Reference	Reference	Reference
Middle (185.6–284.0 mg/day)	9.28 (5.17, 13.39) <0.0001	4.17 (0.25, 8.10) 0.0373	2.42 (−1.67, 6.50) 0.2462
High (284.1–1,097.0 mg/day)	6.39 (2.39, 10.39) 0.0017	−4.18 (−8.09, −0.27) 0.0364	−3.60 (−7.61, 0.41) 0.0782
P for trend	0.004	0.019	0.036

Model 1: no covariates were adjusted.
Model 2: age, gender, and race/ethnicity were adjusted.
Model 3: age, gender, race/ethnicity, education level, marital status, body mass index, smoking behavior, and the existence of diabetes, hypertension, and high cholesterol level, aspartate aminotransferase, alanine aminotransferase, γ- glutamyl transpeptidase, serum albumin, serum creatinine and uric acid were adjusted.
In the subgroup analysis stratified by gender and race/ethnicity, the model is not adjusted for gender and race/ethnicity, respectively.

TABLE 3 Stratified analyses of the association between magnesium intake (mg/day) and controlled attenuation parameter (dB/m).

Subgroup	β (95% CI) P value	*P* for interaction
Age (years)		0.5169
<45	−0.02 (−0.04, 0.00) 0.0765	
45–60	−0.00 (−0.02, 0.02) 0.9278	
>60	−0.01 (−0.04, 0.01) 0.2605	
Gender		0.0389
Men	−0.00 (−0.02, 0.01) 0.7084	
Women	−0.02 (−0.04, −0.01) 0.0091	
Race/ethnicity		0.0495
Mexican American	−0.00 (−0.04, 0.03) 0.9272	
Other Hispanic	0.00 (−0.04, 0.04) 0.9241	
Non-Hispanic White	−0.02 (−0.04, −0.00) 0.0307	
Non-Hispanic Black	0.02 (−0.01, 0.05) 0.1851	
Other Race	0.02 (−0.01, 0.06) 0.1768	
Education level		0.5770
Less than high school	0.01 (−0.04, 0.05) 0.7937	
High school	−0.01 (−0.03, 0.02) 0.5507	
More than high school	−0.02 (−0.03, −0.00) 0.0352	
Marital status		0.3185
Married/living with partner	−0.01 (−0.02, 0.01) 0.3308	
Widowed/ divorced/ separated	−0.03 (−0.06, −0.00) 0.0310	
Never married	−0.02 (−0.04, 0.01) 0.3058	
Smoked at least 100 cigarettes in life		0.0803
Yes	−0.03 (−0.05, −0.01) 0.0130	
No	−0.00 (−0.02, 0.01) 0.7064	
Diabetes		0.0649
Yes	−0.01 (−0.05, 0.03) 0.6527	
No	−0.01 (−0.02, 0.00) 0.1117	
Borderline	−0.10 (−0.18, −0.03) 0.0090	
Hypertension		0.0070
Yes	0.01 (−0.01, 0.04) 0.2381	
No	−0.02 (−0.04, −0.01) 0.0022	
High cholesterol level		0.5610
Yes	−0.01 (−0.03, 0.01) 0.5126	
No	−0.01 (−0.03, 0.00) 0.0689	

Age, gender, race/ethnicity, education level, marital status, body mass index, smoking behavior, and the existence of diabetes, hypertension, and high cholesterol level, aspartate aminotransferase, alanine aminotransferase, γ- glutamyl transpeptidase, serum albumin, serum creatinine, and uric acid were adjusted.
Each stratification adjusted for the above factors except the stratification factor itself.

regression model was employed. The beta values and 95% confidence intervals were calculated using weighted multivariate linear regression analysis between the magnesium intake and CAP. For the purpose of subgroup analysis, stratified multivariate regression analysis was performed, and their interactions were tested. A combination of smooth curve fits and generalized additive models were utilized to investigate the non-linear relationship between CAP and magnesium intake. After non-linearity was identified, we used a recursive method to identify the inflection point in the connection between magnesium intake and CAP, and on either side of this point, we applied a two-piecewise linear regression model. All analyses were conducted using R (http://www.Rproject.org) and EmpowerStats (http://www.empowerstats.com), considering a *P*-value <0.05 as statistically significant.

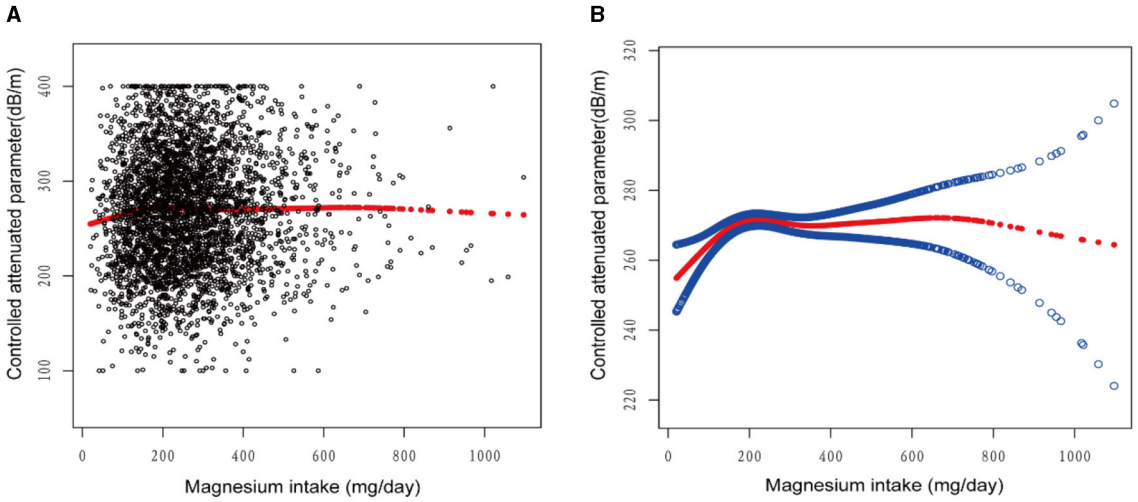

FIGURE 2
The association between magnesium intake and controlled attenuation parameter. **(A)** Each black point represents a sample. **(B)** Solid rad line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. Age, gender, race/ethnicity, education level, marital status, body mass index, smoking behavior, and the existence of diabetes, hypertension, and high cholesterol level, aspartate aminotransferase, alanine aminotransferase, γ- glutamyl transpeptidase, serum albumin, serum creatinine, and uric acid were adjusted.

Results

Our study comprised 5,935 participants. The population characteristics based on magnesium intake tertiles are shown in Table 1. Higher magnesium intake was associated with an older age, male predominance, non-Hispanic White race, higher education and income levels, and married/partnered marital status. Higher cholesterol levels, liver enzyme levels (AST, ALT, GGT), serum albumin, serum creatinine, uric acid and CAP values increased with higher magnesium intake. No significant differences were seen across tertiles for BMI, smoking rates, diabetes or hypertension prevalence.

Table 2 shows the results of the multivariate linear regression analysis. In the unadjusted Model 1, magnesium intake showed no significant association with CAP (β = 0.01, 95% CI: −0.00, 0.02, P = 0.1402). But when age, sex, and race/ethnicity were taken into account, Model 2 showed a significant correlation between increased magnesium intake and decreased CAP (β = −0.02, 95% CI: −0.04, −0.01; P = 0.0003). Even after accounting for extra factors, Model 3′s negative connection persisted (β = −0.01, 95% CI: −0.03, −0.00; P = 0.0477). Analysis by tertiles of magnesium intake in Model 3 showed that the highest tertile had lower CAP than the lowest tertile (β = −3.60, 95% CI: −7.61, 0.41, P = 0.0782), demonstrating a significant linear trend (P for trend = 0.036).

Table 3 details stratified analyses of the association between magnesium intake and CAP. After adjusting for confounding factors in the study, the inverse link between magnesium intake and CAP remained significant for women (β = −0.02, 95% CI: −0.04, −0.01, P = 0.0091), White people (β = −0.02, 95% CI: −0.04, −0.00, P = 0.0307) and people without hypertension (β = −0.02, 95% CI: −0.04, −0.01, P = 0.0070), and interaction tests further confirmed the significance of the differences between groups (all P for interaction < 0.05). In gender, education level, marital status, smoking behavior, diabetes, and high cholesterol subgroups, the interaction effect was not statistically significant, indicating that the inverse association between magnesium intake and liver steatosis deposition remains consistent across these subgroups.

Figures 2–5 illustrate smooth curve fits and generalized additive models, demonstrating an inverted U-shaped relationship between magnesium intake and CAP in women, White people and people without hypertension. As shown in Table 4, in the female subgroup, the standard linear model showed a significant inverse association between magnesium intake and controlled attenuation parameter (CAP) (β = −0.02, 95%CI: −0.04, −0.01, P = 0.0091). The two-piecewise linear regression model revealed a threshold point at 126 mg/day. Below this threshold, there was a significant positive association between magnesium intake and CAP (β = 0.31, 95% CI: 0.13, 0.48, P = 0.0005); above the threshold, a significant inverse association was observed (β = −0.04, 95% CI: −0.06, −0.02, P = 0.0001). Comparing the goodness of fit between the two-piecewise linear regression models and the standard linear model, the log-likelihood ratio test revealed a significant difference (P < 0.001), providing further support for the use of the two-piecewise regression model to capture threshold effects. Similarly, in the non-Hispanic white subgroup and the subgroup without hypertension, comparisons of the goodness of fit between the two-piecewise linear regression models and the standard linear model showed significant differences in the log-likelihood ratio tests (P = 0.001 and P = 0.004, respectively). In the White people, inflection points occur at a magnesium intake of 124.5 mg/day, as evidenced by a significant likelihood ratio (P = 0.001). For magnesium intakes below 124.5 mg/day, each 1 mg/day increase was related to a 0.36 dB/m raise in CAP (95% CI: 0.13, 0.60, P = 0.0026); by comparison, for individuals with a magnesium intake >124.5 mg/day, a 1 mg/day upregulation was connected with a 0.03 dB/m drop in CAP (95% CI: −0.05, −0.01, P = 0.0022). In the subgroup without hypertension, the two-piecewise linear regression model indicated a threshold value of 125 mg/day. When

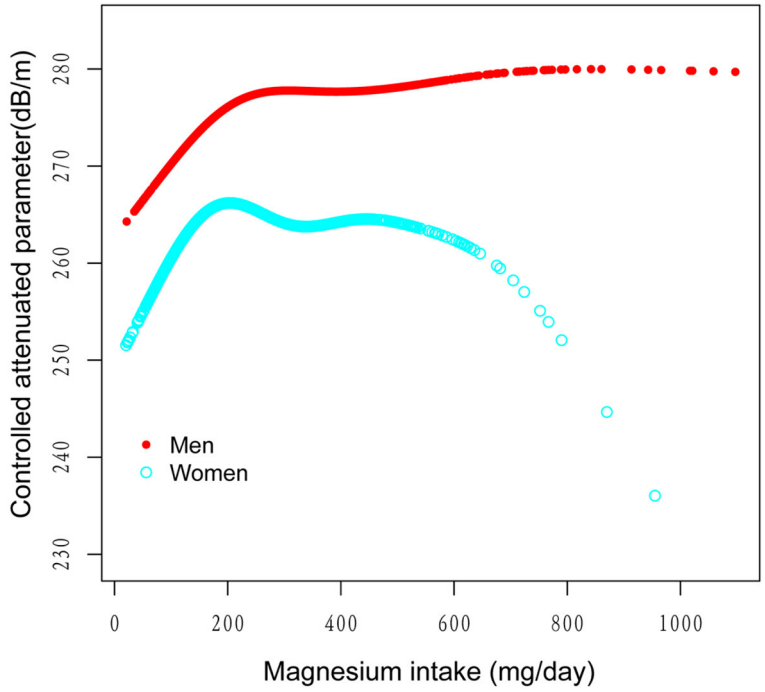

FIGURE 3
The association between magnesium intake and controlled attenuation parameter stratified by gender. Age, race/ethnicity, education level, marital status, body mass index, smoking behavior, and the existence of diabetes, hypertension, and high cholesterol level, aspartate aminotransferase, alanine aminotransferase, γ- glutamyl transpeptidase, serum albumin, serum creatinine, and uric acid were adjusted.

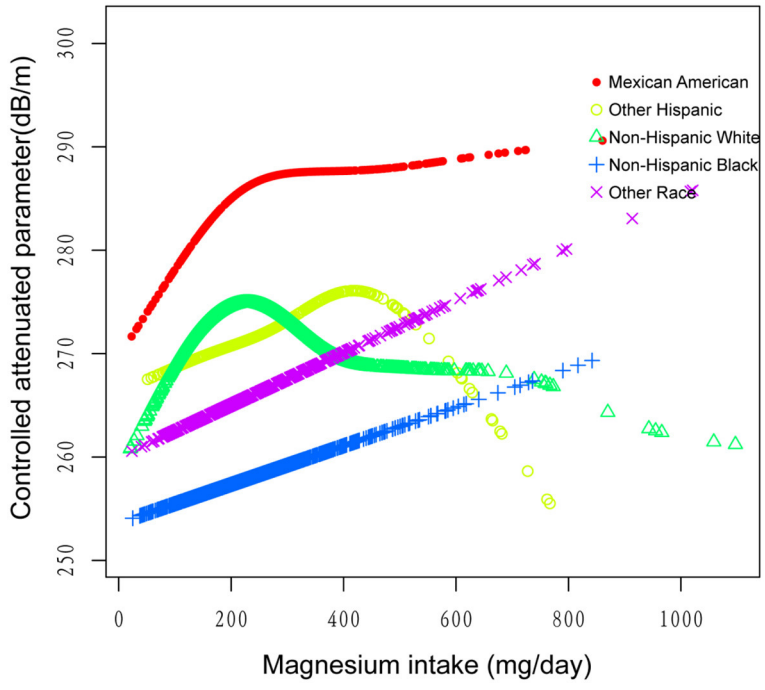

FIGURE 4
The association between magnesium intake and controlled attenuation parameter stratified by race/ethnicity. Age, gender, education level, marital status, body mass index, smoking behavior, and the existence of diabetes, hypertension, and high cholesterol level, aspartate aminotransferase, alanine aminotransferase, γ- glutamyl transpeptidase, serum albumin, serum creatinine, and uric acid were adjusted.

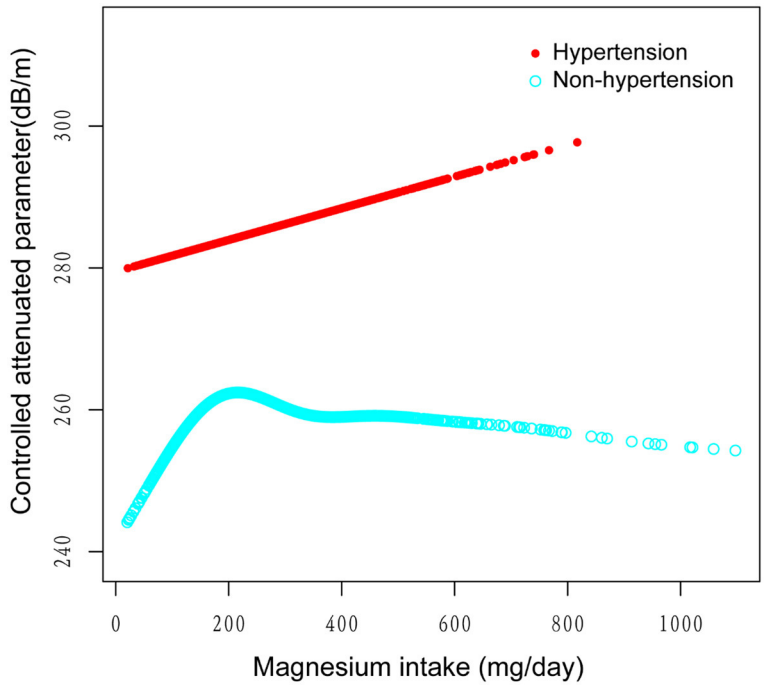

FIGURE 5
The association between magnesium intake and controlled attenuation parameter stratified by the existence of diabetes. Age, gender, race/ethnicity, education level, marital status, body mass index, smoking behavior, and the existence of diabetes, and high cholesterol level, aspartate aminotransferase, alanine aminotransferase, γ- glutamyl transpeptidase, serum albumin, serum creatinine, and uric acid were adjusted.

magnesium intake was below 125 mg/day, each 1 mg/day increase resulted in a 0.25 dB/m increase in CAP (95% CI: 0.07, 0.44, $P = 0.0077$); whereas when magnesium intake exceeded 125 mg/day, each 1 mg/day increase led to a decrease in CAP by 0.03 dB/m (95% CI: −0.04, −0.01, $P = 0.0003$).

Discussion

This study analyzed 2017–2020 NHANES data from the United States to explore the relationship between magnesium intake and liver fat accumulation, using CAP values as a measure. The multivariate regression analysis indicated a trend of decreasing CAP values correlating with increased magnesium intake. This inverse relationship between magnesium intake and hepatic steatosis is consistent with prior research. A study of 226 healthy individuals demonstrated an independent association between lower serum magnesium levels and biopsy-confirmed hepatic steatosis (13). Another analysis of the NHANES III cohort indicated a potential link between higher magnesium intake and lower liver disease mortality in hepatic steatosis patients (21). Furthermore, a study in which mice were fed a magnesium-deficient diet revealed increased levels of hepatic steatosis, swelling, and overall scores in comparison to a control group (22).

In a study on metabolic dysfunction-associated steatotic liver disease in the Korean NHANES database (23), magnesium intake was not mentioned in relation to MASLD, possibly due to variations in study populations and definitions of fatty liver. Our study, on the other hand, accounted for a broader range of confounding factors, potentially offering a more in-depth exploration of the true relationship between the two variables.

Magnesium deficiency is associated with an increased risk of liver steatosis through several pathways. It disrupts fatty acid and triglyceride metabolism, leading to fat accumulation in the liver (22). The deficiency also interferes with insulin signaling and exacerbates inflammation and oxidative stress in the liver (24). Additionally, magnesium deficiency may increase liver adipocytes that produce proteases enhancing angiotensin II, further worsening steatosis (25). Conversely, magnesium supplementation can regulate fatty acid metabolism, promote fatty acid oxidation, and activate the AMP-activated protein kinase-mammalian rapamycin target protein (AMPK-mTOR) pathway to induce autophagy in liver cells. This helps reduce intracellular fat deposition and thereby prevent or improve hepatic steatosis (22).

Our subgroup analysis revealed significant non-linear associations between magnesium intake and CAP in the female, White people, and non-hypertension subgroups, each showing a distinct turning point. Below this point, magnesium intake and CAP were positively correlated, while above it, they exhibited a significant inverse relationship. These findings suggest that the optimal level of magnesium intake may vary by gender, ethnicity, and hypertension status. To our knowledge, this may be the first study to unveil the complex non-linear relationship between magnesium intake and liver fat accumulation, indicating potential gender, ethnic, and hypertension-related differences. These variations may be attributable to genetic risk factors, lifestyle differences, and other elements (26). Further research, involving larger sample sizes and a prospective approach, is necessary for

TABLE 4 Threshold effect analysis of magnesium intake on controlled attenuation parameter using the two-piecewise linear regression model.

Controlled attenuation parameter	Adjusted β (95% CI), *P*-value
Women	
Fitting by the standard linear model	−0.02 (-0.04,−0.01) 0.0091
Fitting by the two-piecewise linear model	
Inflection point	126
Magnesium intake < 126 (mg/day)	0.31 (0.13, 0.48) 0.0005
Magnesium intake > 126 (mg/day)	−0.04 (−0.06, −0.02) 0.0001
Log likelihood ratio	<0.001
Non-Hispanic White	
Fitting by the standard linear model	−0.02 (−0.04, −0.00) 0.0307
Fitting by the two-piecewise linear model	
Inflection point	124.5
Magnesium intake < 124.5 (mg/day)	0.36 (0.13, 0.60) 0.0026
Magnesium intake > 124.5 (mg/day)	−0.03 (−0.05, −0.01) 0.0022
Log likelihood ratio	0.001
Non-hypertension	
Fitting by the standard linear model	−0.02 (−0.04, −0.01) 0.0048
Fitting by the two-piecewise linear model	
Inflection point	125
Magnesium intake < 125 (mg/day)	0.25 (0.07, 0.44) 0.0077
Magnesium intake > 125 (mg/day)	−0.03 (−0.04, −0.01) 0.0003
Log likelihood ratio	0.004

Age, gender, race/ethnicity, education level, marital status, body mass index, smoking behavior, and the existence of diabetes, hypertension, and high cholesterol level, aspartate aminotransferase, alanine aminotransferase, γ- glutamyl transpeptidase, serum albumin, serum creatinine, and uric acid were adjusted.
Each stratification adjusted for the above factors except the stratification factor itself.

validation. The scale of our study, using data from the NHANES cohort, strengthens our findings, given NHANES' nationally representative scope. This discovery holds significant clinical implications by emphasizing the need to consider individual characteristics when assessing the role of magnesium in preventing and managing hepatic steatosis. Nevertheless, our study has limitations; as a cross-sectional study, it establishes only a correlation, not a causal relationship, between magnesium intake and liver fat. Additionally, the magnesium intake data, based on two 24-h dietary recalls, could be influenced by reporting biases. Moreover, as our sample exclusively comprises U.S. participants, this might limit the generalizability of our findings to international populations. Lastly, there may be other potential biases stemming from additional confounding factors. For instance, due to limitations in the NHANES database, not all cardiac metabolic factors are available, leading to an incomplete assessment of cardiac metabolic risk factors.

Conclusions

Our research suggests an inverse relationship between magnesium intake and hepatic steatosis in the majority of Americans. In women, whites, and non-hypertensive individuals, the relationship followed an inverted U-curve, with turning points at 126, 124.5, and 125 mg/day, respectively. The impact of magnesium intake on CAP values varies before and after these turning points. These findings inform clinical nutritional interventions and personalized magnesium intake, underscoring the significance of magnesium research in developing pharmacological approaches to reverse liver steatosis.

Data availability statement

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

Ethics statement

The studies involving humans were approved by National Center for Health Statistics Research Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

XC: Data curation, Investigation, Methodology, Software, Writing – original draft. LF: Investigation, Writing – review & editing. ZZ: Project administration, Writing – review & editing. YW: Methodology, Project administration, 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.

Publisher's note

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Effects of *Portulaca oleracea* (purslane) on liver function tests, metabolic profile, oxidative stress and inflammatory biomarkers in patients with non-alcoholic fatty liver disease: a randomized, double-blind clinical trial

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Background: Non-alcoholic fatty liver disease (NAFLD) is a prevalent chronic liver disease. *Portulaca oleracea* exhibits anti-oxidant, anti-inflammatory, and hepatoprotective effects. This clinical trial aimed to investigate the potential benefits of *Portulaca oleracea* in improving NAFLD.

Methods: This double-blind, randomized clinical trial enrolled 70 patients with NAFLD assigned to either the intervention group ($n = 35$) or placebo group ($n = 35$) using stratified block randomization. The intervention group received 700 mg *Portulaca oleracea* supplement for eight weeks, while the control group received placebo capsules. In addition, all participants received a calorie-restricted diet. Liver steatosis and fibrosis were assessed using elastography along with liver function and metabolic tests, blood pressure measurements, body composition analysis and dietary records pre-and post-intervention.

Results: The average age of the participants was 44.01 ± 8.6 years, of which 34 (48.6%) were women. The group receiving *Portulaca oleracea* showed significant weight changes, body mass index, fat mass index, and waist circumference compared to the placebo ($p < 0.001$). In addition, blood sugar, lipid profile, liver enzymes aspartate and alanine transaminase, gamma-glutamyl transferase, and systolic blood pressure were significantly improved in the intervention group compared to those in the placebo ($p < 0.05$). During the study, inflammatory and oxidative stress indicators, improved significantly ($p < 0.05$). Based on the elastography results, the hepatorenal ultrasound index and liver stiffness

decreased significantly in the *Portulaca oleracea* group compared to the placebo ($p < 0.001$).

Conclusion: The present clinical trial showed that receiving *Portulaca oleracea* supplement for eight weeks can improve the condition of liver steatosis and fibrosis in patients with NAFLD.

KEYWORDS

non-alcoholic fatty liver, liver steatosis, *Portulaca oleracea*, oxidative stress, inflammation, purslane

1 Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide. The global prevalence of this disease is estimated to be between 25 and 40% in high-risk groups and is rapidly increasing (1). The prevalence of NAFLD has increased in recent years along with the obesity epidemic, type 2 diabetes, hypertension, and hypercholesterolemia (2). NAFLD encompasses a spectrum of liver diseases including steatosis, non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma (1).

Approximately 25–40% of NASH patients progress to advanced liver fibrosis; 20–30% of this group will develop cirrhosis and its associated complications. Numerous long-term studies have shown that the mortality rate is 60% in patients with NAFLD with advanced fibrosis and 9% in patients with NAFLD without advanced fibrosis (3).

The management of non-alcoholic fatty liver disease is based on treating the associated liver and metabolic disorders, such as obesity, dyslipidemia, and insulin resistance. Lifestyle modifications, including dietary changes and physical activity aimed at gradual weight loss, are the mainstay of NAFLD management (4, 5), and therapy with the primary goal of improving liver status is recommended only for those with non-alcoholic steatohepatitis or liver fibrosis (6). Interestingly, several recent studies have revealed that dietary components or medicinal plants (with pharmacological capabilities) could be considered substitutes for traditional NAFLD management methods (7–12). Among them, special attention has been paid to supplements that can effectively reduce oxidative stress, which is known to cause hepatic lipotoxicity and inflammation, one of the leading causes of disease pathogenesis (13). However, given the prevalence and complications of the disease, there is still a need for therapeutic supplements to reduce inflammatory status and oxidative stress and improve the lipid profile and nutritional status of these patients (14, 15). *Portulaca oleracea* is an important medicinal plant is *Portulaca oleracea*, that is a rich source of biologically active compounds, including omega-3 fatty acids and β-carotene (16), amino acids, α-tocopherols, ascorbic acid, glutathione (GSH) (17), and flavonoids (18). The anti-inflammatory activity of purslane has been confirmed in several studies (19–24). Hepatoprotective (25–29) and anti-diabetic effects (30, 31) of *Portulaca oleracea* extract have also been reported in animal studies. Therefore, Based on its anti-inflammatory and antioxidant properties as well as its ability to reduce the expression of pro-fibrogenic cytokines and decrease collagenolytic activity, it has been shown that *Portulaca oleracea* has anti-steatotic and anti-fibrotic effects on the liver (32, 33).

Various studies have investigated the effects of *Portulaca oleracea* on different aspects (34). However, to our knowledge, no human study has been conducted on the effect of aerial part extracts on non-alcoholic fatty liver fibrosis. Significant animal studies have been carried out on the aerial parts of *Portulaca oleracea*, demonstrating its beneficial effects on fatty liver in mice, including antioxidant, anti-inflammatory, and liver-protective properties attributed to its alkaloids, flavonoids, terpenoids, sterols, omega-3 unsaturated fatty acids, and numerous vitamin and mineral components (22).

Given the current lack of effective treatments for fatty liver disease and the wide range of pharmacological properties attributed to *Portulaca oleracea* due to its demonstrated anti-inflammatory and antioxidant properties, this study aims to investigate the effects of *Portulaca oleracea* extract supplementation on various aspects of NAFLD, including evaluating its impact on liver steatosis and fibrosis degrees. Secondary objectives involve assessing its effects on dietary intake, weight, body composition, serum lipid profiles, insulin resistance, fasting blood sugar (FBS) levels, inflammatory markers, liver function tests, and oxidative stress levels. We expect that supplementation will lead to improvements in these parameters, potentially reducing the severity of NAFLD. Confirming the efficacy of *Portulaca oleracea* extract in human subjects could introduce a novel therapeutic strategy for NAFLD management, emphasizing the importance of integrating traditional medicine with modern pharmacology for addressing complex diseases effectively.

2 Materials and methods

2.1 Study design and patient selection

The present study was a randomized, double-blind, parallel, and placebo-controlled clinical trial designed to investigate the effects of a hydroethanolic extract of the aerial parts of *Portulaca oleracea* on clinical and laboratory outcomes in patients with non-alcoholic fatty liver disease. This study was conducted between December 2021 and November 2022 in Mashhad, Razavi Khorasan Province, Iran. The study was conducted according to the principles of the Helsinki Declaration and approved by the ethics committee of the Mashhad University of Medical Sciences (ethics code: IR.MUMS.REC.1400.223). The study protocol have been recently published (35). At the outset of the study, written informed consent was obtained from all participants after providing them with complete information regarding the study procedures.

Participants were recruited from among patients with non-alcoholic fatty liver disease who had been referred to the nutrition clinic and met the inclusion criteria.

Inclusion criteria:

- Participants aged between 18 and 65 years were eligible.
- Diagnosis of steatosis was confirmed via two-dimensional elastography.
- Participants demonstrated willingness to participate in the study.
- Non-alcoholic fatty liver patients classified with F0 and F1 grades were included.

Exclusion criteria:

- Participants who were pregnant or lactating were excluded.
- Individuals with a history of diabetes, autoimmune disorders, cancer, liver failure, viral hepatitis, liver surgery, or kidney disorders (GFR <50) were excluded.
- Participants with a known allergy to *Portulaca oleracea* or herbal supplements were excluded.
- Individuals using hepatotoxic medications such as amiodarone, tamoxifen, sodium valproate, or Methotrexate were excluded.
- Participants with a body mass index (BMI) exceeding 40 were excluded.
- Those with alcohol consumption exceeding 30 g/day for men or 20 g/day for women were excluded.
- Individuals with a history of bariatric surgery were excluded.
- Non-alcoholic fatty liver patients classified with F2, F3, or F4 grades were excluded.
- Participants using multivitamins, antioxidants such as vitamins C and E, silymarin, or herbal medicines regularly were excluded.
- Those who did not comply with weight loss programs in the preceding three months were excluded.

Additionally, participants who became pregnant, developed sensitivity to *P. oleracea* extract or placebo, or exhibited less than 70% compliance with their assigned treatment during the study period were excluded from further analysis.

2.2 Randomization and blinding

To minimize potential biases and enhance comparability between study groups, participants were randomly allocated to either the intervention or control groups using a stratified block randomization method. This process ensured balanced distribution across key demographic factors, including age (18–40 years and 40–65 years) and sex (male/female). Randomization was achieved through four blocks in equal proportions.

Participants assigned to the intervention group received two capsules of *Portulaca oleracea* extract daily, each containing 350 mg of hydroethanolic extract derived from the aerial parts of *Portulaca oleracea*. These capsules were administered with meals (breakfast and dinner) for a duration of eight weeks, alongside a prescribed dietary regimen tailored for NAFLD management.

Conversely, participants assigned to the control group received two placebo capsules daily with meals for the same duration of eight weeks. These placebo capsules contained an inert compound, avicel, and were identical in appearance to the *Portulaca oleracea* capsules. Both intervention and control groups followed the same dietary regimen throughout the study period.

To ensure blinding, neither the participants nor the researchers, except for the pharmacist responsible for capsule preparation, were aware of the group assignments until the completion of the study and subsequent data analysis. Blinding was maintained throughout the study in a double-blind manner. Coded containers were utilized to conceal the random allocation of participants to their respective groups, further preserving the integrity of the blinding process.

2.3 Procedures

2.3.1 Dose selection

The dosage of *P. oleracea* extract (700 mg daily) was determined based on a previous study in rats (22) and a pilot trial involving five individuals to assess potential challenges or adverse effects.

2.3.2 Preparation of capsules

A 70% hydroalcoholic extract was prepared from the aerial parts of *P. oleracea*. The extract was standardized using the Folin-Miran method, and its components were identified using LC–MS/MS. Capsules containing 350 mg of dried extract and 150 mg of Avicel were prepared for the intervention group, while placebo capsules containing only Avicel and a green colorant were prepared for the control group.

2.3.3 Intervention

Seventy patients with non-alcoholic fatty liver disease were enrolled and randomly assigned to either the intervention or control group. The intervention group received *P. oleracea* capsules (350 mg) twice daily for 60 days, while the control group received placebo capsules. Both groups were provided with a hypocaloric diet and physical activity guidelines.

2.3.4 Follow-up

Participants were followed up through weekly telephone calls and visits during the final week of each 30-day period to assess their well-being and monitor progress.

2.4 Data collection and analysis

Data were collected three times: at baseline, on day 30 of the intervention, and on day 60 of the follow-up visit. A questionnaire was used to collect demographic data. Additionally, blood pressure (via a Riester Nova.1032), body mass index, body composition (via a bioimpedance device, "TANITA"), height (via a standard meter), weight (via a portable scale, "Balas"), gastrointestinal complications, and blood pressure were measured at baseline, on the 30th day, and at the end of the intervention period. Transient elastography was performed at the beginning and end of the trial to assess the state of the liver. At baseline, on the 30th day, and at the end of the intervention, participants completed a 3-day food diary and the International Physical Activity Questionnaire (IPAQ). To reduce bias, the responder was asked to clarify any inconsistent answers. Ten milliliters of venous blood was drawn from each patient at the

beginning and end of the study to assess biochemical factors such as CBC-diff (using Sysmex KX21), lipid profiles, FBS, serum insulin, hepatic enzymes such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total and direct bilirubin (via Auto analyzers), as well as inflammatory and oxidative stress markers such as high-sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), Glutathione peroxidases (GPx), and Malondialdehyde (MDA) (using Colorimetry[a]). Serum samples were isolated, and ELISA kits were used to measure the markers.

2.5 Study measurements

The primary outcome, liver steatosis, was assessed using the SuperSonic Aixplorer device and a two-dimensional elastography technique at baseline and the end of the study. Secondary outcomes included biochemical evaluations, food intake, anthropometric measurements, blood pressure, physical activity, and other variables. Fasting blood samples were collected at baseline and the end of the study to measure serum levels of liver enzymes, lipid profile, glucose, insulin, and markers of oxidative stress and inflammation. Participants completed a three-day food record at the beginning of the study and at the end of each month to evaluate dietary intake. Height, weight, body composition, and waist circumference were measured at baseline, day 30, and the end of the intervention period. Blood pressure was measured at each visit using a calibrated and standardized mercury sphygmomanometer. The International Physical Activity Questionnaire-Short Form (IPAQ) was used to collect data on participants' physical activity levels at baseline, day 30, and the end of the intervention period. Demographic and socioeconomic information, as well as gastrointestinal symptoms, were assessed using specific questionnaires.

Detailed information on the methodology, including comprehensive procedures for capsule preparation and intervention, safety considerations, and an in-depth description of the study measurements, is available in the Supplementary materials.

2.6 Statistical analyses

In this study, the sample size was determined using the A'Hern RP method and the formula for comparing two proportions related to a qualitative trait, specifically hepatic steatosis, which is applicable to Phase 2 clinical trials (24). Based on the results obtained from treating patients with non-alcoholic fatty liver disease using an anti-oxidant supplement (36), it was assumed that the intervention group would exhibit improvements in hepatic steatosis. With a significance level (alpha) of 0.05, power (beta) of 0.2 (equivalent to 80 percent power), and utilizing the formula for comparing two proportions associated with the qualitative trait of hepatic steatosis, a minimum of 30 participants per group was deemed necessary. The sample size was increased to 36 individuals per group, with a maximum potential dropout rate of 20%.

Following the completion of data collection, all gathered data were entered into the statistical software SPSS version 26 for further analysis. The dataset in this study comprised qualitative and quantitative variables of both continuous and discrete natures. Descriptive and inferential statistical methods were used for data analysis. Before conducting the analyses, the normality of the distribution of quantitative variables was assessed using the Shapiro–Wilk test. Parametric statistical tests were applied to variables that exhibited a normal distribution, whereas non-parametric equivalents were used for variables that did not meet the normality assumption.

Descriptive statistics such as mean, standard deviation, and frequency distribution were used to summarize the demographic information and individual characteristics of the study participants. Quantitative data that followed a normal distribution are presented as mean ± standard deviation, whereas non-normally distributed quantitative data are reported using the median and interquartile range. Qualitative data were summarized using frequency counts and percentages.

To account for missing data, the Last Observation Carried Forward (LOCF) method was employed to impute the missing values using the last observed measurement. The distribution of qualitative variables was examined using the chi-square test for comparisons between and within the groups. Independent sample t-tests were conducted for normally distributed quantitative variables for between-group comparisons and paired t-tests were used for within-group comparisons. In cases where the distribution of quantitative variables was non-normal, non-parametric tests, such as the Mann–Whitney U and Wilcoxon signed-rank tests, were employed.

To control for potential confounding factors such as baseline values, changes in energy intake, physical activity, and body weight, analysis of covariance (ANCOVA) was used. Qualitative variables are presented as frequency counts and percentages. In contrast, quantitative variables with skewness less than one were reported as mean ± standard deviation, and those with skewness greater than or equal to one were presented as median (interquartile range). A statistical significance level of less than 5 percent ($p < 0.05$) was considered statistically significant.

3 Results

3.1 Characteristics of patients

A total of 240 patients were screened for eligibility and 82 met the inclusion criteria. Ten patients declined to participate in the study, and 72 patients were randomly assigned to the intervention ($n = 36$) and control ($n = 36$) groups. One patient was excluded from each group because of pregnancy or unwillingness to continue the study. Finally, 70 patients (purslane group, $n = 35$; placebo group, $n = 35$) completed the trial (Figure 1).

The baseline patient characteristics are shown in Table 1. The mean age of the patients was 44.01 ± 8.6, of which 51.4% were male. Most of the patients were overweight or obese, and only two patients had a normal BMI. The physical activity level (PAL) of 55.7% of the patients was moderate, and only two had vigorous PAL. There were no significant differences between the purslane and placebo groups in terms of age, sex, BMI, PAL, smoking, and comorbidities, including type 2 diabetes, cardiovascular diseases, hyperlipidemia, and hypertension. Moreover, regarding energy, macronutrient, and micronutrient intake, no significant differences were observed between the two groups (Table 2).

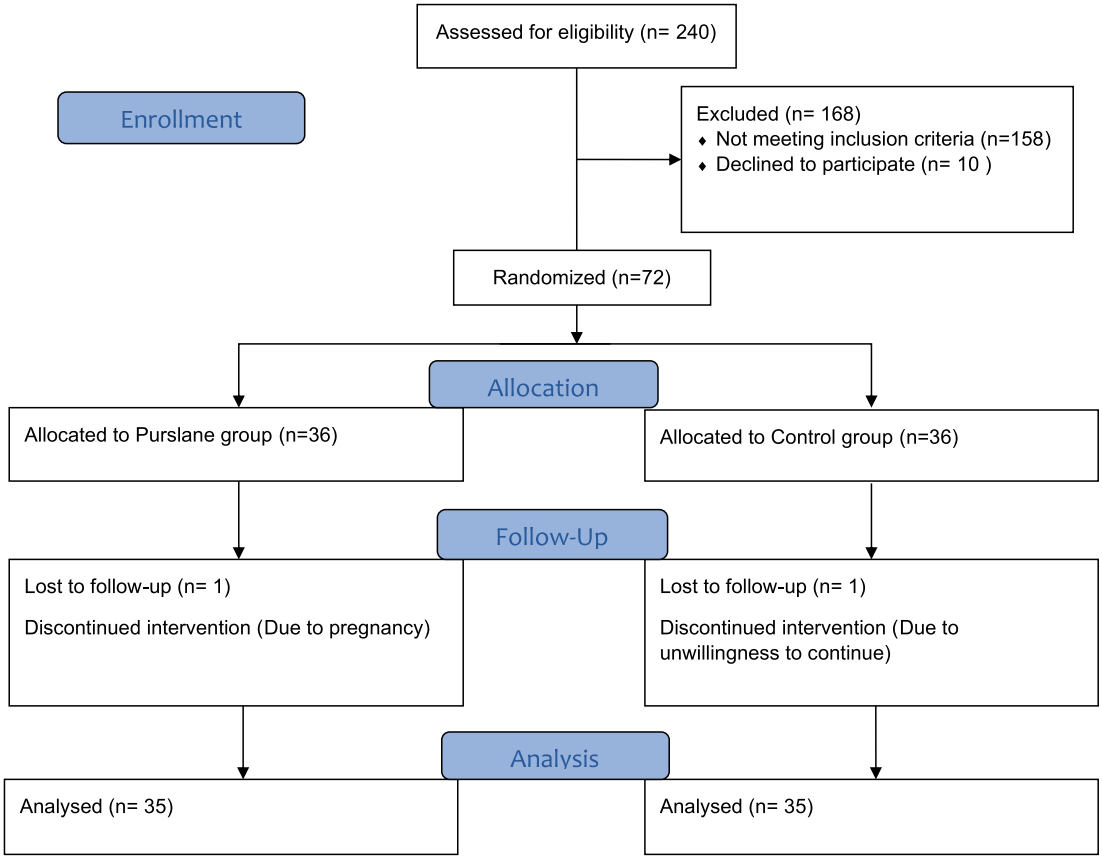

FIGURE 1
Consort flow diagram.

3.2 Anthropometric measurements and blood pressure

Table 3 demonstrates the anthropometric measurements and blood pressure of the participants at baseline and at weeks 4 and 8. Weight, BMI, waist circumference (WC), and fat mass index (FMI) significantly decreased after 8 weeks in the purslane group compared to those in the control group (p-value <0.001) and remained significant after adjusting for baseline values (p-value <0.001). The control and intervention groups experienced reduced systolic and diastolic blood pressures; however, this reduction was significantly greater in the intervention group after adjusting for baseline values, weight, energy intake, and PAL.

3.3 Liver function and glycemic indices

The changes in liver function and glycemic indices of the two groups after 8 weeks are shown in Table 4. Although there were no significant changes in ALP levels in the two groups, the levels of AST, ALT, GGT, and total and direct bilirubin significantly decreased in the purslane group compared to those in the placebo group. Regarding the glycemic indices, a significant decrease was observed in FBS levels and TyG index after treatment with purslane for 8 weeks. The grades of liver steatosis at baseline and at the end of the intervention are shown in Figure 2. A significant improvement in liver steatosis was observed in the purslane group compared with that in the placebo group.

3.4 Lipid profile, inflammation, and oxidative stress

Table 5 illustrates the patients' lipid profile changes, oxidative stress, and inflammatory markers. There was a significant improvement in the total cholesterol (TC), triglycerides (TG), and high-density lipoproteins (HDL) levels in the purslane group compared to those in the placebo group. In contrast, no significant change was observed in low-density lipoproteins (LDL) levels (p-value =0.05). In terms of oxidative stress, MDA levels did not change significantly after the intervention; however, a significant increase in superoxide dismutase (SOD), GPxs, and catalase levels was observed in the intervention group. Regarding inflammatory markers, purslane supplementation significantly decreased IL-6 and hs-CRP levels and ESR (p-value <0.001) (see Table 6).

4 Discussion

The findings of our study showed that supplementation with 700 mg of purslane aerial part extract along with a restricted-calorie diet for eight weeks led to an improvement in liver steatosis and liver

TABLE 1 Baseline characteristics of participants.

Characteristics	Purslane group	Placebo group	*p*-value
Age (years)	43.8 ± 7.6	44.2 ± 9.6	0.85#
Weight	85.97 ± 11.36	88.93 ± 13.17	0.31
Height (m)	1.67 ± 1.65	1.76 ± 1.81	0.53
Sex, *n* (%)			
Female	17 (48.6)	17 (48.6)	1.00
Male	18 (51.4)	18 (51.4)	
PAL, *n* (%)			
Mild	13 (37.1)	16 (45.7)	0.76
Moderate	21 (60)	18 (51.4)	
High	1 (2.9)	1 (2.9)	
BMI (kg/m^2), *n* (%)			
Normal	2 (5.7)	0 (0)	0.22
Overweight	15 (42.9)	20 (57.1)	
Obese	18 (51.4)	15 (42.9)	
Type 2 diabetes, *n* (%)	2 (6.3)	4 (11.4)	0.56
CVD, *n* (%)	2 (6.1)	3 (8.6)	0.71
Hyperlipidemia, *n* (%)	13 (40.6)	11 (31.4)	0.61
Hypertension, *n* (%)	5 (15.2)	6 (17.1)	0.67
Smoking, *n* (%)	3 (8.6)	7 (20)	0.37

Data are presented as Mean ± SD or number (percent). *p* extracted of Chi-Square except (#), which is obtained from two independent sample *t* test. $p < 0.05$ is considered as statistically significant.

function indicators (AST, ALT, GGT, and total and direct bilirubin) in patients with NAFLD. In addition, inflammatory factors (IL-6, hs-CRP, and ESR) and oxidative stress factors (SOD, GPx, and CAT) improved significantly. On the other hand, anthropometric indices such as weight, BMI, FMI, WC, metabolic indices (FBS, TyG, TG, HDL, TC), and DBP were also recovered in the purslane recipient group.

In contrast with intervention group, in patients receiving placebo, a slight increase in liver stiffness and hepatorenal index was observed, which was statistically significant. It is possible that nonadherence to the diet and physical activity recommended in the plan among some people in the placebo group could be the reason for this difference.

El-Sayed et al. (51) investigated the antidiabetic activity of purslane seeds in patients with type 2 diabetes. They observed that treatment with 10 g of purslane seed powder for eight weeks significantly decreased total and direct bilirubin, ALT, AST, GGT, TG, TC, LDL-c, FBS, insulin, body weight, and BMI and increased albumin and HDL-c levels. The control group (receiving 1,500 mg of metformin) showed the same results as the purslane group, except for albumin, direct bilirubin, HDL-c, and TG levels. These data show that purslane seeds improve liver function in patients with diabetes compared to metformin. In addition, purslane seeds have been shown to reduce blood glucose, insulin resistance, blood lipids, liver protection, and weight loss due to the content of polyunsaturated fatty acids (PUFA), flavonoids, polysaccharides, antioxidants, and vitamins (52). Various studies have suggested that purslane can significantly reduce the severity of non-alcoholic fatty liver steatosis owing to its hypoglycemic and hypolipidemic properties (53–55). Studies have also shown that obesity and related hormonal changes, including increased insulin synthesis, peripheral insulin resistance, increased leptin synthesis, and decreased adiponectin synthesis by adipose tissue, lead to a decrease in fatty acid oxidation and endothelial dysfunction (56), followed by an increase in blood sugar (57). Increased fasting blood glucose is independently associated with decreased adiponectin levels, which plays a significant role in reducing insulin resistance (58). Purslane can effectively improve liver steatosis through weight loss, increased adiponectin levels, increased fatty acid oxidation, and reduced insulin resistance (59). Ranaei et al. (60) investigated the effects of 400 mg/kg purslane extract on sirtuin levels and insulin resistance in rats with NAFLD. After eight weeks, purslane extract improved insulin resistance in NAFLD by increasing sirtuin levels. Sirtuin levels are associated with insulin resistance, which can predispose patients to the development of NAFLD (61). In addition, the activation of sirtuin by the polyphenols in purslane acts as an upstream regulator in the LKB1/AMPK signaling axis, suppressing Acetyl-CoA Carboxylase (ACC) and Fatty Acid Synthase (FAS) expression and reducing fat accumulation in liver cells (62, 63).

Damavandi et al. (59) investigated the effects of purslane extract in patients with NAFLD. Purslane extract at a dose of 300 mg daily for 12 weeks significantly decreased the ALT, AST, GGT, FBS, HOMA-IR, TG, and LDL-c levels in the purslane group. However, in contrast to our study, at the end of the study, no significant changes were observed in the grade of hepatic steatosis, insulin, liver enzymes, total bilirubin, lipid profile, and blood pressure compared to the control group.

A study by Wainstein et al. (64) reported that purslane extract (three capsules: 180 mg/day) for 12 weeks reduced BMI and body weight in patients with type 2 diabetes. Purslane is known to affect metabolism and body weight (54). In this regard, and in line with our study, the study by Esmaillzadeh et al. (65) showed that a daily intake of 10 g of purslane seeds for five weeks could improve anthropometric indicators (weight, BMI, and waist circumference).

In the present study, purslane supplementation significantly improved SOD levels. We also observed a significant increase in the levels of catalase (CAT) and GPx in the purslane group. In line with our results, in a study conducted by Sousou et al. (32) on rats with liver fibrosis, supplementation with purslane aerial part extract at a dose of 400 mg/kg per day caused a significant decrease in AST, ALT, ALP, GGT, total bilirubin, MDA, and tumor necrosis factor α (TNF-α), and a significant increase in SOD, CAT, GPx, and GSH levels. In this study, we investigated the anti-oxidant effects of purslane hydroethanolic extract against biliary obstruction caused by liver fibrosis in rats and observed that this compound inhibited oxidative stress, reduced the expression of profibrogenic cytokines, collagenolytic activity, and activated hepatic stellate cells.

Purslane, known for its hypolipidemic effects, influences liver function, inflammation, and oxidative stress through various mechanisms. Fresh purslane upregulates cholesterol 7a-hydroxylase (CYP7A1) and low-density lipoprotein receptor (Ldlr) to combat the harmful effects of a high-fat diet on the liver (66). Moreover, purslane's bioactive compounds, such as omega-3 fatty acids and antioxidants, may improve liver function, reduce inflammation, and combat oxidative stress through enhancing antioxidant enzymes like catalase and glutathione peroxidase, as shown in rat studies (67). Additionally, *Portulaca oleracea* L. flavone (POL-F) from purslane inhibits inflammatory responses by reducing Nitric oxide (NO) secretion and modulating phosphoinositide 3-kinases (p-PI3K) and p-AKT

TABLE 2 Energy, macronutrient, and micronutrient intake at baseline and at the end of week 8.

Dietary variables	Baseline	After 8 weeks	*p*-value*	Change	*p*-value**
Total energy (kcal)					
Placebo	2524.83 ± 318.82	1899.27 ± 301.46	<0.001	−625.56 ± 131.38	0.55
Purslane	2466.74 ± 294.10	1820.51 ± 264.75	<0.001	−646.23 ± 133.53	
Protein (g)					
Placebo	87.90 ± 18	82 ± 21.10	0.26	−5.80 ± 26.90	0.94
Purslane	89 ± 24.10	82.60 ± 16.50	0.22	−6.40 ± 26.50	
Carbohydrate (g)					
Placebo	352.20 ± 59.30	317.90 ± 61.40	0.01	−34.30 ± 66.30	0.91
Purslane	374.50 ± 52.60	341.50 ± 61.10	0.001	−32.90 ± 37	
Fat (g)					
Placebo	71.09 ± 25.90	66.45 ± 23.30	0.38	−4.64 ± 27.10	0.83
Purslane	73.80 ± 22	67.30 ± 19.70	0.20	−6.50 ± 22.80	
Fiber (g)					
Placebo	18.70 ± 5.70	16.20 ± 6.30	0.12	−2.50 ± 8.30	0.34[&]
Purslane	18.37 ± 4.10	17.70 ± 4.60	0.54	−0.67 ± 5.90	
Cholesterol (mg)					
Placebo	227 ± 105	217 ± 109	0.68	−10 ± 135	0.62
Purslane	236 ± 91	211 ± 103	0.28	−25 ± 118	
SFA (g)					
Placebo	21.87 ± 8.80	19.37 ± 6.80	0.06	−2.5 ± 6.90	0.23
Purslane	22.73 ± 8.70	17.05 ± 8.90	0.002	−5.68 ± 8.4	
Vitamin E (mg)					
Placebo	8.64 ± 7.15	6.70 ± 4.40	0.27[#]	−1.95 ± 8.90	0.78[&]
Purslane	9.96 ± 5.60	7.40 ± 4.10	0.06[#]	−2.56 ± 6.70	

Data are presented as Mean ± SD. p * is extracted from Paired t-test and p ** is extracted from two independent sample t test. p [&] is extracted from Mann–Whitney and p [#] is extracted from Wilcoxon. $p < 0.05$ is considered as statistically significant.

signaling pathways (68). Purslane seed extracts, particularly the methanol extract, exhibit anti-inflammatory properties by reducing TNF-α and IL-1β production through antioxidant pathways (69).

The results of our study showed that supplementation with 700 mg of purslane aerial part extract along with a restricted calorie diet for eight weeks led to a significant decrease in TC and TG levels and a significant increase in HDL-c levels in the purslane group compared with the placebo group. Sabzghabaee et al. (70) studied the antidyslipidemic effects of purslane in 74 obese adolescents. The results showed that the TG, LDL-C, and TC levels decreased significantly in the purslane group. In addition, the differences in LDL-C and TG parameters between the two groups were statistically significant. Purslane has positive effects on serum lipid profile, which may be attributed to its polyphenolic and antioxidant compounds. The uniqueness of purslane as the richest plant source of omega-3 PUFA has been well established. Purslane also contains high amounts of vitamins E and C, beta-carotene, and various flavonoids with anti-atherogenic activity (70).

We observed that supplementation with 700 mg of purslane aerial part extract, along with a restricted calorie diet for eight weeks, led to a significant reduction in DBP. High blood pressure is a known risk factor for NAFLD (71). Consistent with our study, Esmaillzadeh et al. (65) have shown the effects of purslane seeds on lowering blood pressure by receiving 10 g of purslane seeds daily for five weeks. Purslane contains PUFA and omega-3 fatty acids, vitamin E, vitamin C, beta-carotene, alkaloids, flavonoids, and polysaccharides (70). Purslane is the richest source of omega-3, and 100 g of fresh purslane leaves (one serving) contain approximately 300–400 mg of omega-3 (17). Evidence confirms the positive relationship between omega-3 consumption and blood pressure control (72).

4.1 Strength and limitations

In the current study, several items can be mentioned as the strengths of this research, which are as follows: In this research, a stratified block design was used to randomly assign the participants to the studied groups. This design resulted in a homogeneous distribution of characteristics between the study groups and the control of confounders. Moreover, to correctly estimate the effect of the intervention, detailed evaluations of potential confounders were made during the study, and their effects were adjusted in statistical models. In this study, the two-dimensional elastography method, which is more accurate than ultrasound and other conventional methods, was used to evaluate liver fibrosis and steatosis.

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TABLE 3 Anthropometric measurements and blood pressure at baseline and at the end of intervention.

Variables	Baseline	Week 4	Week 8	Change	*p*-value (group)	*p*-value (time)	*p*-value (group/time)	*p*-value[&]
Weight (kg)								
Placebo	88.9 ± 13	87.9 ± 13	87.5 ± 13	−1.4 ± 1.9	0.17	<0.001	<0.001	<0.001
Purslane	85.9 ± 11	84.1 ± 11	82.1 ± 11	−3.8 ± 2.4				
Waist circumference (cm)								
Placebo	108.4 ± 7.9	107.1 ± 8	106.3 ± 8	−2.08 ± 3	0.21	<0.001	<0.001	<0.001
Purslane	107.9 ± 8.9	104.2 ± 9	101.9 ± 9	−5.98 ± 3				
BMI (kg/m^2)								
Placebo	30.5 ± 4	30.2 ± 4	30 ± 4	−0.52 ± 0.7	0.89	<0.001	<0.001	<0.001
Purslane	30.7 ± 4	30.1 ± 4	29.4 ± 4	−1.37 ± 0.9				
Fat mass index (kg/m^2)								
Placebo	8.4 ± 3	8 ± 3	8 ± 3	−0.4 ± 0.6	0.60	<0.001	<0.001	<0.001
Purslane	9.1 ± 3	8.5 ± 3	8 ± 3	−1.07 ± 0.7				
Total body water (%)								
Placebo	52.2 ± 6	53.2 ± 6	53 ± 6	0.77 ± 1.7	0.41	<0.001	0.02	0.05
Purslane	50.7 ± 5	51.7 ± 5	52.4 ± 5	1.69 ± 1.4				
Fat-free mass (kg)								
Placebo	64.4 ± 10	64.7 ± 11	64.2 ± 10	−0.24 ± 1.5	0.14	0.02	0.36	0.12
Purslane	61 ± 10	60.7 ± 10	60.4 ± 10	−0.56 ± 1.4				
Trunk fat (%)								
Placebo	25.7 ± 7	24.3 ± 8	24.7 ± 7	−0.98 ± 2.6	0.86	<0.001	0.015	0.06
Purslane	26.5 ± 5	25.1 ± 5	23.9 ± 6	−2.57 ± 2.6				
Systolic blood pressure (mmHg)								
Placebo	136.8 ± 15	134 ± 13	130.5 ± 13	−6.25 ± 9.5	0.24	<0.001	0.064	0.03
Purslane	135.8 ± 13	130.1 ± 11	125.4 ± 11	−10.27 ± 10				
Diastolic blood pressure (mmHg)								
Placebo	87.3 ± 8	84.6 ± 7	82.8 ± 7	−4.45 ± 6.6	0.052	<0.001	0.04	0.01
Purslane	85.9 ± 8	81.5 ± 7	77.7 ± 6	−8.2 ± 8.4				

Data are presented as Mean ± SD. *p* is extracted from Repeated Measures. *p* [&] is presented adjusted model by weight and physical activity with ANCOVA. $p < 0.05$ is considered as statistically significant.

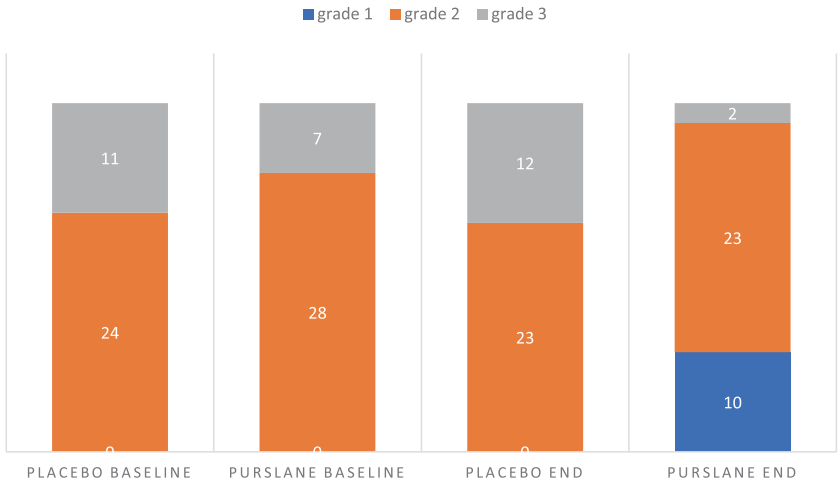

FIGURE 2
The grade of liver steatosis at baseline and at the end of intervention in placebo and purslane groups.

TABLE 4 Glycemic indices and liver function at baseline and at week 8.

Variables	Baseline	Week 8	Change	*p*-value*	*p*-value#	*p*-value&
FBS (mg/dl)						
Placebo	103.4 ± 20	103.8 ± 20	0.34 ± 7	0.78	<0.001	<0.001
Purslane	98.4 ± 16	92.9 ± 16	−5.54 ± 6	<0.001		
Insulin (uIU/ml)						
Placebo	19.42 ± 11.5	19.63 ± 11.5	0.21 ± 1.8	0.50	0.22	0.37
Purslane	18.6 ± 9.9	16.86 ± 9.5	−1.74 ± 9.3	0.27		
HOMA-IR						
Placebo	5.05 ± 3	5.78 ± 4.7	0.72 ± 4	0.29	0.08	0.13
Purslane	4.59 ± 3	3.87 ± 2.3	−0.71 ± 2.6	0.12		
TyG index						
Placebo	8.5 ± 0.5	8.49 ± 0.5	−0.01 ± 0.1	0.64	<0.001	<0.001
Purslane	8.79 ± 0.5	8.61 ± 0.5	−0.18 ± 0.1	<0.001		
ALP (U/L)						
Placebo	186.7 ± 42.5	187.4 ± 41.3	0.77 ± 5.5	0.41	0.21	0.29
Purslane	190 ± 41.6	188.4 ± 37.8	−1.6 ± 12	0.33		
AST (U/L)						
Placebo	27.37 ± 14.6	26.57 ± 14.3	−0.8 ± 1.7	0.01	<0.001	<0.001
Purslane	26.66 ± 10.6	20.9 ± 7.5	−5.74 ± 4.5	<0.001		
ALT (U/L)						
Placebo	31.11 ± 16.5	31.4 ± 17	0.28 ± 5.2	0.74	<0.001	<0.001
Purslane	32.11 ± 14.1	25.1 ± 10.8	−7.02 ± 5.9	<0.001		
GGT (U/L)						
Placebo	34.57 ± 12.9	33.88 ± 12.8	−0.68 ± 2.8	0.15	0.002	<0.001
Purslane	32 ± 14	27.65 ± 10.9	−4.34 ± 5.9	<0.001		
AST/ALT						
Placebo	0.91 ± 0.2	0.87 ± 0.2	−0.04 ± 0.1	0.04	0.06	0.07
Purslane	0.87 ± 0.2	0.88 ± 0.2	0.01 ± 0.1	0.62		
Total bilirubin (mg/dl)						
Placebo	1.23 ± 0.3	1.21 ± 0.3	−0.01 ± 0.1	0.28	<0.001	<0.001
Purslane	1.23 ± 0.4	0.99 ± 0.3	−0.23 ± 0.2	<0.001		
Direct bilirubin (mg/dl)						
Placebo	0.25 ± 0.1	0.29 ± 0.2	0.03 ± 0.2	0.32	0.01	0.03
Purslane	0.3 ± 0.1	0.24 ± 0.05	−0.06 ± 0.1	<0.001		
Liver stiffness (kPa)						
Placebo	5.11 ± 0.56	5.26 ± 0.55	0.15 ± 0.21	<0.001	<0.001	<0.001
Purslane	5.36 ± 0.45	4.68 ± 0.55	−0.68 ± 0.36	<0.001		
Hepatorenal Index						
Placebo	1.83 ± 0.25	1.88 ± 0.26	0.05 ± 0.15	0.055	<0.001	<0.001
Purslane	1.88 ± 0.25	1.58 ± 0.2	−0.3 ± 0.18	<0.001		

Data are presented as Mean ± SD. p * is extracted from Paired t-test and p # is extracted from 2 independent sample t test. p & is presented adjusted model by weight and physical activity with ANCOVA. $p < 0.05$ is considered as statistically significant.

4.2 Future work

Given the promising results observed, future research endeavors should aim to conduct longitudinal studies that extend beyond the 8-week period to assess the durability of treatment effects provided by *Portulaca oleracea* extract on liver health in patients with NAFLD. It would be valuable to investigate the interactions between purslane extract and conventional NAFLD

TABLE 5 lipid profile, oxidative stress, and inflammatory markers at baseline and at week 8.

Variables	Baseline	Week 8	Change	*p*-value*	*p*-value#	*p*-value&
TC (mg/dl)						
Placebo	173.49 ± 23.1	176.89 ± 21.3	3.4 ± 9.8	0.05	< 0.001	< 0.001
Purslane	183.54 ± 35.4	171.86 ± 28.6	−11.68 ± 17	< 0.001		
TG (mg/dl)						
Placebo	107.37 ± 50.2	107.66 ± 52.5	0.28 ± 9.9	0.86	< 0.001	0.005
Purslane	149.43 ± 60.8	131.83 ± 56.4	−17.6 ± 21.9	< 0.001		
HDL (mg/dl)						
Placebo	41.77 ± 5.3	42.54 ± 5.3	0.77 ± 1.8	0.02	< 0.001	< 0.001
Purslane	42.6 ± 6.7	46.63 ± 6.9	4.02 ± 4.5	< 0.001		
LDL (mg/dl)						
Placebo	89.66 ± 12.9	91.29 ± 13	1.62 ± 6	0.12	0.05	0.06
Purslane	92.26 ± 18.3	88.74 ± 14.2	−3.51 ± 8.3	0.18		
MDA (nmol/ml)						
Placebo	0.83 ± 0.19	0.86 ± 0.2	0.03 ± 0.11	0.07	0.051	0.062
Purslane	0.89 ± 0.23	0.81 ± 0.25	−0.08 ± 0.23	0.05		
SOD (U/ml)						
Placebo	235.34 ± 9.3	224.03 ± 10.3	−11.3 ± 9	< 0.001	< 0.001	< 0.001
Purslane	227.45 ± 14	244.94 ± 11	17.48 ± 14.8	< 0.001		
GPx (U/ml)						
Placebo	29.01 ± 6.8	27.69 ± 6.5	−1.31 ± 3.5	0.03	< 0.001	< 0.001
Purslane	26.96 ± 7	33.51 ± 8.1	6.54 ± 3.9	< 0.001		
Catalase (U/ml)						
Placebo	4.21 ± 1.25	3.62 ± 1.35	−0.59 ± 0.54	< 0.001	< 0.001	< 0.001
Purslane	3.76 ± 1.14	6.07 ± 1.39	2.3 ± 0.78	< 0.001		
IL-6 (pg/ml)						
Placebo	12 ± 2.06	12.54 ± 2.1	0.53 ± 1.89	0.10	< 0.001	< 0.001
Purslane	12.95 ± 1.7	10.94 ± 1.8	−2 ± 1.68	< 0.001		
hs-CRP (mg/L)						
Placebo	2.6 ± 1.09	2.9 ± 1.09	0.29 ± 0.42	0.05	< 0.001	< 0.001
Purslane	3.13 ± 2.05	1.93 ± 1.13	−1.19 ± 1.64	< 0.001		
ESR 1h						
Placebo	14.69 ± 8.2	13.51 ± 6.23	−1.17 ± 3.4	0.05	< 0.001	0.003
Purslane	14.89 ± 8.7	10.46 ± 6.23	−4.42 ± 4.2	< 0.001		
ESR 2h						
Placebo	24.97 ± 13.14	23.89 ± 21	−1.08 ± 3.89	0.10	< 0.001	< 0.001
Purslane	25.8 ± 12.24	19.8 ± 10.7	−6 ± 4.39	< 0.001		

Data are presented as Mean ± SD. p * is extracted from Paired t-test and p # is extracted from 2 independent sample t test. p & is presented adjusted model by weight and physical activity with ANCOVA. $p < 0.05$ is considered as statistically significant.

treatments to explore potential synergistic effects. Furthermore, identifying and isolating specific bioactive compounds within purslane extract responsible for its hepatoprotective properties could pave the way for the development of new therapeutic agents. Expanding the participant pool to include a wider range of ethnicities and backgrounds would enhance the external validity of the findings. Moreover, given the complexity of NAFLD as a disease that intersects with metabolic syndrome, future studies might also benefit from exploring the impact of purslane extract across different facets of metabolic health.

5 Conclusion

The collective findings from these studies underscore the therapeutic potential of *Portulaca oleracea*, commonly known as

TABLE 6 Determining the peaks of metabolites in the hydroalcoholic extract of *Portulaca Oleracea* using LC–MS in positive mode.

Peak number	Name of the compound	t_R (min)	M + H (*m/z*)	References
1	Portulacanone D	26/9	299/76	(37)
2	Noradrenaline	37/0	170/7	(38)
3	Dopa	15/0	198/12	(39)
4	Oleraceins A	62/5	504/66	(39)
5	Oleraceins B	9/5	533/76	(39)
6	Oleraceins C	64/1	666/06	(39)
7	Oleraceins D	13/1	696/84	(39)
8	Adenosine	19/8	268/8	(39)
9	(3R)-3,5 –Bis (3-methoxy-4-hydroxyphenyl)-2,3 -dihydro-2 (1H)-pyridinone	89/3	342/36	(40)
10	Aurantiamide acetate	36/4	445/8	(41)
11	Cyclo (L-tyrosinyl-L-tyrosinyl)	67/7	327/24	(41)
12	Portuloside A	72/2	332/22	(42)
13	Portulene	66/3	337/02	(29)
14	Lupeol	5/66	427/5	(29)
15	(3S)-3 -O-(*β*-D-Glucopyranosyl)-3,7 -dimethylocta-1,6-dien-3-ol	8/67	318/12	(43)
16	Friedelane	54/9	413/34	(44)
17	Quercetin	39/4	303/18	(45)
18	Myricetin	55/1	318/24	(45)
19	Genistin	65/4	433/20	(46)
20	Indole-3-carboxylic acid	77/8	162/90	(37)
21	Palmitic acid	62/2	256/14	(47)
22	Stearic acid	37/8	285/18	(47)
23	Caffeic acid	65/8	181/08	(48)
24	Riboflavin	35/0	376/62	(49)
25	Vitamin C	28/5	177/00	(50)
26	*α*-Tocopherol	67/1	431/22	(47)
27	Hesperidin	76/8	611/58	(50)
28	Portulacerebroside A	64/6	843/18	(44)
29	*β*-Sitosterol	48/7	415/32	(29)
30	*β*-Carotene	37/5	538/74	(47)

purslane, in managing non-alcoholic fatty liver disease (NAFLD) and improving metabolic health. Purslane supplementation was shown to significantly enhance liver function, reduce inflammatory and oxidative stress markers, and improve metabolic parameters such as lipid profiles and blood glucose levels. These beneficial effects are attributed to purslane's rich composition of bioactive compounds, including antioxidants, omega-3 fatty acids, and various phytochemicals, which collectively contribute to its hepatoprotective, anti-inflammatory, and antidiabetic properties. Given the promising outcomes, purslane presents a viable natural therapeutic agent for addressing NAFLD and potentially other metabolic disorders. However, further research is warranted to explore the long-term benefits, optimal dosages, and underlying mechanisms of action of purslane supplementation.

Data availability statement

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

Ethics statement

The studies involving humans were approved by School of Medicine, Mashhad University of Medical Sciences Biomedical Research Ethics Committee (IR.MUMS.REC.1400.223). 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

NM: Conceptualization, Data curation, Formal analysis, Writing – original draft. HaB: Data curation, Writing – original draft. SB: Data curation, Writing – original draft. HoB: Writing – original draft. FR: Data curation, Investigation, Writing – review & editing. AO: Data curation, Investigation, Software, Writing – original draft. LG: Data curation, Methodology, Validation, Writing – review & editing. MN: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Writing – review & editing. VA: Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1371137/full#supplementary-material

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Glossary

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	body mass index
CBC-diff	complete blood count with differential count
CRP	C-reactive protein
FBS	fasting blood sugar
GPx	glutathione peroxidase
HbA1c	hemoglobin A1c
IL	interleukin
IPAQ	international physical activity questionnaire
MDA	malondialdehyde
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NF-κβ	nuclear factor kappa β
PI3K	phosphoinositide 3-kinases
RCT	randomized clinical trial
SOD	super oxide dismutase
TNF-α	tumor necrosis factor α
ESR	erythrocyte sedimentation rate
GGT	gamma-glutamyl transferase
TG	triglycerides
TC	total cholesterol
LDL	low-density lipoproteins
HDL	high-density lipoproteins

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The association between serum vitamin A concentrations and virus hepatitis among U.S. adults from the NHANES database: a cross-sectional study

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Objective: According to the present study, the relationship between vitamin A (VA) levels and hepatitis virus carriage has been unclear and controversial. This study aimed to determine the potential relationship between serum VA levels and viral hepatitis and to provide ideas for future clinical treatments.

Methods: A cross-sectional study was performed using the 2005–2006 and 2017–2018 National Health and Nutrition Examination Survey (NHANES) datasets. Multiple linear regression and logistic regression were adopted to analyze the association between serological hepatitis B surface antigen (HBsAg) or hepatitis C RNA (HCV-RNA) positivity and VA levels. There were 5,351 HBsAg-related responders and 242 HCV-RNA-related responders, including 52 HBsAg (+) and 104 HCV-RNA (+) responders.

Results: Compared with HBsAg (–) and HCV-RNA (–) respondents, HBsAg (+) and HCV-RNA (+) respondents tended to have lower serum VA levels, respectively [1.63 (1.33 ~ 2.01) vs. 1.92 (1.57 ~ 2.34), $P < 0.001$; 1.54 (1.25 ~ 1.83) vs. 1.78 (1.46 ~ 2.26), $P < 0.001$]. A greater percentage of responders in the subclinical VA deficiency (SVAD) group were HBsAg (+) and HCV-RNA (+) than were those in the normal VA (VAN) group [2.4% (9/374) vs. 0.9% (43/4977), $p = 0.003$; 61.5% (16/26) vs. 40.7% (88/215), $p = 0.043$]. According to the results of the multiple regression analyses of the different models, the serum VA concentration was negatively correlated with HBsAg (+) and HCV-RNA (+) status ($\beta = -0.14$, 95% CI = −0.30 to −0.01, $p = 0.066$; $\beta = -0.29$, 95% CI = −0.50 ~ −0.09, $p = 0.005$, respectively). Compared to those with SVAD, patients with VAN were less likely to be serologically HBsAg (+) or HCV-RNA (+) (OR = 0.53, 95% CI = 0.25 ~ 1.10, $p = 0.089$; OR = 0.39, 95% CI = 0.18 ~ 0.84, $p = 0.016$, respectively).

Conclusion: Our study provides evidence that patients who are HBsAg (+) or HCV-RNA (+) have a high incidence of SVAD. Moreover, HBsAg and HCV-RNA positivity are negatively correlated with VA levels, and patients with SVAD are more likely to carry HBsAg (+) or HCV-RNA (+). These findings suggest that the relationship between hepatitis viruses and vitamin A needs to be validated by more basic studies and clinical large-sample randomized controlled trials to provide ideas for new therapeutic targets.

KEYWORDS

serum VA, HBsAg, HCV-RNA, NHANES, virus hepatities

1 Introduction

Vitamin A (VA) refers to a group of small fat-soluble molecules composed of a cyclic ring, a polyene side chain, and a polar end group (1). VA is crucial for many important biological functions, including reproduction, embryological development, cellular differentiation, growth, immunity, and vision. VA deficiency (VAD) is common and has been shown to be associated with infection, especially viral infection (2). In our previous study, we observed that most children with hand, foot and mouth disease (HFMD) presented with reduced VA levels, which was associated with their decreased immunity and exacerbation of the disease. The data showed that the reduction of IFN-α and EV71-IgM concentrations was positively correlated with the reduction of VA levels (3). At the same time, our study revealed that VAD was associated with severe sepsis, septic shock and increased PRISM scores (4). Furthermore, VAD may be a marker of death in children with severe sepsis. More than 80% of VA is stored in the liver in the form of retinyl esters (5). The human liver not only functions as a storehouse for VA but also needs retinol for its normal functions. However, whether there is a correlation between VA and hepatitis virus infection is currently unclear.

Retinol is a natural metabolite of VA, and plasma retinol levels indicate VA status (6). The serum retinol concentration, which is positively associated with the dietary retinol concentration, has been shown to have an independent effect on hepatocellular carcinoma (7). According to clinical research, in patients with liver disease, the levels of VA, retinol-binding protein (RBP), and prealbumin (PA) are markedly decreased and are highly significantly correlated with a wide range of concentrations (8, 9). Moreover, in patients with acute hepatitis, as the disease progresses, the plasma concentrations of VA, RBP, and PA increase (8, 10). In contrast, Saron et al. (11) reported lower serum retinol levels in individuals with chronic liver diseases, which was related to the severity of the condition. These findings suggest that VA levels vary among different liver diseases and may be related to disease progression, etiology, and nutritional status. Hepatic stellate cells within liver lobules store approximately 80% of the total body VA in lipid droplets in the cytoplasm (12). These cells also play a pivotal role in the regulation of VA homeostasis (13–15). The burden of liver disease caused by hepatitis B virus (HBV) infection worldwide is great, with approximately 250 million people chronically infected globally (16, 17). Chronic HBV infection is particularly prevalent in Asia, especially in China (18). Piyathilake (19) showed that HBV infection was also a strong predictor of VAD. HBV infection can impact hepatic cells, thereby affecting VA metabolism and storage. The association between VA deficiency and high aflatoxin B1 albumin (AF-ALB) levels may result in impairment of the host immune response, which increases susceptibility to infectious diseases (19).

Hepatitis C virus (HCV) is a human pathogen responsible for acute and chronic liver disease (20) and chronically infects an estimated 71.1 million individuals worldwide (21). In addition to being a simple hepatic viral infection, HCV is now thought to cause metabolic alterations, as much of its life cycle is closely associated with lipid metabolism (22). Several lines of evidence indicate the involvement of HCV in the retinoid pathway (23). VA and other retinoids have recently been demonstrated to inhibit measles virus, EV71 (3) and HCV *in vitro* (24). These findings indicate that HCV infection can affect VA levels, which in turn affects the body's immunity, further aggravating the patient's condition.

The aim of this study was to analyze vitamin A levels and hepatitis virus carriage to explore the potential relationship between viral hepatitis and vitamin A and to provide new ideas for clinical treatment.

2 Methods

2.1 National health and nutrition examination survey

The data analyzed were collected from the 2005–2006 and 2017–2018 NHANES survey cycles.[1] The NHANES is a nationwide survey conducted annually for the purpose of collecting health and diet information from a representative, non-institutionalized U.S. population. The NHANES is unique in that it combines interviews, physical examinations, and laboratory evaluations to obtain a large amount of quantitative and qualitative data (25). Briefly, the survey examines approximately 5,000 persons each year from various counties across the U.S. who are divided into a total of 30 primary sampling units (PSUs), 15 of which are visited annually. All participants provided written informed consent in agreement with the Public Health Service Act prior to any data collection. Household questionnaires, telephone interviews, and examinations conducted by healthcare professionals and trained personnel were utilized to collect the data.

2.2 Study participants and exclusion criteria

The 2005–2006 and 2017–2018 NHANES cycles collected data on 19,602 individuals. In the analysis, the researchers excluded 5,536 individuals without VA data and all children under the age of 18. After excluding data from non-compliant respondents, we collected 5,351 respondents with both hepatitis B surface antigen (HBsAg) and VA (Supplementary Figure S1) and 242 respondents with both hepatitis C RNA (HCV-RNA) and VA (Supplementary Figure S2). Serum VA levels were compared between HBsAg or HCV-RNA serology-positive and -negative respondents to determine whether there was a difference in the serum VA concentration between HBsAg-and HCV-RNA-negative respondents. According to the World Health Organization, the blood vitamin A (retinol) concentration $<0.70\,\mu mol/L$ is considered as VAD in children and adults. In practice, however, few adults reach this level of deficiency, so in this article we chose to use subclinical vitamin A deficiency (SVAD) as the study index, with a value of $<1.12\,\mu mol/L$. The concentration of vitamin A detected in serum is the retinol concentration, which we express here as serum VA. Among these remaining individuals, those with valid serum VA concentrations and complete information on demographic, anthropometric, questionnaire, and laboratory variables, including sex, age, body mass index (BMI), the ratio of family income to poverty (PIR), ethnicity, education, cigarette use, alcohol use, diabetes status, high blood pressure status, daily dietary energy/vitamin A/retinol intake, blood counts, C-reactive protein (CRP), and liver and kidney function parameters, were included in the analysis.

1 https://wwwn.cdc.gov/Nchs/Nhanes/2005–2006/2017-2018/TST_H.htm

2.3 Assessment of serum VA

The serum VA concentration is available in the datasets and is included as an exposure variable expressed as a continuous variable. NHANES-examined participants aged 6 years and older were eligible. In the analysis, serum specimens were processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, for analysis. The vials were frozen at −30°C until they were shipped to the National Center for Environmental Health for testing. The serum VA concentration was measured using modified high-performance liquid chromatography with a photodiode array detection method. The NHANES quality assurance and quality control protocols met the 1988 Clinical Laboratory Improvement Act mandates.

2.4 Assessment of hepatitis serology

HBsAg was tested by using the VITROS HBsAg test, the VITROS HBsAg kit on the VITROS ECi/ECiQ Immunodiagnostic System and VITROS 3600 Immunodiagnostic System, and the VITROS Immunodiagnostic Product HBsAg Calibrator. The principle of the test is based on an immunoassay technique consisting of a simultaneous reaction of HBsAg in the sample with the mouse monoclonal anti-HBs antibody coated on the wells and the mouse monoclonal anti-HBs antibody labeled with horseradish peroxidase (HRP) in the conjugate. The unbound conjugate is removed by washing and reagents consisting of luminescent substrates (luminescent phenol derivatives and peroxides) and electron transfer agents are added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminescent phenol derivative to generate light. The electron transfer agent increases the intensity and prolongs the duration of the light. The light signal is read out with the VITROS ECi/ECiQ immunodiagnostic system. Ultimately, the amount of bound HRP conjugate indicates the amount of HBsAg in the sample (26). Hepatitis C ribonucleic acid was tested by using the COBAS Amplicon HCV Monitor test. The COBAS Amplicon HCV Monitor version 2 0 (v2.0) is an *in vitro* nucleic acid amplification test for the quantification of HCV-RNA in human serum or plasma on a COBAS Amplicon analyzer (27).

2.5 Defining demographic variables

Covariates related to serum VA, as well as potential confounders, were included based on the results of the literature searches. Participants were classified according to age, PIR, BMI, highest level of education, cigarette use, alcohol use, high blood pressure status, and diabetes status. Age was categorized according to international standards. PIR and BMI were classified by the triple fraction method. The participants' levels of education were based on their responses during the home interview. High blood pressure status and diabetes status were recorded as yes or no response from the home interview. Diabetes status was defined as a fasting serum glucose >126 mg/dL, having answered "yes" to taking diabetic medications, or having been diagnosed by a physician with diabetes. A high blood pressure status was defined by whether a person was told that he/she has high blood pressure by a physician, if the blood pressure was >140/90 mmHg, and/or if that person is currently taking antihypertensive drugs. Alcohol use was divided into three categories: "non-drinker," "moderate drinker," and "heavy drinker." Cigarette use was divided into two categories: "<100″ and "≥100″.

2.6 Statistics

Continuous variables are reported as the mean ± standard deviation (SD) or median with interquartile range (IQR). Categorical variables are presented as counts and percentages. Continuous variables were compared between groups using Mann–Whitney *U*-test or Student's *t*-test, as appropriate. Categorical variables were compared using the chi-square test or Fisher's exact test. A $p < 0.05$ was considered to indicate statistical significance. All the statistical analyses were performed by using STATA version 17. The association between vitamin A levels and the incidence of hepatitis was first estimated using a univariate logistic regression model or linear regression model. Multivariate logistic regression models or linear regression models were used to further evaluate the associations between them.

Logistic regression analysis using the Enter method was performed to assess the association between VAD and HBsAg/HCV-RNA in the different models. In the adjusted models, Model 1 made no adjustments, Model 2 adjusted for demographic variables, and Model 3 adjusted for variables with Model 2 and $P < 0.05$ according to Student's *t*-test and Mann–Whitney *U*-test. Linear regression analyses were also conducted in the same way as those used in the logistic regression models.

3 Results

3.1 Study population

3.1.1 Participants with HBsAg/HCV-RNA

The characteristics of the study subjects were divided according to HBsAg/HCV-RNA and VA levels and are presented in Table 1. The overall weighted incidence of hepatitis C was 43.0% (104/242), with males and females accounting for 69.2 and 30.8%, respectively, of the total HCV-RNA (+) population ($p = 0.007$). Notably, the VA level was significantly lower in HBsAg (+)/HCV-RNA (+) patients than in serologically virus-negative patients [1.63 (1.33 ~ 2.10) μg/L vs. 1.92 (1.57 ~ 2.34) μg/L, $P < 0.001$; 1.54 (1.25 ~ 1.83) μg/L vs. 1.78 (1.46 ~ 2.26) μg/L, $P < 0.001$]. The serum ALB concentration was significantly lower in HBsAg (+)/HCV-RNA (+) patients than in serologically virus-negative patients [(3.99 ± 0.36) g/dL vs. (4.14 ± 0.41) g/dL, $P < 0.001$; (3.84 ± 0.43) g/dL vs. (4.06 ± 0.36) g/dL, $P < 0.001$].

3.1.2 Participants with SVAD

The characteristics of the study subjects with SVAD (VA < 1.12 μmol/L) are shown in Supplementary Tables S1, S2. Compare to the normal vitamin A (VAN) group, SVAD was lower in the daily dietary intake, including energy intake (kcal) [1800 (1,340 ~ 2,438) vs. 2013(1,464 ~ 2,681), $P < 0.001$], vitamin A (RAE) (mcg) [342(159 ~ 594) vs. 464(253 ~ 776), $P < 0.001$], and retinol (mcg) [223(92 ~ 450) vs. 314(160 ~ 554), $P < 0.001$] among participants with hepatitis B. The overall weighted prevalence of hepatitis B was 2.4% in SVAD group

TABLE 1 Weighted characteristics of the study participants (US adults), according to HBsAg or HCV-RNA seropositivity.

Hepatitis B and C related indicators						
	HBsAg(+)	HBsAg(−)	*p*-value	HCV-RNA(+)	HCV-RNA(−)	*p*-value
No.	52	5,299		104	138	
Sex (%)			$P = 0.658^{b}$			$P = 0.007^{b}$
Male	27(51.9%)	2,588(48.8%)		72(69.2%)	72(52.2%)	
Female	25(48.1%)	2,711(51.2%)		32(30.8%)	66(47.8%)	
Age (year)	57(40.3–64)	45(28–63)	$P = 0.070^{a}$	54(46–60)	58(45–66)	$P = 0.019^{a}$
Age group (%)			$P = 0.007^{b}$			$P = 0.095^{b}$
<45	16(30.8%)	2,636(49.7%)		22(21.2%)	32(23.2%)	
(45, 60)	19(36.5%)	1,170(22.1%)		60(57.7%)	45(32.6%)	
>60	17(32.7%)	1,493(28.2%)		22(21.2%)	61(44.2%)	
Race (%)			$P < 0.001^{c}$			$P = 0.116^{c}$
Mexican American	1(1.9%)	1,078(20.3%)		8(7.7%)	18(13.0%)	
Other Hispanic	4(7.7%)	177(3.3%)		4(3.8%)	11(8.0%)	
Non-Hispanic White	6(11.5%)	2,411(45.5%)		43(41.3%)	56(40.6%)	
Non-Hispanic Black	19(36.5%)	1,289(24.3%)		43(41.3%)	39(28.3%)	
Other race	22(42.3%)	344(6.5%)		6(5.8%)	14(10.1%)	
PIR	1.68(1.06–4.86)	2.25(1.16–4.08)	$P = 0.224^{a}$	1.32(0.75–2.41)	1.69(0.95–3.46)	$P = 0.006^{a}$
Education (%)			$P = 0.143^{c}$			$P = 0.143^{c}$
< High school	13(25%)	1,325(25%)		30(28.8%)	27(19.6%)	
High school graduate/some college	24(46.2%)	2,991(56.4%)		66(63.5%)	93(67.4%)	
College graduate or above	15(28.8%)	983(18.6%)		8(7.7%)	18(13.0%)	
BMI (kg/m^{-2})	27.68 ± 6.57	28.52 ± 6.80	$P = 0.203^{d}$	27.58 ± 6.24	28.76 ± 7.30	$P = 0.316^{d}$
BMI group (%)			$P = 0.282^{b}$			$P = 0.491^{b}$
<25	18(34.6%)	1,710(32.3%)		35(33.7%)	48(34.8%)	
(25, 30)	21(40.4%)	1,751(33.0%)		36(34.6%)	39(28.3%)	
>30	12(23.1%)	1,760(33.2%)		28(26.9%)	45(32.6%)	
Energy intake (kcal)	1721(1368–2,352)	2,000(1456–2,665)	$P = 0.053^{a}$	2,346(1539–3223)	1,998(1176–2743)	$P = 0.055^{a}$
Vitamin A, RAE (mcg)	316(213–620)	455(24–768)	$P = 0.089^{a}$	444(209–741)	420(213–764)	$P = 0.773^{a}$
Retinol (mcg)	201(73–331)	311(156–550)	$P = 0.002^{a}$	324(134–563)	296(117–556)	$P = 0.512^{a}$
High blood pressure (%)			$P = 0.091^{b}$			$P = 0.037^{b}$
Yes	18(34.6%)	1,604(30.3%)		38(36.5%)	69(50%)	
No	33(63.5%)	3,682(69.5%)		66(63.5%)	69(50%)	
Refusal	1(1.9%)	13(0.2%)		0(0%)	0(0%)	
Diabetes (%)			$P = 0.010^{b}$			$P = 0.057^{b}$
Yes	12(23.1%)	508(9.6%)		12(11.5%)	26(18.8%)	
No	40(76.9%)	4,687(88.5%)		92(88.5%)	108(78.3%)	
Refusal	0(0%)	104(2.0%)		0(0%)	4(2.9%)	
Alcohol use (%)			$P = 0.864^{b}$			$P = 0.288^{b}$
Never or rarely	8(26.7%)	958(25.0%)		21(23.3%)	35(31.3%)	
Sometimes	17(56.7%)	2,085(54.4%)		49(54.4%)	60(53.6%)	
Often	5(16.7%)	792(20.7%)		20(22.2%)	17(15.2%)	
Cigarette use (%)			$P = 0.564^{b}$			$P < 0.001^{b}$
<100	29(56.9%)	2,529(52.8%)		11(10.7%)	51(38.1%)	
≥100	22(43.1%)	2,260(47.2%)		92(89.3%)	83(61.9%)	

(Continued)

TABLE 1 (Continued)

	Hepatitis B and C related indicators					
	HBsAg(+)	HBsAg(−)	*p*-value	HCV-RNA(+)	HCV-RNA(−)	*p*-value
Drugs						
Serum VA (μmol/L)	1.63(1.33–2.01)	1.92(1.57–2.34)	$P<0.001^a$	1.54(1.25–1.83)	1.78(1.46–2.26)	$P<0.001^a$
SVAD			$P=0.003^b$			$P=0.043^b$
Yes	9(17.3%)	365(6.9%)		16(15.4%)	10(7.2%)	
No	43(82.7%)	4,934(93.1%)		88(84.6%)	128(92.8%)	
Hemoglobin (g/dL)	14.10 ± 1.28	14.19 ± 1.59	$P=0.141^d$	14.34 ± 1.89	13.98 ± 1.54	$P=0.102^d$
Platelet count (1,000 cells/μL)	236.50(176.00–271.75)	266.00(225.00–314.00)	$P<0.001^a$	229.00(181.00–284.75)	241.00(195.75–297.75)	$P=0.191^a$
White blood cell count (1,000 cells/μL)	6.40(4.93–8.35)	7.10(5.90–8.60)	$P=0.058^a$	6.80(5.70–8.46)	7.10(5.90–8.40)	$P=0.549^a$
Segmented neutrophils percent (%)	55.65 ± 10.62	58.32 ± 10.16	$P=0.869^d$	54.33 ± 11.17	58.56 ± 10.55	$P=0.124^d$
Lymphocyte percent (%)	34.10(27.00–38.90)	29.60(24.00–35.8)	$P=0.012^a$	31.55(25.45–39.20)	29.65(23.58–35.80)	$P=0.047^a$
C-reactive protein (mg/L)	0.73(0.28–1.99)	0.24(0.08–0.62)	$P<0.001^a$	0.41(0.09–1.79)	1.33(0.39–3.57)	$P<0.001^a$
Albumin (g/dL)	3.99 ± 0.36	4.14 ± 0.41	$P<0.001^d$	3.84 ± 0.43	4.06 ± 0.36	$P<0.001^d$
Total bilirubin (μmol/L)	10.26(5.13–13.68)	10.26(8.55–13.68)	$P=0.142^a$	10.26(6.84–13.68)	8.55(5.13–11.97)	$P=0.004^a$
Alanine aminotransferase ALT (IU/L)	24(18.00–31.00)	20(16.00–28.00)	$P=0.032^a$	41(28.00–65.75)	19(12.00–26.00)	$P<0.001^a$
Aspartate aminotransferase AST (IU/L)	24(20.00–30.00)	23(19.00–27.00)	$P=0.222^a$	40(29.00–71.75)	20(18.00–25.00)	$P<0.001^a$
Alkaline phosphatase (ALP) (IU/L)	74.00(62.00–90.00)	69.00(56.00–85.00)	$P=0.044^a$	73.50(62.25–95.50)	79.00(62.00–94.50)	$P=0.528^a$
Creatinine (mg/dL)	0.88(0.71–1.01)	0.90(0.73–1.00)	$P=0.643^a$	0.90(0.80–1.00)	0.90(0.75–1.10)	$P=0.428^a$
Blood urea nitrogen (mg/dL)	13(10.00–17.00)	12(9.00–15.00)	$P=0.025^a$	13(9.00–16.00)	14(10.50–17.50)	$P=0.039^a$
Uric acid (μmol/L)	321.20(279.60–380.70)	309.30(255.80–374.70)	$P=0.332^a$	339.00(281.08–397.08)	327.10(249.80–389.60)	$P=0.102^a$
Total calcium (mg/dL)	9.39 ± 0.35	9.46 ± 0.38	$P=0.083^d$	9.24 ± 0.44	9.39 ± 0.45	$P=0.026^d$
Cholesterol (mg/dL)	190.33 ± 47.23	196.94 ± 43.91	$P=0.397^d$	177.35 ± 38.24	189.31 ± 42.75	$P=0.036^d$
HDL-cholesterol (mmol/L)	1.40(1.06–1.76)	1.34(1.11–1.66)	$P=0.837^a$	1.36(1.10–1.70)	1.29(1.03–1.59)	$P=0.363^a$
LDL-cholesterol (mmol/L)	2.91(2.13–3.84)	2.85(2.22–3.49)	$P=0.901^a$	2.53(2.19–3.28)	2.87(2.41–3.49)	$P=0.169^a$
Glycohemoglobin (%)	6.16 ± 1.51	5.54 ± 1.01	$P<0.001^d$	5.61 ± 1.14	5.93 ± 1.26	$P=0.173^d$

Data are presented as means (SD), median (quartile 1, quartile 3) or number (percentage). SVAD, Subclinical vitamin A deficiency; VA, vitamin A; PIR, The ratio of family income to poverty; BMI, Body mass index; HBsAg, Hepatitis B surface antigen; HCV-RNA, Hepatitis C RNA. [a]Mann-Whitney *U*-test. [b]Chi-squared test. [c]Fisher exact test. [d]Student's *t*-test.

higher than 0.9% in VAN group (Supplementary Figure S3A). The results also showed that SVAD was more common in women than in men [78.9% (295/374) vs. 21.1% (79/374), $P<0.001$] among respondents with HBsAg (Supplementary Figure S4). Similar results were obtained for daily dietary intake in the hepatitis C population, but the difference was not significant. The overall weighted incidence of hepatitis C was 61.5% in the SVAD group, which was greater than the 40.7% in the VAN group (Supplementary Figure S3B).

3.2 Associations between VA and the prevalence of HBsAg (+)/HCV-RNA (+)

3.2.1 SVAD and viral hepatitis

As presented in Figure 1, VA was negatively related to the incidence of HBsAg (+)/HCV-RNA (+). The odds ratios (ORs) were 0.49 (95% CI = 0.29 ~ 0.82), 0.35 (95% CI = 0.18 ~ 0.67), and 0.53 (95% CI = 0.25 ~ 1.10) in Model 1, Model 2, and Model 3, respectively, among respondents with HBsAg (+) (Table 2). Moreover, among respondents with HCV-related RNA (+), the ORs were 0.49 (95% CI = 0.31 ~ 0.77), 0.44 (95% CI = 0.27 ~ 0.74), and 0.39 (95% CI = 0.18 ~ 0.84) in Model 1, Model 2, and Model 3, respectively (Table 3).

3.2.2 Viral hepatitis and VA levels

The results from linear regression models are shown in Figure 2. An inverse association between serum VA as a continuous factor and HBsAg was observed ($\beta=-0.23$, 95% CI = −0.41 to −0.06, $p=0.008$; $\beta=-0.29$, 95% CI = −0.47 to −0.12, $p=0.001$; $\beta=-0.14$, 95% CI = −0.30 ~ 0.01, $p=0.066$) (Table 4). Moreover, among the respondents with HCV-related RNA, there was also a negative correlation ($\beta=-0.27$, 95% CI = −0.44 to −0.11; $P=0.001$; $\beta=-0.30$, 95% CI = −0.49 to −0.12; $p=0.002$; $\beta=-0.29$, 95% CI = −0.50 to −0.09; $p=0.005$) (Table 5).

4 Discussion

VA is an immunomodulatory substance, and studies have confirmed its anti-infective effects; deficiency of this substance may lead to an imbalanced inflammatory response and impaired immune

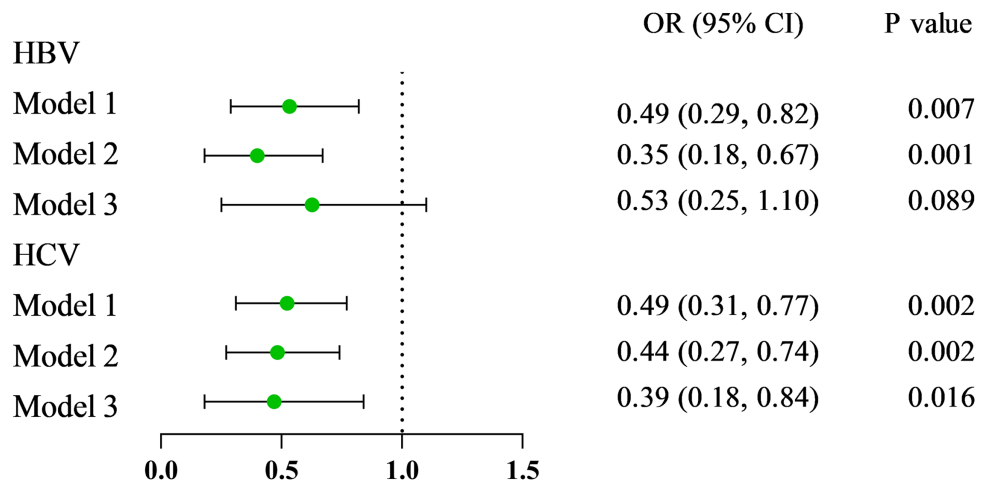

FIGURE 1
The forest plots of the relationship between SVAD and viral hepatitis.

function. HBV and HCV infections are the major causes of acute and chronic hepatitis worldwide and can lead to liver cirrhosis and hepatocellular carcinoma (HCC) (28). Our study showed that the incidence of SVAD is greater in patients with HBV or HCV infection and that serum virus-positive patients have lower levels of VA than serum virus-negative patients.

Several studies have shown that endogenous VA is involved in the body's immunity, and a large amount of loss leads to further aggravation of the disease (29–31). This may create a vicious cycle between viral hepatitis and VA. Moreover, the latest studies have reported that exogenous supplementation of VA may be a target for improving immunity and fighting viral infections (32). However, the results of our data analysis do not explain whether VA deficiency leads to an increase in the rate of viral infections or whether viral infections lead to a decrease in the body's VA levels. This supposition needs to be confirmed by further clinical studies.

In this cross-sectional study of U.S. adults, serum VA was inversely associated with serum viral positivity, and subjects with concurrent SVAD were more likely to be virus positive. Hepatitis B has been reported to affect VA storage in several ways (33). With impaired liver function after hepatitis, the storage of retinyl esters may be reduced, as may the ability of retinyl esters to mobilize hydrolysis and dehydroconversion to retinol, which may explain the low serum retinol concentration in patients with impaired liver function (34). Interestingly, our study showed that the daily intake of VA and retinol in the included population was less than the amount recommended by the World Health Organization and that VA-deficient subjects consumed even less VA (Tables 2, 3). Therefore, there may be a correlation between serum VA levels and viral positivity and between the serum VA concentration and viral intake, and additional detailed details need to be verified in additional clinical trials.

VA and its derivatives play crucial roles in controlling cell growth and differentiation, inhibiting liver cell transformation, inhibiting the proliferation of liver tumor cells, inhibiting the production of proinflammatory cytokines in macrophages, reducing inflammatory reactions, and protecting organisms from oxidative stress (OS) (35, 36). Notably, studies of VA and viral infections, including early childhood hand, foot and mouth disease (3), measles virus (24), and hepatitis C virus, are not uncommon. Furthermore, our previous research has shown that the prevalence of VAD is high in children

TABLE 2 Associations between hepatitis B among participants with SVAD.

Variable	Hepatitis B	
	OR (95% CI)	*p*-value
Serum VA		
Model 1	0.49 (0.29, 0.82)	0.007
Model 2	0.35 (0.18, 0.67)	0.001
Model 3	0.53 (0.25, 1.10)	0.089

Model 1: Non-adjusted. Model 2: Adjustment of sociodemographic variables including sex, race, age and BMI + socioeconomic status. Variables including PIR and education level. Model 3: Adjustment of covariates in model 2 and important variables including Retinol intake, PLT, Lymphocyte percent, CRP, ALB, ALT, ALP, BUN, diabetes, and Glycohemoglobin. OR, Odds ratio; CI, 95% confidence interval; PIR, The ratio of family income to poverty; BMI, Body mass index; PLT, Platelet count; CRP, C-reactive protein; ALB, Albumin; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; BUN, Blood urea nitrogen; SVAD, Subclinical vitamin A deficiency.

TABLE 3 Associations between hepatitis C among participants with SVAD.

Variable	Hepatitis C	
	OR (95% CI)	*p*-value
Serum VA		
Model 1	0.49 (0.31, 0.77)	0.002
Model 2	0.44 (0.27, 0.74)	0.002
Model 3	0.39 (0.18, 0.84)	0.016

Model 1: Non-adjusted. Model 2: Adjustment of sociodemographic variables including sex, race, age and BMI + socioeconomic status. Variables including PIR, and education level. Model 3: Adjustment of covariates in model 2 and important variables including Lymphocyte percent, CRP, ALB, ALT, AST, BUN, Total calcium, Cholesterol, and high blood pressure. OR, Odds ratio; CI, 95% confidence interval; PIR, The ratio of family income to poverty; BMI, Body mass index; CRP, C-reactive protein; ALB, Albumin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BUN, Blood urea nitrogen; SVAD, Subclinical vitamin A deficiency.

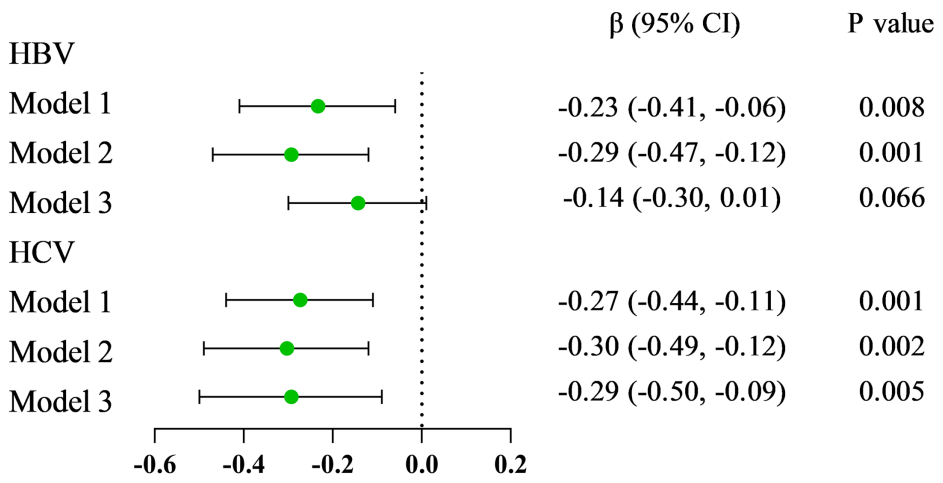

FIGURE 2
The forest plots of the relationship between viral hepatitis and VA levels.

TABLE 4 Associations between serum VA among participants with hepatitis B.

Variable	HBsAg (–)	HBsAg (+)	*p*-value
Serum VA			
Model 1 *β* (95% CI)	0	−0.23 (−0.41, −0.06)	0.008
Model 2 *β* (95% CI)	0	−0.29 (−0.47, −0.12)	0.001
Model 3 *β* (95% CI)	0	−0.14 (−0.30, 0.01)	0.066

Model 1: Non-adjusted. Model 2: Adjustment of sociodemographic variables including sex, race, age and BMI + socioeconomic status. Variables including PIR and education level. Model 3: Adjustment of covariates in model 2 and important variables including Energy intake, REA, Retinol intake, Cigarette use, Segmented neutrophils percent, Lymphocyte percent, CRP, ALB, TB, ALT, AST, ALP, Creatinine, BUN, UA, Cholesterol, HDL-Cholesterol, and high blood pressure. HBsAg, hepatitis B surface antigen; CI, 95% confidence interval; PIR, The ratio of family income to poverty; BMI, Body mass index; REA, Vitamin A intake; CRP, C-reactive protein; ALB, Albumin; TB, Total bilirubin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; Cr, Creatinine; BUN, Blood urea nitrogen; UA, Uric acid; VA, Vitamin A.

TABLE 5 Associations between serum VA among participants with hepatitis C.

Variable	HCV-RNA (–)	HCV-RNA (+)	*p*-value
Serum VA			
Model 1 *β* (95% CI)	0	−0.27 (−0.44, −0.11)	0.001
Model 2 *β* (95% CI)	0	−0.30 (−0.49, −0.12)	0.002
Model 3 *β* (95% CI)	0	−0.29 (−0.50, −0.09)	0.005

Model 1: Non-adjusted. Model 2: Adjustment of sociodemographic variables including sex, race, age and BMI + socioeconomic status. Variables including PIR and education level. Model 3: Adjustment of covariates in model 2 and important variables including WBC, CRP, AST, and BUN. HCV-RNA, hepatitis C RNA; CI, 95% confidence interval; PIR, The ratio of family income to poverty; BMI, body mass index; WBC, White blood cell count; CRP, C-reactive protein; AST, Aspartate aminotransferase; BUN, Blood urea nitrogen; VA, vitamin A.

with sepsis and that VAD may be a marker of mortality in critically ill children with sepsis (4). A wealth of data indicates that patients with these viral infections exhibit low VA levels, which may be related to an immunocompromised status and reduced VA stores following liver damage. However, additional in-depth basic research is needed to clarify the specific underlying mechanisms involved. Overall, this approach can prompt clinicians to pay attention to patients' VA levels when encountering viral infections or severe infections to provide new ideas for later treatment protocols.

For the past few years, several studies have shown that transretinoic acid can be used to treat hepatitis C. Vitamin A upregulates type 1 IFN receptor expression and enhances the anti-HCV replication effects of IFN-α (37). In a cohort of previously unresponsive patients with chronic HCV infection, all-trans retinoic acid (ATRA) had direct antiviral effects and strong additive or synergistic effects with polyethylene glycol IFN (38). However, little research has been conducted on VA for the treatment of hepatitis B. In contrast, there are several reported side effects of VA on the liver in previous articles, revealing that VA is a proven hepatotoxic agent that can cause conditions ranging from acute hepatitis to cirrhosis, particularly short-term high dose use of VA (39). We are currently conducting trials on the efficacy and safety of VA-assisted therapy for children with sepsis to analyze whether VA supplementation improves the clinical prognosis in patients with sepsis. VA is necessary for an adequate immune response to infection. Several studies have shown that VAD leads to increased systemic inflammation (40, 41). The decreased VA levels under inflammatory conditions may be related to low levels of retinol binding protein (RBP) and leakage of RBP into the extravascular space (42). In addition, some studies have reported that the plasma retinol concentration decreases during inflammation and sepsis, and retinol is secreted into the urine (43). In western Kenya, there was a significant positive association with infection among VA-deficient (RB$P < 0.83$ μmol/L) infants (44). Infants with iron deficiency were less likely to have infection based on the CRP level, while infants with VAD were five times more likely to have infection. The decrease in plasma AST activity in patients with urolithiasis might be attributed to supplementation with the vitamin ADEK. Patients with fat malabsorption may benefit from the supplementation of the fat-soluble vitamin ADEK (45). Conversely, it has also been found that serum VA was positively associated with the degree of non-alcoholic fatty liver disease (NAFLD), especially in the non-obese population (46). This finding is contrary to our findings. It also suggests us that various liver diseases, hepatitis and liver injury (trauma) may affect serum VA concentration. This needs to

be confirmed by more prospective studies and explored at the basic research level.

5 Limitations

This study was subject to certain constraints. First, this was a cross-sectional investigation, precluding the determination of causality. Second, serum vitamin A (VA) levels were assessed only once, and any fluctuations within individuals might influence the observed association. However, the half-life of VA is extended in humans (approximately 140 days), and serum VA levels within the typical range are tightly controlled in individuals (47, 48). Third, despite meticulously considering various related covariates, we cannot exclude all potential residual confounding factors in observational studies. Finally, decreased serum vitamin A levels in hepatitis patients may be associated with decreased hepatic stellate cell retinyl ester storage as well as mobilization capacity and with general susceptibility of the population to hepatitis viruses, and whether decreased vitamin A levels result in a population with impaired immune function more susceptible to transition to chronic patients or carriers needs to be confirmed by more rigorous basic studies.

6 Conclusion

In this study, we found that patients with hepatitis viral infections had low levels of VA, that the rate of viral infection was high in patients with SVAD, and that the serum VA concentration was inversely associated with seropositivity for hepatitis B and C viruses. Our findings suggest that, in patients with hepatitis B or C viral infections, we should pay attention to their serologic VA levels, and timely supplementation may be twice as effective as conventional treatment.

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

ML: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft. JF: Data curation, Methodology, Software, Visualization, Writing – original draft. XZ: Methodology, Investigation, Validation, Writing – review & editing. QF: Investigation, Writing – review & editing, Project administration, Software. YJ: Writing – review & editing, Funding acquisition, Validation. SC: Funding acquisition, Validation, Writing – review & editing, Conceptualization, Resources, Supervision.

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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.2024.1387461/full#supplementary-material

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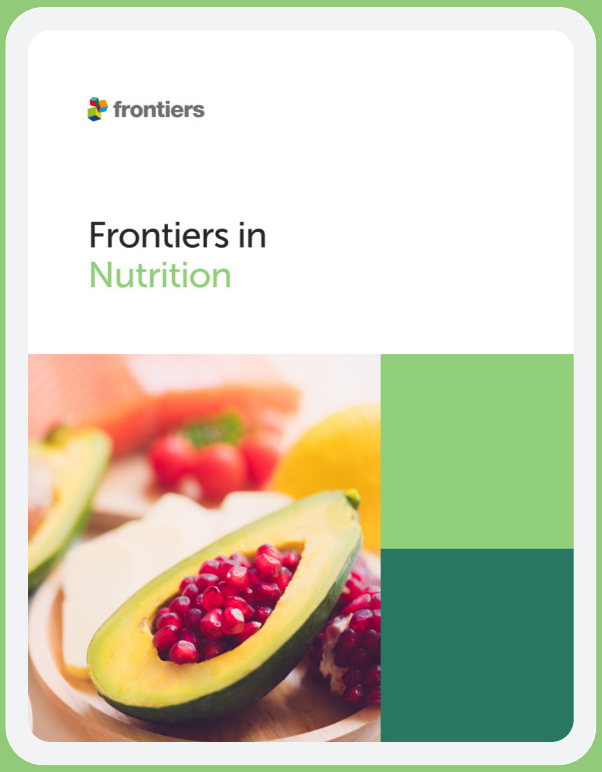

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