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Metabolic dysfunction-associated steatotic liver disease (MASLD) - pathogenesis, prevention and treatment

Edited by
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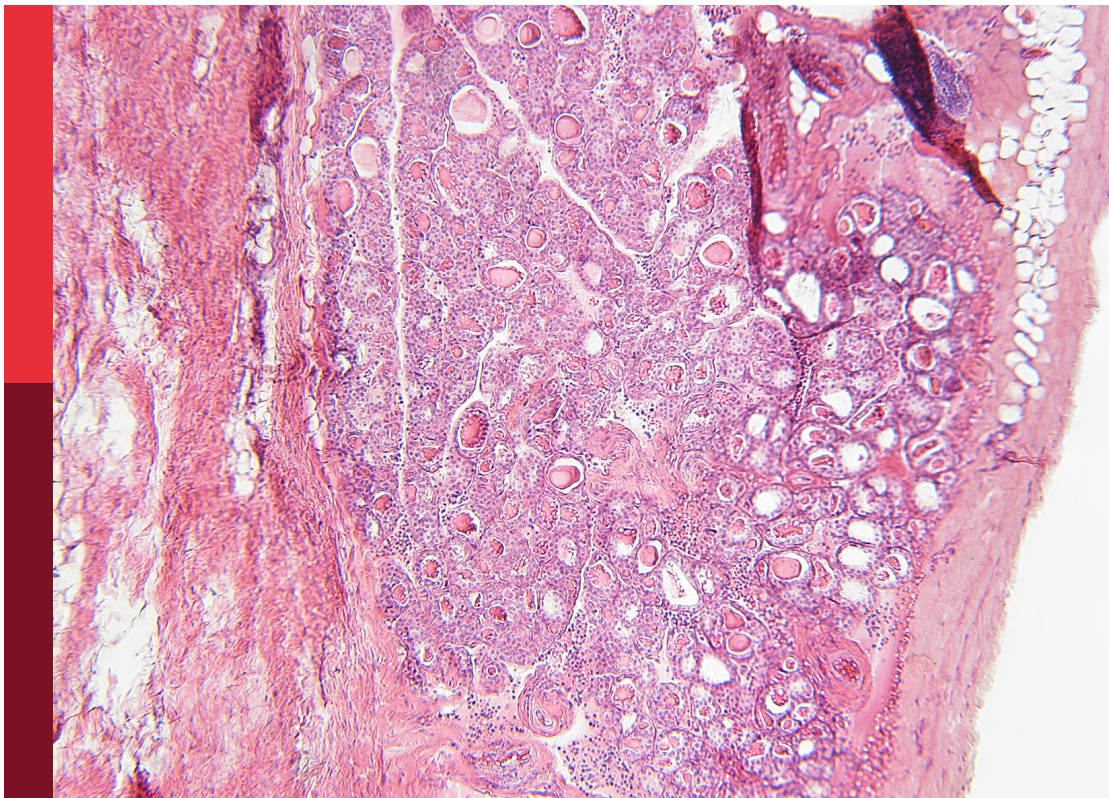
Published in
Frontiers in Endocrinology



ISSN 1664-8714
ISBN 978-2-8325-6979-5
DOI 10.3389/978-2-8325-6979-5

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Metabolic dysfunction-associated steatotic liver disease (MASLD) - pathogenesis, prevention and treatment

Topic editors

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Citation

Surma, S., Bułdak, Ł., eds. (2025). *Metabolic dysfunction-associated steatotic liver disease (MASLD) - pathogenesis, prevention and treatment*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6979-5

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frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 24 September 2025
DOI 10.3389/fendo.2025.1703019

OPEN ACCESS

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RECEIVED 10 September 2025
ACCEPTED 15 September 2025
PUBLISHED 24 September 2025

CITATION
Surma S and Bułdak Ł (2025) Editorial: Metabolic dysfunction-associated steatotic liver disease (MASLD) - pathogenesis, prevention and treatment. *Front. Endocrinol.* 16:1703019. doi: 10.3389/fendo.2025.1703019

Editorial: Metabolic dysfunction-associated steatotic liver disease (MASLD) - pathogenesis, prevention and treatment

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KEYWORDS

MASLD, metabolic abnormalities, prevention, treatment, CV risk

Editorial on the Research Topic
Metabolic dysfunction-associated steatotic liver disease (MASLD) - pathogenesis, prevention and treatment

Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) represents one of the most serious health challenges facing the modern world and is currently considered the most common chronic liver disease, with a prevalence of approximately one-third of the adult population (1–3). The nomenclature change from NAFLD to MASLD was necessary to emphasize its close association with metabolic disorders such as obesity, insulin resistance, type 2 diabetes, dyslipidemia, and hypertension, which not only increase the risk of developing the disease but also determine its progression and complications (1). MASLD is an insidious disease, often developing asymptomatically, and its diagnosis is often made only in advanced stages, when metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, cirrhosis, or hepatocellular carcinoma develop. Furthermore, MASLD significantly increases the risk of cardiovascular and renal diseases, which in clinical practice are the leading causes of death in this group of patients (2, 3). The global burden of MASLD continues to grow, generating significant health and economic consequences, including the costs of treating complications, decreased productivity, and premature death (2, 3). In this context, an interdisciplinary approach encompassing basic research, clinical diagnostics, pharmacotherapy development, and public health interventions is crucial. This Research Topic, titled "Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) – Pathogenesis, Prevention, and Treatment," comprises thirteen studies that collectively provide a multidimensional picture of the disease, highlighting pathogenic mechanisms, risk factors, diagnostic tools, therapeutic options, social determinants, and implications for healthcare systems.

Several studies have made significant contributions in the area of pathogenesis and risk factors. Shen et al. conducted a large population-based study that demonstrated that elevated aldosterone levels are associated with a higher prevalence of MASLD in patients with arterial hypertension. This finding highlights the role of the endocrine system in the development and progression of the disease and suggests the possibility of incorporating parameters related to mineralocorticoid metabolism into future risk assessment algorithms.

TABLE 1 Summary of articles published in Research Topic.

Author; year; ref	Topic	Key findings	Implication
Shen et al.; 2024	Aldosterone and MASLD	High aldosterone levels increase the incidence of MASLD in patients with HT	Possibility of inclusion in risk assessment
Chen et al.; 2024	AIP and MASLD	High AIP associated with MASLD in the NHANES population	Atherosclerosis prediction using AIP in MASLD patients
Meroni et al.; 2024	MASLD and HCC	Cardiometabolic factors accelerate cancer progression	The need to monitor the oncological risk of patients with MASLD
Hong et al.; 2025	GP73 and fibrosis in MASLD	A new biomarker of fibrosis in MASLD	Enrichment of non-invasive diagnostic methods
Alkaabi et al.; 2024	Predictive tools	Fib-4 and Hamaguchi effective in T2DM	Application in primary care and diabetology
Sharma et al.; 2024	FibroScan and Framingham	MASLD as an indicator of cardiometabolic risk in young adults	Possibility of screening and early optimization of cardiovascular risk
Suzuki et al.; 2025	Pemafibrate in T2DM patients	Improvement of steatosis index and lipid profile in T2DM	New pharmacotherapy option
Oe et al.; 2025	Tirzepatide (case report)	Improving the outcomes of patients with severe MASH	Proof of concept for GIP/GLP-1 efficacy; new pharmacotherapy option
Taiwo et al.; 2025	Metabolomics in T1DM patients with MASLD	Specific metabolic disorders	Personalization of MASLD diagnostics in patients with T1DM
Hi et al.; 2025	Children's body structure	Segmental adipose tissue as a predictor of MASLD	Early pediatric prevention
Wang et al.; 2025	Working time	Long hours and type of work increase the risk of MASLD	Considering occupational factors in MASLD risk assessment
Zhu et al.; 2024	Folate and vitamin B12 and mortality in MASLD	Deficiencies increase mortality in MASLD	Simple supplementation intervention
Anastasaki et al.; 2024	Algorithm for the diagnosis and management of MASLD in primary care practice	The use of algorithms helps in the management of MASLD	Improving diagnosis and care

Chen et al. focused on the atherogenic index of plasma (AIP), which reflects the balance between lipid fractions in serum. Analysis of NHANES data showed that a high AIP is associated with an increased incidence of MASLD, further supporting the importance of lipid abnormalities in the development of this condition. Meroni et al. in a literature review of patients with MASLD and hepatocellular carcinoma, presented evidence indicating that the presence of cardiometabolic factors significantly accelerates neoplastic progression and worsens prognosis, highlighting the urgent need for comprehensive monitoring of patients with MASLD for oncological risk. Diagnosing MASLD remains a challenge, especially since the disease develops insidiously and affects a large population. Hong et al. demonstrated that Golgi protein 73 (GP73) may be a useful marker of liver fibrosis, capable of supporting the assessment of disease severity without the need for biopsy. Alkaabi et al. assessed the effectiveness of simple predictive tools, such as Fib-4 and the Hamaguchi score, in identifying MASLD in patients with type 2 diabetes, which has important implications for clinical practice in diabetes, enabling rapid and accessible methods for early detection of the disease. Sharma et al. used FibroScan elastography among medical students and correlated its results with 30-year Framingham risk scores, demonstrating that MASLD can be considered an early indicator of overall cardiometabolic risk even in young populations. This creates potential opportunities for early screening of young people and implementation of interventions aimed at reducing the risk of premature heart attack (4).

In terms of therapy and prevention, this Research Topic provides inspiring data. Suzuki et al. demonstrated that the use of pemafibrate in patients with type 2 diabetes led to improvement in both the fatty liver index and lipid profile, indicating that this drug may play an important role in the treatment of MASLD. Oe et al. described the case of a patient with severe MASLD, in whom switching from standard GLP-1 agonist therapy to tirzepatide led to significant clinical and biochemical improvement. This paper provides proof of concept for the potential efficacy of new dual GIP/GLP-1 agonists in the treatment of advanced MASLD. Taiwo et al. addressed the issue of MASLD in the context of type 1 diabetes, demonstrating characteristic metabolomic abnormalities. This is an important finding, as most studies have focused on type 2 diabetes, yet patients with T1DM also constitute a risk group requiring a tailored diagnostic and therapeutic approach. Hu et al. focused on children and adolescents, demonstrating that the distribution of body fat, not just overall BMI, determines the risk of MASLD. These results indicate the need to implement targeted preventive measures already in childhood, which could limit the progression of the

disease in adulthood. Social and environmental factors are another area that cannot be ignored. Wang et al. using NHANES data from 1999–2014, demonstrated that long work hours and the specific nature of employment significantly increase the risk of MASLD. These results highlight the need for public health interventions that also take into account working conditions and the lifestyle of the population. Zhu et al. demonstrated that low folate and vitamin B12 levels are associated with increased mortality in patients with MASLD, suggesting that supplementation and monitoring vitamin status may be simple and effective interventions to improve prognosis. Anastasaki et al. developed and tested an algorithm for primary care in a European setting that allows for early diagnosis and more effective management of MASLD. Implementation of such solutions could significantly improve the quality of care for patients in the general population.

In summary, the articles collected in this Research Topic demonstrate MASLD as a disease with a complex, systemic pathogenesis and a wide spectrum of complications, requiring a multifaceted approach (Table 1). On the one hand, the development of biomarkers and non-invasive diagnostic methods allows for earlier detection of the disease, while advances in pharmacotherapy and personalized treatment offer new therapeutic options. The importance of social and environmental factors, which play a key role in shaping the risk of MASLD and should be considered in preventive measures, cannot be overlooked. Future research on MASLD should focus on integrating clinical, molecular, and epidemiological data to create personalized treatment and prevention strategies. Developing screening programs for high-risk populations and health education across various age and social groups is also essential. This collection of thirteen articles represents a significant step forward in MASLD research, providing scientific evidence that can translate into clinical practice and health policy. We believe that these results will contribute to improving the diagnosis, treatment and prevention of MASLD and, consequently, to reducing the global burden of this rapidly growing epidemic.

Author contributions

SS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. ŁB: Supervision, Writing – original draft, Writing – review & editing.

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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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 14 May 2024
DOI 10.3389/fendo.2024.1405828

OPEN ACCESS

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RECEIVED 23 March 2024
ACCEPTED 30 April 2024
PUBLISHED 14 May 2024

CITATION
Chen Y, Lu C, Ju H, Zhou Q and Zhao X (2024) Elevated AIP is associated with the prevalence of MAFLD in the US adults: evidence from NHANES 2017–2018. *Front. Endocrinol.* 15:1405828. doi: 10.3389/fendo.2024.1405828

Elevated AIP is associated with the prevalence of MAFLD in the US adults: evidence from NHANES 2017–2018

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Background: Atherogenic Index of plasma (AIP) is closely related to metabolic abnormalities. But as of now, there is no definitive conclusion on the dose-response relationship pattern between AIP and metabolic associated fatty liver disease (MAFLD).

Objective: The objective of this study was to provide a fresh insight for understanding the intrinsic link between AIP and the prevalence of MAFLD by exploring the dose-response pattern between AIP and MAFLD.

Methods: A total of 9254 participants received the survey and 1090 participants were finally included according to the screening criteria. To evaluate the association between AIP and the prevalence of MAFLD based on weighted multivariate logistic regression. Sensitivity analysis of the association between AIP and MAFLD was performed using propensity score matching (PSM). Restrictive cubic splines (RCS) were used to identify patterns of dose-response relationships between AIP and MAFLD, and receiver operator characteristic (ROC) curves were used to evaluate the predictive ability of AIP and traditional lipid parameters for MAFLD.

Results: In this study, a total of 563 participants were found to have MAFLD. The results of weighted multivariate logistic regression analysis demonstrated that, after adjusting for sex and age, participants in the highest quartile (Q4) of AIP had a significantly increased risk of developing MAFLD compared to those in the lowest quartile (Q1) (Model 2: OR = 9.03, 95% CI 4.75–17.17). A similar trend was observed in the fully adjusted model (Model 3: OR = 3.85, 95% CI 1.55–9.52). The RCS analysis revealed a linear dose-response association between AIP and MAFLD(*P* for crude non-linearity = 0.087). This association remained significant after accounting for potential confounding variables(*P* for adjusted non-linearity = 0.663). The ROC curve results suggest that AIP performs better than traditional lipid indicators in predicting MAFLD (AUC = 0.732, 95%CI 0.705–0.758).

Conclusion: A linear dose-response relationship exists between AIP and MAFLD, suggesting that as AIP increases, so does the risk of developing MAFLD.

KEYWORDS

metabolic syndrome, atherogenic index of plasma(AIP), hepatic steatosis, metabolic associated fatty liver disease (MAFLD), restricted cubic spline (RCS), NHANES

Introduction

In recent years, more and more attention has been paid to MAFLD characterized by hepatic steatosis and metabolic abnormalities (1, 2). According to statistics, the combined prevalence of MAFLD is 39.22% (3), especially in economically developed countries and regions. With the systematic definition of MAFLD diagnostic criteria (1), there is increasing recognition of the important role of metabolic abnormalities in such diseases. Unlike NAFLD, MAFLD is not a diagnosis of exclusion, and its definition identifies more patients with metabolic abnormalities and hepatic steatosis, thus having greater clinical applicability (1, 4, 5). Existing studies have indicated that MAFLD is superior to non-alcoholic fatty liver disease(NAFLD) in terms of identifying cardiovascular adverse events, risk of liver disease progression, and risk of all-cause mortality (4, 6, 7). This advantage might be attributed to the frequent presence of metabolic abnormalities observed among patients with MAFLD (8). With the growing prevalence of obesity and diabetes worldwide, the trend of the MAFLD pandemic may also worsen in the future (9, 10). Therefore, accurately identifying underlying MAFLD patients is crucial for improving their prognosis.

AIP is a novel lipid marker proposed by Dobiásová. It is obtained by performing logarithmic transformation on the ratios of triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) (11). AIP demonstrates an inverse relationship with lipoprotein particle size, leading to its recognition as a dependable proxy for small dense low-density lipoprotein cholesterol (sd-LDL-C) (11). Recent studies have shown that AIP outperforms traditional lipid markers in predicting the risk of cardiovascular disease (12–16). Interestingly, AIP is not only strongly associated with atherosclerosis, but also reflects the severity of insulin resistance(IR) in humans (17). Previous findings have shown that AIP is strongly associated with metabolic diseases, such as hypertension, diabetes, obesity, and atherosclerotic cardiovascular disease (15, 18–20). In view of the close association between AIP and indicators of human metabolic status (21), it is essential to investigate the potential connection between AIP and MAFLD to better find a convenient and easily accessible index for screening patients with MAFLD. Previous studies have verified the association between AIP and the prevalence of MAFLD among Chinese and Iranian individuals (22, 23). However, studies assessing the association between AIP and MAFLD prevalence among the American population remain scarce, and there is currently no research exploring the dose-response relationship between AIP and MAFLD prevalence.

Therefore, utilizing a cross-sectional analysis of NHANES (2017–2018) data, this study endeavors to illuminate the association between AIP and MAFLD prevalence among the general population in the United States. Furthermore, it explores the potential dose-response relationship patterns between AIP and MAFLD, offering a fresh perspective for assessing their association.

Methods

Study population

The NHANES, which commenced in the early 1960s and underwent a transformation in 1999, is a carefully planned study that assesses the comprehensive health and nutritional well-being of American adults and children (24). It has evolved into a continuous endeavor with a dynamic focus on diverse health and nutrition metrics to cater to evolving necessities. Each year, this survey meticulously examines a representative sample of approximately 5,000 individuals from across the nation. Data was gathered through face-to-face interviews and extensive health checks at mobile centers, employing a complex sampling method that ensured a representative cross-section of the population. Specifically, the study utilized information from the NHANES 2017–2018, encompassing 9,254 individuals. However, after rigorous screening, only 1,090 participants were deemed suitable for the analytical purposes of the research. The National Center for Health Statistics(NCHS) Research Ethics Review Committee has approved the survey and all participants have signed an informed consent form. The detailed screening process for selecting the final participants in this study is illustrated in Figure 1.

Covariates

Covariates used in this study mainly included sex, age, race, education levels, economic status, BMI, smoking habits, alcohol consumption, waist and hip circumference, physical activity status, as well as health conditions like hypertension, diabetes, and hyperlipidemia. Economic status is gauged using the family income-to-poverty ratio (PIR), with classifications of < 1.0, 1.0–

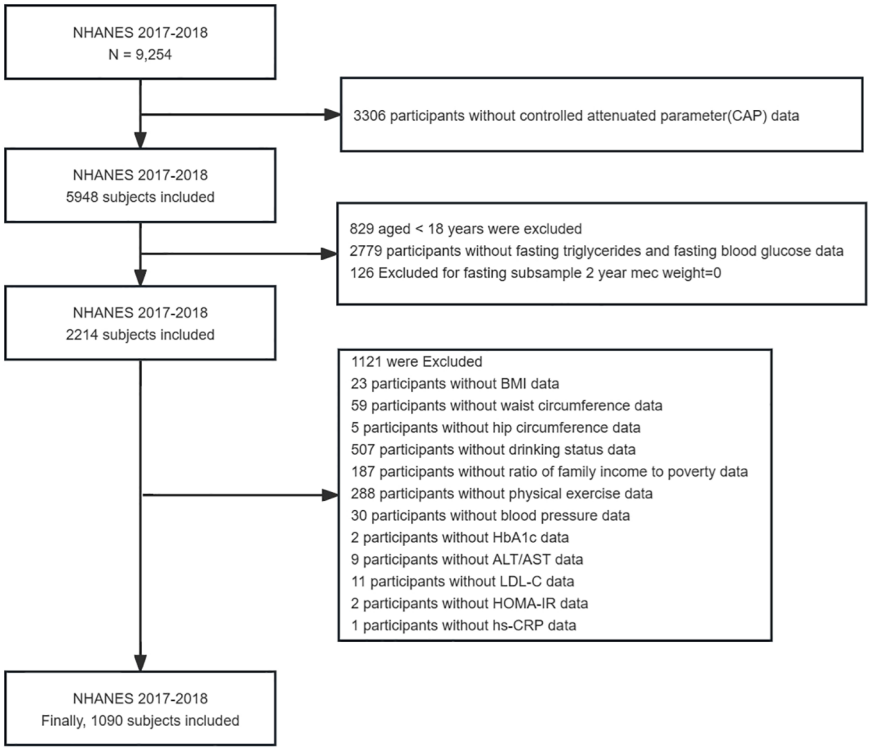

FIGURE 1
Flow chart of participants selection from the NHANES 2017–2018.

3.0, and > 3.0. The Body Mass Index (BMI) is calculated by dividing the weight in kilograms by the squared height in meters. Subsequently, it is classified into three categories: normal (BMI less than 25 kg/m^2 overweight (BMI ranging from 25 to less than 30 kg/m^2 and obese (BMI of 30 kg/m^2 or higher). Participants were divided into never smokers, former smokers, and current smokers based on their smoking history and current smoking status (25). Drinking status is determined based on specific criteria: heavy drinking is defined as consuming three or more drinks per day for women, four or more drinks per day for men, or engaging in binge drinking on at least five days per month. Moderate drinking is characterized by having two or more drinks per day for females, three or more drinks per day for males, or binge drinking on at least two days per month. Mild drinking is designated for those who do not meet the criteria for heavy or moderate drinking, while never drinking refers to individuals who have consumed less than twelve drinks in their lifetime (26). The data on the use of lipid-lowering drugs by participants was obtained from questionnaire survey. The lipid-lowering drugs in this study mainly included drugs that have a significant impact on TG and HDL-C, such as statins, fibrates, and ezetimibe. Physical activity status was divided into groups according to whether moderate intensity exercise was performed for 150 minutes or more in a week (27). Moderate-intensity exercise was defined as having MET values between 3 and 6, and since NHANES designed questions in the PAQ questionnaire all had MET > 3, these items were considered at least equivalent to moderate-intensity exercise. The investigators asked the participants according to the "vigorous/moderate recreational activity", "vigorous/moderate recreational activity", and "walk or bicycle" in the PAQ questionnaire, and if the participants answered "YES" according to the description of the question, they were further asked about the number of times they performed this exercise per week and the time of each exercise.To calculate their total weekly exercise time, simply multiply the frequency of weekly workouts by the length of each session. Finally, we summed the exercise time for all items to calculate the total time participants spent performing moderate intensity exercise for one week. Exercise time at Vigorous intensity was finally multiplied by 2 to convert to exercise time at Moderate intensity. Detailed questionnaire content is available in the NHANES website. Hypertension is diagnosed based on several factors, including a self-reported history of the condition, currently taking medication to lower blood pressure, or exhibiting an average systolic blood pressure that is 140 mmHg or above, and/or a diastolic blood pressure averaging 90 mmHg or more. The diagnostic criteria for diabetes mellitus are as follows: a self-reported physician diagnosis, fasting glucose levels of 7.0 mmol/L or above, glycosylated hemoglobin (HbA1c) levels of 6.5% or higher, and/or the current use of diabetes medications. Hyperlipidemia is determined by assessing various parameters, including LDL-C levels of 130 mg/dL or higher (equivalent to 3.37 mmol/L or above), total cholesterol levels of 200 mg/dL or higher (5.18 mmol/L or above), TG levels of 150 mg/dL or higher (1.7 mmol/L or above), and HDL-C levels below 40 mg/dL for men (less than 1.04 mmol/L) or below 50 mg/dL for women (less than 1.30 mmol/L). Additionally, the use of lipid-lowering medications is also considered in the determination of hyperlipidemia (28). The

laboratory test measures include HbA1c, fasting glucose, hs-CRP, fasting TG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, ALT, AST, and HOMA-IR. HOMA-IR is determined by multiplying fasting insulin with fasting plasma glucose and then dividing the result by 22.5 (29). All routine biochemical tests were executed in strict adherence to the standards specified in the NHANES Laboratory/Medical Technologist Procedure Manual.

Calculation of AIP

AIP is derived from the ratio of fasting TG to HDL-C after undergoing logarithmic transformation, calculated as AIP = lg(TG/HDL-C) (11). The participants were categorized into four quartile groups according to their AIP values: Q1 (-1.25, -0.37), Q2 (-0.37, -0.15), Q3 (-0.15, 0.08), and Q4 (0.08, 0.85).

MAFLD diagnosis

The diagnosis of MAFLD is conducted based on the 2020 consensus of the national expert panel. In brief, participants with hepatic steatosis who also exhibit any one of the following conditions: overweight/obesity, type 2 diabetes, or metabolic dysregulation, are diagnosed with MAFLD (1).

In this study, the existence of hepatic steatosis was confirmed using imaging methods. It is worth mentioning that the NHANES (2017–2018) survey employed a new method by combining ultrasound and vibration-controlled transient elastography (VCTE) to assess liver function. Qualified NHANES health professionals performed these evaluations on suitable participants at the NHANES Mobile Examination Center (MEC), utilizing FibroScan® technology. This device measures ultrasonic attenuation, which correlates with hepatic steatosis, and documents controlled attenuation parameters (CAP). Previous studies have demonstrated the reliability of CAP in determining the presence of hepatic steatosis, with CAP values of 248 dB/m or higher indicating the presence of hepatic steatosis (30). In addition, we collected liver stiffness measurements(LSM) to assess liver fibrosis. The extent of fibrosis can be classified into three categories: F2, F3, and F4, with thresholds of 8.2, 9.7, and 13.6 kPa, respectively (31, 32).

Statistical analysis

As recommended by the NHANES guidelines (33), we considered both complex sampling designs and sampling weights in the process of analyzing NHANES data. Because we used the index in fasting state (fasting TG) in this study, we chose fasting subsample MEC Weight (2017–2018). Basic characteristics were presented as counts and percentages (%) for categorical variables and as medians (interquartile range) for continuous variables. Chi-square test was used for differences between groups for categorical variables and Kruskal-Wallis test for differences between groups for continuous variables. Weighted logistic regression was employed to evaluate the relationship between AIP and MAFLD, resulting in the establishment of three models. Model 1 did not adjust for any confounding factors, Model 2 adjusted for sex and age, and Model 3 adjusted for race, education level, PIR, BMI, smoking status, drinking status, hyperlipidemia, hypertension, diabetes, physical activities status, ALT, AST, and lipid-lowering drugs on the basis of Model 2. Sensitivity analyses were performed using further matching propensity score (PSM) to assess whether the association between AIP and MAFLD was stable and reliable. A propensity score calculated for five demographic factors: sex, age, race, educational level, and PIR in the MAFLD and no MAFLD groups, matched 1 to 1 according to the score, with a caliper set at 0.01. Weighted multivariate logistic regression was performed on the data after PSM to assess whether the association between AIP and MAFLD remained significant. The dose-response relationship between AIP and MAFLD was visualized with RCS with three knots, and the predictive accuracy of AIP was assessed using ROC curves. All statistical analyses were performed using R (version 4.2.1, R Core Team 2020, Vienna, Austria) and MedCalc (version 20.022, MedCalc Software Ltd, Ostend, Belgium), considering a *P*-value less than 0.05 as statistically significant.

Result

Characteristics of participants

A total of 1090 participants were included in this study, including 556 males and 534 females. Participants were divided into Q1-Q4 groups according to AIP quartiles. Compared with the lowest quartile (Q1) group, participants with higher AIP were generally male, Mexican American, current smokers, hyperlipidemia, and diabetes patients, with higher BMI, waist circumference, fasting blood glucose, HOMA-IR, hs-CRP, TC, TG, LDL-C, ALT levels, and lower HDL-C levels(all *P*-values < 0.05), while there were no differences in age, drinking status, PIR, and AST (all *P*-values > 0.05), and the baseline characteristics of the participants are detailed in Table 1.

Association between AIP and the presence of MAFLD

The association between AIP and MAFLD prevalence was evaluated based on weighted logistic regression. In the logistic regression model without adjustment, the risk of MAFLD gradually increased in the quartiles with higher AIP compared with the lowest quartile (Q1) (*P* for trend < 0.0001). Multivariate logistic regression models(Model3) adjusting for potential confounders such as sex, age, race, education level, PIR, BMI, smoking status, drinking status, hyperlipidemia, hypertension, diabetes, physical activities status, ALT, AST, and lipid-lowering drugs, showed that the ORs for participants in Q2, Q3, and Q4 compared to those in Q1 based on their AIP were 2.00 (95% CI 1.03 to 3.91), 2.63 (95% CI 1.39 to 4.96), and 3.85 (95% CI 1.55 to 9.52),

10.3389/fendo.2024.1405828

TABLE 1 Baseline characteristics of the study population based on AIP quartile grouping.

Variable	Total (N=1090)	Q1 (-1.25,-0.37)	Q2 (-0.37,-0.15)	Q3 (-0.15,0.08)	Q4 (0.08,0.85)	P value
Age (years)	44.00 (30.00,58.00)	38.00 (27.00,54.00)	41.00 (27.00,58.00)	47.00 (29.00,62.00)	47.00 (36.00,57.00)	0.06
Sex, n (%)						< 0.0001
Female	534 (49.06)	176 (64.19)	157 (53.35)	111 (42.91)	90 (34.46)	
Male	556 (50.94)	96 (35.81)	116 (46.65)	161 (57.09)	183 (65.54)	
RACE, n (%)						0.01
Non-Hispanic Black	239 (10.19)	91 (14.87)	70 (12.32)	57 (9.35)	21 (3.81)	
Mexican American	154 (8.99)	23 (5.15)	38 (8.50)	38 (9.39)	55 (13.29)	
Non-Hispanic White	390 (66.05)	88 (68.31)	93 (63.55)	102 (65.06)	107 (66.98)	
Other Race	307 (14.77)	70 (11.66)	72 (15.63)	75 (16.20)	90 (15.92)	
Education levels, n (%)						0.01
< high school	151 (7.22)	26 (3.73)	35 (7.06)	37 (6.71)	53 (11.75)	
= high school	266 (27.11)	60 (23.48)	56 (19.71)	80 (33.36)	70 (31.97)	
> high school	673 (65.66)	186 (72.79)	182 (73.23)	155 (59.94)	150 (56.28)	
Smoking status, n (%)						0.001
Never	657 (59.76)	190 (67.48)	173 (60.03)	162 (63.09)	132 (47.62)	
Former	239 (24.29)	59 (25.08)	58 (28.44)	54 (18.19)	68 (25.59)	
Current	194 (15.95)	23 (7.44)	42 (11.54)	56 (18.72)	73 (26.79)	
Drinking status, n (%)						0.19
Never	118 (7.16)	35 (4.60)	30 (8.68)	26 (10.37)	27 (5.25)	
Mild	483 (45.21)	117 (43.70)	128 (42.59)	122 (47.35)	116 (47.26)	
Moderate	236 (22.18)	74 (28.40)	54 (24.25)	57 (17.26)	51 (18.29)	
Heavy	253 (25.45)	46 (23.30)	61 (24.48)	67 (25.02)	79 (29.20)	
BMI (kg/m^2)	27.80 (23.80,32.90)	24.30 (21.70,28.50)	27.30 (23.40,32.00)	29.20 (25.60,34.30)	30.70 (27.30,35.40)	< 0.0001
BMI category, n (%)						< 0.0001
Normal weight (< 25)	329 (31.55)	142 (55.83)	99 (35.60)	55 (19.97)	33 (12.64)	
Over weight (25–30)	326 (29.17)	66 (24.82)	77 (29.88)	89 (31.65)	94 (30.78)	
Obesity (≥ 30)	435 (39.27)	64 (19.35)	97 (34.53)	128 (48.38)	146 (56.59)	
Waist circumference, (cm)	97.40 (85.60,110.40)	86.10 (78.00,95.80)	94.50 (84.50,107.70)	101.20 (92.50,112.50)	105.30 (97.10,116.60)	< 0.0001
Hip circumference, (cm)	104.10 (97.70,114.60)	100.30 (94.20,106.80)	102.50 (97.30,116.10)	107.00 (101.00,116.00)	107.20 (101.20,118.80)	< 0.0001
Hyperlipidemia, n (%)	706 (62.48)	101 (33.12)	140 (48.20)	204 (76.23)	261 (94.70)	< 0.0001
DM,n (%)	186 (12.63)	17 (2.18)	38 (8.32)	52 (15.57)	79 (25.34)	< 0.0001
Hypertension, n (%)	413 (33.88)	71 (21.29)	89 (27.16)	126 (45.36)	127 (42.61)	0.002
MAFLD, n (%)	563 (50.50)	70 (23.02)	115 (46.34)	164 (61.90)	214 (73.27)	< 0.0001
LSM (kPa)	4.80 (4.00,6.10)	4.80 (4.00,5.80)	4.80 (3.90, 6.10)	4.70 (4.00, 6.10)	5.40 (4.20,6.20)	0.06
Lipid-lowering drugs, n (%)	168 (14.10)	31 (11.22)	31 (7.89)	50 (19.34)	56 (17.99)	0.05
Physical activities status, n (%)						0.03
< 150min/week	162 (13.52)	28 (6.18)	41 (16.61)	45 (18.95)	48 (13.10)	
≥ 150min/week	928(86.48)	244 (93.82)	232 (83.39)	227 (81.05)	225 (86.90)	

(Continued)

TABLE 1 Continued

Variable	Total (N=1090)	Q1 (-1.25,-0.37)	Q2 (-0.37,-0.15)	Q3 (-0.15,0.08)	Q4 (0.08,0.85)	P value
PIR, n (%)						0.24
< 1	177(10.97)	39 (8.10)	37 (8.16)	51 (14.23)	50 (13.56)	
1–3	451(33.54)	96 (28.01)	129 (36.88)	111 (35.87)	115 (34.01)	
> 3	462(55.49)	137 (63.89)	107 (54.96)	110 (49.90)	108 (52.43)	
Laboratory data						
HbA1c (%)	5.40(5.20,5.70)	5.30 (5.10,5.50)	5.40 (5.10,5.60)	5.40 (5.20,5.80)	5.50 (5.30,6.00)	< 0.0001
FBG (mmol/L)	5.66(5.33,6.11)	5.44 (5.16,5.77)	5.55 (5.27,6.00)	5.83 (5.38,6.27)	5.88 (5.55,6.55)	< 0.0001
HOMA-IR	2.21(1.37,4.05)	1.42 (0.88,1.95)	1.99 (1.26,3.19)	2.86 (1.77,4.25)	3.79 (2.25,6.57)	< 0.0001
hs-CRP (mg/L)	1.57(0.73,3.72)	0.83 (0.48,1.95)	1.64 (0.81,3.85)	2.09 (1.02,4.73)	2.32 (1.01,4.64)	0.002
TC (mmol/L)	4.65(4.11,5.40)	4.45 (3.96,5.04)	4.55 (4.06,5.28)	4.76 (4.14,5.46)	5.04 (4.37,5.79)	< 0.001
HDL-C (mmol/L)	1.37(1.14,1.68)	1.78 (1.55,1.99)	1.45 (1.24,1.71)	1.27 (1.16,1.45)	1.06 (0.96,1.19)	< 0.0001
TG (mmol/L)	0.95(0.63,1.48)	0.53 (0.43,0.63)	0.81 (0.67,0.93)	1.16 (0.99,1.39)	1.94 (1.66,2.35)	< 0.0001
LDL-C (mmol/L)	2.77(2.25,3.34)	2.38 (2.07,2.92)	2.77 (2.30,3.13)	2.97 (2.33,3.49)	3.08 (2.48,3.65)	< 0.001
ALT (U/L)	19.00(14.00,27.00)	16.00 (12.00,22.00)	17.00 (13.00,22.00)	20.00 (14.00,31.00)	24.00 (17.00,35.00)	< 0.001
AST (U/L)	20.00(16.00,24.00)	19.00 (17.00,24.00)	20.00 (16.00,22.00)	20.00 (16.00,24.00)	20.00 (17.00,26.00)	0.27

BMI, body mass index; DM, diabetes mellitus; MAFLD, metabolic associated fatty liver disease; PIR, poverty income ratio; LSM, liver stiffness measurement; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity c-reactive protein; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

respectively (*P* for trend = 0.006). When AIP was treated as a continuous variable, a strong positive association between AIP and the prevalence of MAFLD remained (Model3: OR = 4.71, 95%CI 1.70–13.01), as detailed in Table 2. Subsequently, in order to further validate the stability of the results and reduce the impact of demographic factors on the results, we used PSM under weighted conditions to adjust demographic characteristics such as gender, age, race, educational level, and PIR between MAFLD patients and those without MAFLD, and the comparison of demographic data between the two groups after adjustment is detailed in Supplementary Table 1. The results of multivariate logistic regression after PSM still suggested a positive association between AIP and the prevalence of MAFLD (Model3: OR = 3.12, 95%CI 1.15–8.42), as detailed in Table 3.

In addition, we analyzed the association between AIP and the degree of liver fibrosis, but the results showed no significant association between AIP and liver fibrosis, as detailed in Supplementary Table 2.

Dose-relationship between AIP and MAFLD

The RCS was used to assess the dose-response relationship between AIP and MAFLD and to clarify the pattern of this dose-response

TABLE 2 Association of AIP as a continuous variable and quartiles with MAFLD.

AIP	Number (%)	Model1		Model2		Model3	
	563	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
as continuous variable	–	14.28 (8.14–25.06)	< 0.0001	14.06 (7.96–24.82)	< 0.0001	4.71 (1.70–13.01)	0.01
Q1 (-1.25,-0.37)	70 (12.43)	REF		REF		REF	
Q2 (-0.37,-0.15)	115 (20.43)	2.89 (1.55–5.38)	0.003	2.96 (1.57–5.60)	0.004	2.00 (1.03–3.91)	0.04
Q3 (-0.15,0.08)	164 (29.13)	5.43 (3.24–9.12)	< 0.0001	5.34 (3.13–9.10)	< 0.0001	2.63 (1.39–4.96)	0.01
Q4 (0.08,0.85)	214 (38.01)	9.17 (5.13–16.39)	< 0.0001	9.03 (4.75–17.17)	< 0.0001	3.85 (1.55–9.52)	0.01
P for trend		< 0.0001		< 0.0001		0.006	

Model 1 was the crude model; Model 2 was adjusted for sex and age; Model 3 was adjusted for sex, age, race, education level, PIR, BMI, smoking status, drinking status, hyperlipidemia, hypertension, DM, physical activities status, ALT, AST, and lipid-lowering drugs.
AIP, atherogenic index of plasma; MAFLD, metabolic associated fatty liver disease; OR, odds ratio; CI, confidence interval; PIR, poverty income ratio; BMI, body mass index; DM, diabetes mellitus; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

TABLE 3 Association of AIP as a continuous variable and quartiles with MAFLD after PSM.

AIP	Number(%)	Model1		Model2		Model3	
	427	OR(95%CI)	*P*	OR(95%CI)	*P*	OR(95%CI)	*P*
as continuous variable	–	10.22(5.10–20.51)	< 0.0001	11.42(5.83–22.38)	< 0.0001	3.91(1.40–10.90)	0.01
Q1(-1.25,-0.36)	56(13.12)	REF		REF		REF	
Q2(-0.36,-0.16)	89(20.84)	2.71(1.30–5.64)	0.01	2.79(1.31–5.93)	0.01	1.80(0.83–3.92)	0.13
Q3(-0.16,0.07)	122(28.57)	4.70(2.43–9.09)	< 0.001	4.98(2.61–9.47)	< 0.001	3.02(1.49–6.08)	0.004
Q4(0.07,0.85)	160(37.47)	7.40(3.83–14.31)	< 0.0001	8.00(4.02–15.89)	< 0.0001	3.12(1.15–8.42)	0.03
P for trend		< 0.0001		< 0.0001		0.014	

Model 1 was the crude model; Model 2 was adjusted for sex and age; Model 3 was adjusted for sex, age, race, education level, PIR, BMI, smoking status, alcohol drinking, hyperlipidemia, hypertension, DM, physical activities status, ALT, AST, and lipid-lowering drugs.
AIP, atherogenic index of plasma; MAFLD, metabolic associated fatty liver disease; PSM, Propensity score matching; OR, odds ratio; CI, confidence interval.

relationship. The results showed a linear dose-response relationship between AIP and MAFLD (*P* for adjusted non-linearity = 0.663), whether adjusted for confounders or not, as shown in Figure 2.

Due to significant differences in MAFLD prevalence among different age groups and genders, we re-evaluated the dose-response relationship between AIP and MAFLD within these subgroups. The results indicated that a linear dose-response relationship between AIP and MAFLD persisted in both age subgroups (<60 years: *P* for adjusted non-linearity = 0.851, ≥60 years: *P* for adjusted non-linearity = 0.879) and gender subgroups (Female: *P* for adjusted non-linearity = 0.711, Male: *P* for adjusted non-linearity = 0.824). These findings are detailed in Figure 3.

Predictive value of AIP for the MAFLD

ROC curves were used to evaluate the value of AIP and traditional lipid parameters (TG, HDL-C, TC, LDL-C) in predicting the risk of MAFLD. The results showed that AIP (AUC = 0.732, 95%CI 0.705–0.758) predicted the risk of MAFLD better than individual lipid parameters, as shown in Figure 4. The optimal cut-off level for AIP was -0.21 (sensitivity = 74.07%, specificity = 62.81%). Differences between AIP and traditional lipid parameters in predicting the risk of MAFLD are detailed in Supplementary Table 3.

Discussion

In this cross-sectional analysis of NHANES 2017–2018, we found a strong association between AIP and MAFLD prevalence. Our study demonstrates for the first time that there is a linear dose-response pattern between AIP and MAFLD, that is, as AIP increases, so does the risk of MAFLD. Finally, the ROC curves analysis demonstrated that AIP is superior to traditional lipid markers in predicting the risk of MAFLD.

Similar to NAFLD, hepatic steatosis is also one of the key features of MAFLD. However, previous studies have demonstrated that individual lipid parameters lack specificity in identifying NAFLD (34, 35), and there remains controversy regarding the evidence of the role of individual lipids in promoting hepatic fat accumulation (7, 36). The advantage of AIP compared with

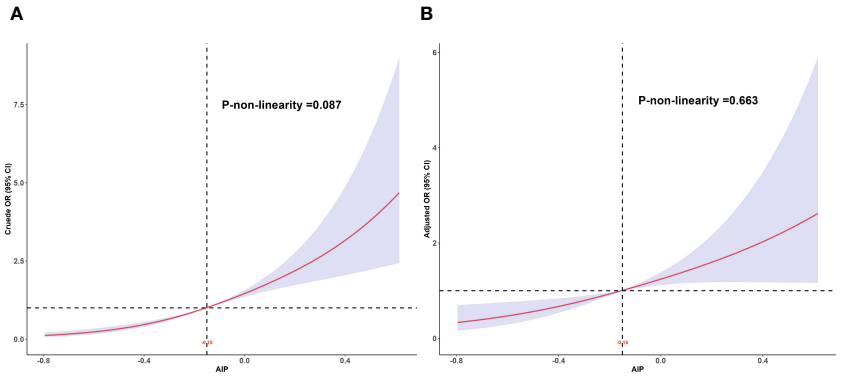

FIGURE 2
Dose-response relationship between AIP and MAFLD. **(A)** Unadjusted dose-response relationship between AIP and MAFLD; **(B)** Adjusted dose-response relationship between AIP and MAFLD. Adjusted factors include sex, age, race, education level, PIR, BMI, smoking status, drinking status, hyperlipidemia, hypertension, DM, physical activities status, ALT, AST, and lipid-lowering drugs. AIP, atherogenic index of plasma; MAFLD, metabolic associated fatty liver disease; PIR, poverty income ratio; BMI, body mass index; DM, diabetes mellitus; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

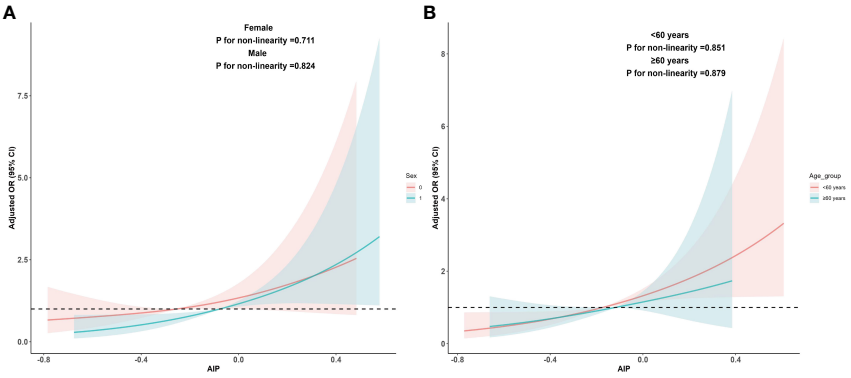

FIGURE 3
Dose-response relationship between AIP and MAFLD in sex and age subgroups. **(A)** Adjusted dose-response relationship between AIP and MAFLD in sex subgroups; **(B)** Adjusted dose-response relationship between AIP and MAFLD in age subgroups. Adjusted factors include sex, age, race, education level, PIR, BMI, smoking status, drinking status, hyperlipidemia, hypertension, DM, physical activities status, ALT, AST, and lipid-lowering drugs. When analyzing sex groups, sex factors should be excluded from confounding factors, and the same applies when analyzing age subgroups. AIP, atherogenic index of plasma; MAFLD, metabolic associated fatty liver disease; PIR, poverty income ratio; BMI, body mass index; DM, diabetes mellitus; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

traditional blood lipid indicators is that it combines two blood lipid indicators, TG and HDL-C, in the form of ratio, which can reflect more information about blood lipid metabolism and is more stable than a single blood lipid index. TG is one of the common indicators of atherosclerosis, especially in recent studies, which have found that TG-rich lipoprotein cholesterol plays a significant role in increasing residual cardiovascular risk (37). In contrast, HDL-C is considered to have anti-inflammatory and anti-atherosclerotic effects (38–40). Therefore, to some extent, the AIP can be regarded as an indicator reflecting the balance between atherogenesis and anti-atherogenesis in the body. Previous studies have suggested that MAFLD may mediate the development and progression of atherosclerosis (41, 42). Therefore, the strong association between AIP and MAFLD may be related to their common involvement in the process of atherosclerosis in the body. In addition, IR also plays an important role in hepatic steatosis. When IR occurs in the body, on the one hand, it leads to accumulation of free fatty acids in liver tissue and increases lipid synthesis (43); on the other hand, it also leads to reduced adiponectin availability and weakens its ability to regulate fat metabolism. At the same time, reduced availability of adiponectin further aggravates body IR, causing a vicious cycle (2, 44). Previous studies have already confirmed a close association between AIP and IR (17, 45). From this, it can be inferred that the association between AIP and MAFLD may be explained based on the strong associations between AIP and atherosclerosis as well as IR. However, further studies are still needed to validate these hypotheses. Shin et al. (21) found that AIP was associated with obesity index, blood glucose, and lipid profile in Korean men in a study based on data from the Korea National Health and Nutrition Examination Survey (KNHANES) from 2013 to 2014, and these real-world evidence also directly indicated that AIP was closely related to multiple metabolic indexes of the body, not only to lipids.

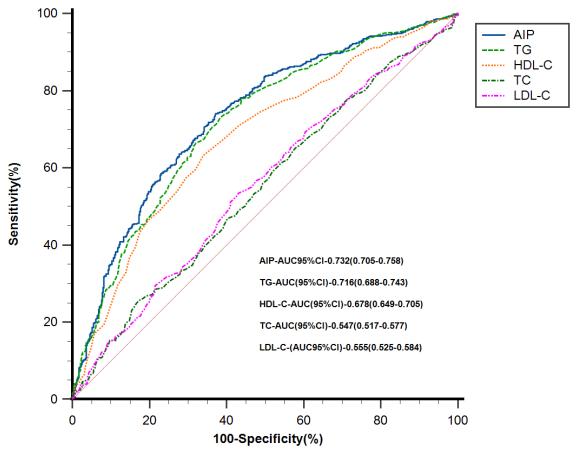

FIGURE 4
ROC analysis of AIP and traditional lipid parameters for predicting the risk of MAFLD. AIP, atherogenic index of plasma; MAFLD, metabolic associated fatty liver disease; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; CI, confidence interval.

To the best of our knowledge, this is the first study to investigate the dose-response relationship between AIP and MAFLD in the adult population of the United States. Although several previous studies have described the association between AIP and MAFLD, none of these studies explored the dose-response relationship between AIP and MAFLD, and the emphasis of these studies was different. A cross-sectional analysis of 864 Chinese participants by Duan et al. showed a positive association between AIP and MAFLD, and combined AIP with BMI and waist circumference to construct a new index that can improve the predictive ability of MAFLD (22). Samimi et al. showed AIP to be a valid predictor of MAFLD in patients with type 2 diabetes, they were more concerned about the relationship between AIP and MAFLD in patients with type 2 diabetes mellitus (T2DM) and this study was mainly conducted in Iranian population (23). Recently, Wang et al. explored the

association between AIP and MAFLD on the basis of NHANES survey data (46). Similar to the two previous studies, this study did not explore the dose-response relationship between AIP and MAFLD. Furthermore, when constructing the final model, the study did not consider the impact of exercise and alcohol consumption on the outcome. In our study, we diagnosed MAFLD according to the recommendations of the 2020 international expert panel consensus (1). However, unlike Wang et al.'s study, which utilized a diagnostic threshold of CAP ≥ 285 dB/m for hepatic steatosis, we employed a lower threshold of CAP ≥ 248 dB/m (30), which could identify more underlying patients with MAFLD (34, 47). In addition, to avoid the effect of diet on TG as much as possible, we used TG measured in the fasting state. Considering the significant sex and age differences in the prevalence of MAFLD (48, 49), we explored whether the linear dose-response relationship between AIP and MAFLD remains in the sex and age subgroups separately, as well as the specific pattern of dose-response relationship in the sex and age subgroups. The results showed that the linear dose-response relationship between AIP and MAFLD remained stable across sex and age subgroups.

It has been shown that MAFLD increases the risk of acute myocardial infarction (AMI) and stroke by 35% and 26%, respectively (50), and the results of Chung et al. suggest that MAFLD with DM can be a strong predictor of all-cause mortality and disease-specific mortality (51). It is therefore highly desirable to identify underlying MAFLD patients in clinical work. However, limited by the actual situation of clinical work, it is difficult for clinicians to conduct universal screening of patients with MAFLD. Our results suggest a strong positive association between AIP and MAFLD, and AIP is superior to traditional lipid parameters in predicting the prevalence of MAFLD. Therefore, clinicians may be able to raise concerns about patients with abnormally elevated AIP and consider MAFLD-related diagnostic tests if necessary.

This study has the following limitations: 1. Based on the design of a cross-sectional study, this study cannot identify whether there is a causal association between AIP and MAFLD; 2. The conclusions of this study apply only to the US adult population; 3. The conclusions of this study warrant further validation in a larger cohort study.

Conclusion

The findings of this study revealed a robust positive association between AIP and MAFLD. This association proved significant, irrespective of whether AIP was treated as a continuous variable or categorized using quartiles. At the same time, potential linear dose-response relationships between AIP and MAFLD have been elucidated, that is, the risk of MAFLD also increased as the AIP value increased. Given that MAFLD significantly increases the risk of adverse outcomes for patients, particularly in terms of cardiovascular and cerebrovascular diseases, physicians may consider using AIP to screen for MAFLD in order to better assess the underlying risks for these patients. However, further validation of this conclusion is warranted through larger-scale cohort studies.

Data availability statement

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

Ethics statement

The studies involving humans were approved by National Center for Health Statistics Research Ethics Review Board (Protocol number: 2018-01). 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

YC: Conceptualization, Writing – original draft, Writing – review & editing. CL: Data curation, Writing – review & editing. HJ: Data curation, Writing – review & editing. QZ: Writing – review & editing. XZ: Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was funded by Dalian Medical University, Interdisciplinary Research Cooperation Project Team Funding of Abnormal blood pressure regulation and Hypertension (JCHZ2023014).

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1405828/full#supplementary-material

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frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 23 May 2024
DOI 10.3389/fendo.2024.1411706

OPEN ACCESS

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RECEIVED 03 April 2024
ACCEPTED 10 May 2024
PUBLISHED 23 May 2024

CITATION
Meroni M, Longo M and Dongiovanni P (2024) Cardiometabolic risk factors in MASLD patients with HCC: the other side of the coin. *Front. Endocrinol.* 15:1411706. doi: 10.3389/fendo.2024.1411706

Cardiometabolic risk factors in MASLD patients with HCC: the other side of the coin

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Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) constitutes the commonest cause of chronic liver disorder worldwide, whereby affecting around one third of the global population. This clinical condition may evolve into Metabolic Dysfunction-Associated Steatohepatitis (MASH), fibrosis, cirrhosis and hepatocellular carcinoma (HCC), in a predisposed subgroup of patients. The complex pathogenesis of MASLD is severely entangled with obesity, dyslipidemia and type 2 diabetes (T2D), so far so nutritional and lifestyle recommendations may be crucial in influencing the risk of HCC and modifying its prognosis. However, the causative association between HCC onset and the presence of metabolic comorbidities is not completely clarified. Therefore, the present review aimed to summarize the main literature findings that correlate the presence of inherited or acquired hyperlipidemia and metabolic risk factors with the increased predisposition towards liver cancer in MASLD patients. Here, we gathered the evidence underlining the relationship between circulating/hepatic lipids, cardiovascular events, metabolic comorbidities and hepatocarcinogenesis. In addition, we reported previous studies supporting the impact of triglyceride and/or cholesterol accumulation in generating aberrancies in the intracellular membranes of organelles, oxidative stress, ATP depletion and hepatocyte degeneration, influencing the risk of HCC and its response to therapeutic approaches. Finally, our pursuit was to emphasize the link between HCC and the presence of cardiometabolic abnormalities in our large cohort of histologically-characterized patients affected by MASLD (n=1538), of whom 86 had MASLD-HCC by including unpublished data.

KEYWORDS

MASLD, HCC, metabolic dysfunctions, cholesterol, carotid plaques

1 Introduction

Due to the global spreading of Metabolic Syndrome (MetS), the newly re-defined Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) represents the greatest challenge for the global Health systems of the 21st century. This concept is even more relevant when we consider that it may constitute the etiopathological substrate for the

development of advanced stages of the disease, passing towards Metabolic Dysfunction-Associated Steatohepatitis (MASH), to fibrosis, cirrhosis and hepatocellular carcinoma (HCC). As assumed by the novel nomenclature, several metabolic alterations may be causative of steatosis, thus being more inclusive of cardiometabolic risk factors as abdominal obesity, dyslipidemia, hypertension, and hyperglycemia. Indeed, the new terminology implies body mass index (BMI) >= 25 kg/m^2 or increased waist circumference (WC >94 cm for men and 80 cm for women); raised plasma triglycerides (TGs>= 1.70 mmol/L or lipid lowering treatments); reduced HDL cholesterol (<= 1.0 mmol/L or lipid lowering agents); high blood pressure (>= 130/85 mmHg or therapy with anti-hypertensives); and enhanced fasting serum glucose (>= 100 mg/dL) or glucose intolerance (1).

A total of 10–20% of HCC cases is attributable to MASLD (2), thus projecting the proportion of the annual incidence of the liver cancer worldwide and requiring a growing attention in surveillance programs (3, 4). With the advances in antiviral therapies, MASLD-related HCC is becoming also the sixth most common tumor globally and the second indication for liver transplantation in the United States (5). Intriguingly, MASLD-HCC may even occur in the absence of cirrhosis, further delaying its diagnosis (6, 7).

Nutritional and lifestyle habits as well as the metabolic status of patients may participate in determining the risk of HCC and modifying its prognosis (8). However, the mechanisms underlying this association are not completely elucidated. Thus, the present mini-review aims to deepen the role of metabolic comorbidities in predisposing to liver cancer in MASLD patients, emphasizing the correlation between HCC and the presence of cardiovascular abnormalities.

2 Common genetic signature between MASLD-HCC and lipid disorders

Ever increasing evidence testifies that the evolution of MASH towards HCC, with or without cirrhosis, is prompted by a plethora of common and rare inherited variations (9). Genetic variants which predispose to HCC are those in genes related to hepatic lipid handling including the *Patatin-like phospholipase domain containing 3* (*PNPLA3*), the *Transmembrane 6 superfamily member 2* (*TM6SF2*) and *Membrane bound-o-acyltransferase domain-containing 7* (*MBOAT7*), widely reported to be responsible for fatty loading of the liver (10). These polymorphisms alone and more so when combined in polygenic risk scores (PRSs), have been extensively associated with glucose reprogramming, tumorigenesis and metabolic switching towards aerobic glycolysis, mitochondrial failure and oxidative stress due to hepatic lipid overload (10, 11). In details, the prevalence of the three at-risk variants was 2.5-fold enriched in patients in the MASLD-HCC compared to non-carriers and their cumulative presence was associated with enhanced MASLD-HCC risk (11). Moreover, by applying a mendelian randomization approach, Dongiovanni and colleagues firstly reported that the co-presence of *PNPLA3*, *TM6SF2*, *MBOAT7*, and *GCKR* at-risk alleles, aggregated into a PRS, causally determines an increased susceptibility to develop severe chronic liver diseases, which reached about 13.4-fold for MASLD-HCC risk, as a consequence of their ability to induce hepatic fat accumulation (12). Accordingly, it has been demonstrated that the impact of each genetic variant on the odd to develop HCC is directly proportional to their ability to predispose to fatty liver (12, 13). For instance, the genetic variation encoding the p.I148M substitution in PNPLA3 which is the major inherited determinant of hepatic fat accumulation, is enabled to predispose to an until 5-fold higher risk to develop HCC even independently of fibrosis (14). Likewise, the common rs641738 close to *MBOAT7* has been pointed out as a modifier of the susceptibility to develop HCC even in the absence of cirrhosis as a consequence of its ability to induce giant lipid droplets (LDs) into the hepatocytes and other rare loss-of-function variants in the same gene have been found enriched in patients with MASLD-HCC (15). Therefore, a close relationship between circulating/hepatic lipids and hepatocarcinogenesis exists and genetic variants influencing them may be helpful in discriminating those MASLD patients at increased risk of HCC.

In keeping with this notion, other variants implicated in the remodeling of lipids and their release favor the switching from fatty liver towards cancer. Indeed, similarly to what occur in patients carrying the p.E167K allele of TM6SF2, even rare mutations with large effect size in the Apolipoprotein B (*APOB*) gene, have been correlated with hypobetalipoproteinemia and a protection against cardiovascular complications, but with more severe liver injuries (15). Indeed, ApoB participates to the VLDL gathering and modulates their secretion and loss-of-function variants blunt circulating lipoprotein concentrations. This event triggers severe fat depots' formation due to VLDL retention and progressive MASLD up to HCC (16). As a consequence, a significant enrichment in pathogenic and truncating mutations in *APOB* gene has been identified in MASLD-HCC patients (15). In line with a possible causative role of lipid retention in hepatocytes in fostering HCC onset, even somatic mutations in *APOB* frequently occur during hepatocarcinogenesis (17). Hence, *APOB* alterations have been recently outlined as a prognostic biomarker for HCC (18).

Similarly, other variants influencing fasting lipids have been reported to increase the susceptibility to severe liver injuries. In particular, Dongiovanni and colleagues demonstrated that the proprotein convertase subtilisin/kexin type 7 (*PCSK7*) rs236918 G > C gain-of-function mutation coupled atherogenic dyslipidemia and acute coronary syndrome with MASH-fibrosis in a large histologically-characterized cohort of MASLD patients (19). Accordingly, it has been recently reported that this variant is enabled to modulate ApoB protein levels reinforcing the hypothesis of a possible role for PCSK7 in modulating lipid metabolism, LDs formation and liver damage (20). Likewise, genetically-determined aberrant expressions of PCSK9, another member of the proprotein convertase subtilisin/kexin family, have been correlated with hereditary hypercholesterolemia, severe steatosis and cardiovascular abnormalities (21–23). However, the impact on hepatocarcinogenesis of inherited variants in these two genes is still unexplored.

Moreover, the rs599839 A>G variant localized in the 1p13.3 locus related to lipid traits reduced the risk of coronary artery

disease (CAD) and dyslipidemia, favoring in turn the incidence of liver cancer, poor prognosis, reduced overall survival and advanced tumor stages in HCC patients. The likely mechanism behind this association is due to the overexpression of the oncogene Proline And Serine Rich Coiled-Coil 1 (*PSRC1*), and of Sortillin 1 (*SORT1*), both involved in distinct pathways. The former regulates cell proliferation participating to microtubule destabilization and spindle assembly, while the latter modulates circulating lipid profiles, reinforcing lipoprotein clearance, turnover and dismissal. Hence, similarly to the aforementioned mutations, it disentangles the risk of atherogenic dyslipidemia and hepatocarcinogenesis, whereby enhancing HCC incidence, but ameliorating serum lipid profile (24). Accordingly, hepatic PSCR1 expression associated with increased HCC recurrence, and its overexpression was observed in hepatoma tissues compared to the adjacent ones and correlated with reduced survival (25, 26). Conversely, the Neurotensin (NTS) rs1800832 variant has been described to enhance the susceptibility to coronary heart diseases, and predispose to advanced fibrosis and HCC in MASLD patients. This polymorphism modulates circulating pro-NTS, a peptide mainly released from entero-endocrine N cells of the gastrointestinal tract which facilitates intestinal fatty acid absorption and its effects are mediated by three neurotensin receptors (NTSRs), one of which is SORT1. Exaggerated NTS expression has been described in human HCC samples, where it mediates hepatocyte proliferation and epithelial mesenchymal transition (EMT), being tumors more aggressive and promoting tumor invasion, spreading and metastasis (27). Even more, elevated pro-NTS circulating levels were significantly correlated with the presence of HCC (28). Therefore, we could hypothesize that other inherited mutations exerting large effects on lipoprotein metabolism may be useful to classify patients with MASLD according to their relative risk of developing cardiovascular *vs*. liver-related events.

Other genetic risk factors may play a crucial role in the transition from simple steatosis to MASH and cancer, whereby modulating the lipid composition and the size of the LDs. For instance, the rs35568725 (p.S251P) variant in Perilipin-2 (*PLIN2*) gene, which regulates the stability and the remodeling of LDs and VLDL lipidation, predisposes to severe insulin resistance (IR) and atherosclerosis. This mutation conveys the risk of severe hepatic disease favoring the accumulation of small LDs in hepatocytes (29, 30). The pro-carcinogenic role of PLIN2 is still under investigation. However, it has been found up-regulated in HCC samples, gaining prognostic value and influencing adverse outcomes (31). Likewise, also PLIN5, another member of the perilipin family, is highly expressed in livers isolated from HCC patients and diethylnitrosamine (DEN)-treated mice (32). The rs884164 in *PLIN5* downregulates its protein expression, blunting the number of contacts between mitochondria and LDs and lipid degradation. This variant is associated with a poorer outcome following myocardial ischemia, and enhanced oxidative stress in cardiomyocytes (33, 34). Alongside, other inherited defects in autophagic processes, mainly in lipophagy, accelerates LD accumulation (35). Although the impact of the variants in *PLIN2* and *PLIN5* genes on liver cancer is still under definition, we cannot rule out that they may define disease progression to cancer and therapeutic response, due to their contribution to LD number, size and composition. Indeed, it has been hypothesized that LDs sequester anti-cancer drugs, hampering their efficacy. Moreover, since augmented LD biogenesis have been reported in different neoplastic conditions, this makes LDs novel suitable targets for anticancer drugs and for the development of new dyes for cancer cells imaging (36, 37).

3 Role of hyperlipidemia in HCC development and evolution

3.1 Fatty acids and lipid droplets metabolism in the modulation of HCC risk

MASLD is a complex disorder, whose pathogenesis involves multi-parallel hits, either environmental or genetic factors. TGs, diglycerides, and ceramides overload trigger endoplasmic reticulum (ER) stress and mitochondrial dysfunction, causing both calcium (Ca2+) and reactive oxygen species (ROS) enrichment, which are directly involved in DNA mutagenesis, activation of oncogenes or inhibition of onco-suppressors (11, 38, 39).

Several studies revealed that hepatic LDs accumulation and *de novo* lipogenesis (DNL) play a crucial role in HCC development. Notably, the inhibition of key enzymes involved in lipogenic pathways, such as stearoyl-CoA desaturase (SCD), fatty acid synthase (FASn), and acetyl-CoA carboxylase (ACC), has been linked to a reduced presence and proliferation of cancer stem cells (CSCs), which are critical for tumor growth and progression, in both *in vitro* and *in vivo* models (40, 41). Moreover, free fatty acids (FFAs) derived from adipose tissue lipolysis, combined with those synthesized through DNL, may accelerate hepatocytes' degeneration, and promote tumor escape mechanisms by activating anti-apoptotic programs. Palmitic acid (PA) treatment affects insulin signaling, enhances β-oxidation, exacerbates ROS content, and turns on c-Jun NH2-terminal kinase (JNK), a constitutively activated factor in HCC. The accumulation of oleic acid (OA) and PA promotes liver cancer by suppressing Pten, an inhibitor of Pi3k/Akt/mammalian target of rapamycin (mTOR) signaling (42).

Notwithstanding, it has been observed that HCC has a wide molecular and phenotypic variability. Indeed, T2D, obesity, or MASLD may induce the development of "oxidative" HCCs, in which FFAs are catabolize through β-oxidation and increase ATP availability. This mechanism may favor cell growth and mainly involve the activation of PPARα, the master regulator of FFA degradation, and of WNT/β-catenin oncogenic cascade (43). In keeping with these findings, Tian et al. has demonstrated that LD breakdown (lipophagy) was unexpectedly involved in carcinogenesis in hepatoma cell lines, MASLD murine models, and MASLD-HCC human samples (44).

Even more, LD formation and their dimension (micro/macro-LDs) hover a crucial role in HCC metastasis, stemness and response to therapy. Interestingly, besides stocking energy sources, LDs work

as a buffering system incorporating lipotoxic species. It has been reported that long-term Sorafenib exposure, the first-line treatment for advanced HCC, impairs β-oxidation, causing the accumulation of FAs. In turn, toxic FAs are incorporated into LDs, favoring their biogenesis and enlargement, and increasing the HCC susceptibility to became drug resistant. This adverse effect has been recently attributed to the upregulation of AKR1C3 protein, which promotes LD shaping and accumulation, insofar as the selective AKR1C3 inhibition improved Sorafenib resistance in HCC cell lines (45).

PLIN1/2/3 proteins mediate LDs–mitochondria crosstalk, regulating LDs' expansion and disposal. Notably, PLIN1, PLIN2 and PLIN3 are differentially expressed during tumorigenesis and usually dwell on the LDs' surface according to the LDs' dimension. PLIN2 and PLIN3 mainly coat small LDs and are commonly overexpressed at early stages of HCC, as the LD dimension allows rapid dynamics between synthesis and consumption to sustain phases of cell proliferation or metabolism. PLIN1 expression is lost during hepatocarcinogenesis and may reflect the differentiation grade of hepatocytes (46).

The activation of SREBP1 via PI3K/Akt/mTOR further contributes to neoplastic steatogenesis and PLINs expression (47). These studies pointed out a differential role of micro/macro-LDs in HCC onset and progression, although details on the mechanisms and respective roles need to be addressed in the future.

3.2 Cholesterol and HCC

Recent clinical and preclinical evidence supported the notion that an excessive cholesterol intake may represent an independent risk factor for liver cancer development in the context of MASLD, even irrespectively of cirrhosis (48–50). However, a precise definition of the likely mechanism behind this association remains to be elucidated. In different rodent models of obesity and diabetes, it has been demonstrated that hyperinsulinemia stimulates induction of new cholesterol synthesis, through sterol regulatory element-binding protein 2 (SREBP2) activation, low-density lipoprotein receptor (LDLR) up-regulation, and the shutdown of its conversion into bile acids, thus leading to the hepatic accumulation of free cholesterol. Cholesterol overload, in turn, favors lipotoxicity into the hepatocytes, mediating the progression to MASH (48). In details, cholesterol over-storage into the organelles as ER and mitochondria generates aberrancies in the intracellular membranes, ROS production, ATP depletion and hepatocyte degeneration. Overall, these events predispose to a more prone pro-inflammatory milieu, priming the transition from uncomplicated steatosis to steatohepatitis and fibrosis (48, 51, 52). Indeed, cholesterol uptake mediated by the scavenger receptor (SR-A) or by CD36 and by lectin-like oxidized LDL receptor-1 (LOX-1), exerts a dual role on Kupffer cells and hepatic stellate cells (HSCs), respectively, thus promoting their activation, the release of pro-inflammatory cytokines, oxidized mtDNA and pro-cancerous factors (53). Accordingly, DEN administration to mice fed a high fat high cholesterol (HFHC) diet induced more severe oxidative DNA damage, non-synonymous somatic mutations, numerous and large liver tumors compared to littermates treated with DEN alone (54). In addition, rodents fed HFHC displayed a pronounced gut microflora dysbiosis and fecal microbiota transplantation in germ-free mice favored the onset of steatosis, inflammation, oxidative stress and cell proliferation. On the contrary, microbiota manipulation to restore the eubiosis as well as cholesterol lowering agents as atorvastatin might be effective for HCC prevention (55).

It has been largely reported that free cholesterol depots are accumulated in patients with MASH, due to the imbalance between its biosynthesis, conversion and excretion and free cholesterol levels correlate with the severity of the disease (56, 57). High cholesterol assumption has been proven to enhance the susceptibility to HCC in a population-based study among 14,407 participants conducted in the United States, but serum cholesterol concentrations were not associated with a higher risk of cirrhosis or liver cancer (49). Conversely, Carr and colleagues found a positive correlation between plasma cholesterol, lipoprotein levels and tumor growth, cell invasion and aggressiveness (58). Likewise, a nested case-control study within the Scottish Primary Care Clinical Informatics Unit (PCCIU) database demonstrated that statins assumption had a protective effect on HCC risk (hazard ratio HR, 0.48; 95% CI, 0.24–0.94) (59). In addition, statin users had 15% lower hazard ratio (HR) to develop a new liver disease, 28% lower HR of liver-related death and a 42% lower HR of HCC, showing a significant hepatoprotection in time and dose dependent manner (60). Similar benefits of statin use have been reported even in patients with chronic hepatitis B (CHB) (61).

Intriguingly, Ma and collaborators demonstrated that cholesterol levels may regulate also tumor immune microenvironment, whereby affecting natural killer and CD8+ T cells (62). In particular, these authors demonstrated that cholesterol-enriched tumors progressively upregulate the programmed death receptor 1 (PD-1), thus inducing CD8+ cell death and the loss of cell growth control. On the contrary, cholesterol-lowering agents, as simvastatin, prime the anti-tumor immunity mimicking the effect of the novel developed PD-1/Programmed death ligand 1 (PD-L1) inhibitors (63).

All-in-all, to further dissect the impact of cholesterol on the incidence of HCC and shed a light on the possible use of lipid biomarkers as a predictive sign of cancer, more investigations will be warranted.

4 Prognostic value of metabolic markers in MASLD-HCC patients

Emerging evidence outlines that metabolic factors, including dyslipidemia, may increase the susceptibility to develop HCC, especially in population with low prevalence of viral hepatitis (64). Indeed, the peculiar pro-inflammatory and lipotoxic milieu that characterizes the hepatic tissue of MASLD patients, influences the risk of HCC and adverse prognosis. However, the magnitude and the direction of this association is still under definition. In addition, except for the total cholesterol, only few studies reported

an association between circulating LDL, HDL and TGs and hepatocarcinogenesis (65, 66). In this context, the idea to possibly exploit lipid biomarkers as an edge of the disease progression, became attractive.

For instance, neutrophil-to-HDL-C ratio (NHR) parameter has been recently proposed as prognostic indicator of mortality in HCC patients, exceeding the power of each single marker alone (67). Indeed, HDL cholesterol may play discrete anti-inflammatory and anti-oxidant functions and its reduction has been correlated with the presence of different metabolic disorders (68, 69), including HCC (70). Conversely, neutrophils constitute a precocious innate immune response to injured tissues, being recruited directly in proximity to the damage and parallelly increasing their count (71). Therefore, the evaluation of this composite marker may be representative of a severe disease status, overall reflecting both inflammation and lipid metabolism. Accordingly, in the last years, various applications for the NHR have been screened, as an index of acute ischemic stroke (AIS) (72), or as a predictor of clinical outcomes of all-causes of cardiovascular mortality (73). In addition, low HDL cholesterol has been recently reported as novel marker to predict HCC in 1,234 MASLD patients by Crudele and collaborators (74). However, further population-based studies are required to better define its sensitivity and sensibility in foreseeing MASLD-HCC.

Given the strong impairment in hepatic parenchyma, an abnormal pattern of serum lipoproteins as ApoA1 and ApoA2, has been observed in patients with HCC (75, 76). For this reason, it has been pointed out ApoA1 as a candidate biomarker for early diagnosis, prognosis, and monitoring of HCC, in patients affected by viral hepatitis C (77). However, one of the main issues is that ApoA1 is not a sensitive or specific biomarker enough to separate HCC from chronic liver diseases, in which hepatic dysfunction occurs. Nonetheless, these findings further corroborate the association between a dyslipidemic profile and the presence and/or the risk of liver cancer (78, 79). Accordingly, we recently demonstrated that the low serum lipoprotein (Lp(a)) concentrations correlated with transaminase elevation and increased risk of developing cirrhosis in MASLD patients, thus representing a novel lipid biomarker to reliably predict severe liver disease (80). Alongside, Lp(a) has been proposed also as an indicator of tumor recurrence after curative resections since it represents an hallmark of the residual hepatic function (81).

Concerning other lipid-related biomarkers, preclinical and *in vitro* studies demonstrate that serum and hepatic PCSK9 levels induced by glucose exposure, alter the response of hepatoma cells to cancer therapy (82). In addition, the high availability of glucose in tumor tissues fosters PCSK9 elevated secretion, which favors, in turn, hypercholesterolemia (82).

Considering the metabolic reprogramming that characterizes hepatoma cells, it is conceivable that lipidomic-based studies may provide novel biomarkers. For instance, a downregulation of plasma phosphatidylcholine species (83) as well as of ceramides (84) has been reported to have an acceptable predictive performance for HCC, whereas the enhancement of Sphingosine 1-phosphate (S1P), the prevalent hepatic sphingolipid, supports tumor growth (85). Likewise, the assessment of the typically aberrant fatty acid metabolism (FAM), which supports cancer cell proliferation, may be useful to construct prognostic risk models for HCC (86).

Alongside novel lipidomics approaches, also recent metabolomics studies on blood samples from subjects with HCC revealed aberrant circulating profiles of glucose, lactic acid, retinol, bile acids and amino acids (including alanine, glutamine, 1-methylhistidine, lysine, and valine), mainly as a consequence of the unique HCC metabolism (87). Notably, a deep understanding of the utility and reliability of these omics biomarkers is necessary to consider them for their introduction into the clinical practice. Even more, further validation analyses in larger cohorts of patients are required to understand whether these serum metabolites may be helpful in diagnosing HCC and in stratifying MASLD-HCC patients according to their prognosis. In this context, an integrated view of the metabo-lipid signature of HCC patients is now emerging as a diagnostic opportunity for Alpha-fetoprotein (AFP) false-negative subjects (88).

5 Cardiovascular risk in patients affected by MASLD-HCC

5.1 Cardiovascular complications, dyslipidemia and type 2 diabetes in MASLD-HCC patients

As extensively reported, the amount and the diameter of circulating LDL cholesterol is one of the primary risk factors that predispose to vascular atherosclerotic diseases in both men and women (89). In details, numerous, small, dense and oxidized LDL particles constitute the more noxious elements in the etiology of CAD. Nevertheless, the causal relationship between HCC and cardiometabolic risk factors is still under investigation. Indeed, conflicting results are available regarding the association between LDL levels and the odd of liver cancer. On one hand, it has been demonstrated that LDL cholesterol loading into intracellular organelles prompts oxidative and ER stress, thus creating a more pro-oncogenic microenvironment (90), on the other, some epidemiological studies described a significant association between relatively low levels of LDL cholesterol (<100 mg/dL) and the risk of incident cancer (91, 92). In keeping with the latter observation, a reduction of the overall mortality in HCC patients with hypertriglyceridemia (HR, 0.38; 95% CI, 0.26 to 0.55) and hypercholesterolemia (HR, 0.50; 95% CI, 0.37 to 0.67) has been reported by Chiang et al. (66).

Notwithstanding, Bertero and colleagues determined that patients affected by cardiovascular diseases (CVD) are more likely prone to develop malignancies, sustaining a possible shared biology (i.e., inherited or acquired predisposing factors, inflammation, stress and angiogenesis) that primes tumors and CVD development (93). Hence a common multi-factorial pathogenic substrate, involving T2D, dyslipidemia, hypertension, and obesity for both diseases has been postulated (94). Accordingly, Banke and collaborators further corroborate this notion, describing that patients with chronic heart failure have an increased susceptibility to develop any-type of cancers, with an incidence ratio of 1.24 (95).

The other side of the coin is that fatal cardiovascular complications and heart involvement in liver cancer is considered a poor prognostic indicator, reducing the survival and cardiac metastasis occurring in 10% of HCC diagnosis (96). Therefore, the overall clinical framework of HCC patients, the use of cholesterol-lowering medications, other therapeutic and dietary indications, and the inference of genetic variations should be rigorously taken into account to evaluate these associations.

A greater unified opinion outlines the causal relationship between poor glycemic control/T2D and the incidence of HCC, thus pointing out the need of achieving the goal of a better glucose management to reduce long-term complications of diabetes in these patients (66, 91, 97). Several epidemiological studies supported this evidence (98–102) and a meta-analysis across 26 studies claimed an almost doubled risk of HCC in patients with T2D of different ethnicity and geographic localization (103).

In particular, T2D represents an independent risk factor for HCC, in both men and women and this association became stronger with prolonged T2D duration and more so in the co-presence of other comorbid metabolic conditions (97). Indeed, hyperglycemia, raised levels of insulin and insulin-like growth factor 1 (IGF1) display proliferative and oncogenic effects and they worsen the already existing cardiometabolic risk factors exasperating chronic inflammation, endothelial dysfunction, oxidative stress and DNA damage thus creating a favorable tumor microenvironment. In this context, intestinal dysbiosis and microbial metabolites may promote the raising of pro-inflammatory, pro-coagulative and pro-fibrotic circulating molecules, as the endotoxins (104, 105). Notably, it has been also reported that T2D and HCC share almost 336 differentially methylated genes (DMGs), including 86 co-methylated DMGs, mainly involved in glycosaminoglycan biosynthesis, fatty acid and metabolic pathways, whereas 250 DMGs with a different methylation direction, enriched in the Sphingolipid metabolism and immune signaling, thus corroborating a common pathophysiology between these two diseases (106). Otherwise, endothelial dysfunction *per se* is responsible for vasoconstriction and platelet aggregation fueling pro-inflammatory mediators release, hypertension, diabetic nephropathy and cardiovascular diseases, fostering a 'vicious cycle' (107). Hence, T2D appears as the common denominator between hepatocarcinogenesis and cardiovascular abnormalities, by predisposing to both advanced lesions, poor outcomes, vascular complications and higher mortality rate (108). Nonetheless, irrespectively of coexisting factors, IR is the major determinant of severe hepatic fibrosis, which is an excellent prognostic indicator of HCC onset (109). Alongside hyperinsulinemia has been reported to be responsible for the induction of more aggressive cancer phenotypes and poor prognosis even in other types of tumors (110).

Finally, the increasing number of metabolic comorbidities, including dyslipidemia, T2D, obesity and hypertension has been associated with enhanced predisposition to HCC, reaching an 8.1-fold increased HCC risk (95% CI, 2.48–26.7) in the co-presence of these four cofactors (97).

5.2 Other cardiometabolic risk factors influencing the risk of MASLD-HCC: the role of abdominal obesity and hypertension in modifying hepatocarcinogenesis

As abovementioned, it is now widely acknowledged that obesity, hypertension, glucose intolerance and T2D frequently co-occur with hepatic steatosis, contribute to its progression and have been associated with both cardiovascular and HCC risk in MASLD patients (111).

Obesity has been increasingly identified as a major driver in the evolution of MASLD up to HCC. Substantial adipose tissue deposits introduce significant complications for the diagnosis and screening of HCC with both invasive and non-invasive techniques. The presence of subcutaneous and visceral fat can hinder the detection of hepatic lesions by ultrasound, the standard protocol for HCC surveillance, as well as it can make challenging the interpretation of images obtained with higher resolution equipment, such as MRI and CT. Additionally, in liver biopsies, the accessibility to the liver can be limited by abdominal fat accumulation, raising the risk of procedural-related complications (112).

Two large population studies carried out in Denmark and US, including 43,965 and 900,000 cases, respectively, have shown that liver cancer development was by around 2-fold higher in obese patients compared to the general population. Furthermore, the relative risk of dying from liver cancer increased by 1.68 times for women and 4.52 times for men with a BMI>30 kg/m^2 compared to a reference group with a median BMI>21.8 kg/m^2 (113, 114). Recently, Rustgi and collaborators conducted a retrospective study in a large cohort of 98,090 newly diagnosed MASLD patients eligible for bariatric surgery, spanning from 2007 to 2017. The authors found that MASLD subjects who underwent bariatric interventions exhibited a lower rate of obesity-related cancer, including HCC, compared to those who did not undergo the surgery (115). These findings were partially corroborated by Saito et al. who tested the efficacy of a multidisciplinary weight loss program (WLP), including the nutritional assessment and physical exercise, in HCC patients with a high BMI (≥25 kg/m^2) before they underwent hepatic resection. Despite WLP did not impact on HCC recurrence or progression, it improved the immune status and liver function (116), thereby supporting that interventions like bariatric surgery and weight loss may potentially aid to mitigate HCC risk and to ameliorate its management.

Hypertension represents the most prevalent cardiometabolic risk abnormality in MASLD patients, diagnosed in up to 50% of cases. The use of antihypertensive drugs, which include angiotensin-converting enzyme inhibitors (ACEi), calcium channel blockers (CCBs), beta-adrenoceptor blockers (BBs), angiotensin receptor blockers (ARBs) and thiazide diuretics, is a critical component in MASLD management, since hypertension showed an independent association with HCC risk and poor prognosis (117). Nonetheless, it is still unclear whether and how

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antihypertensive drugs may modify the risk of HCC or may limit the efficacy of anti-cancer therapies (118, 119). For instance, captopril, an ACEi, reduced hepatic fibrosis and prevented progression towards HCC development in murine models of liver cancer (119). Conversely, Zhang and coworkers reported that ACE inhibitors may compromise the effects of anti-angiogenic drugs in HCC mouse models (120), thus supporting the necessity for a rigorous monitoring and control of hypertension in MASLD-HCC patients to optimize treatment efficacy and outcomes.

A large meta-analysis collecting data from 1976 to 2017 in 12 countries (a total of 526,336 participants) have shown that hypertension awareness, treatment, and control have been improved in high-income states (121). However, in the past decades, an alarming data has emerged concerning the ever-increasing percentage of MASLD patients who suffered of uncontrolled hypertension, which *per se* represents a negative predictor of CVD-related mortality (121, 122). Multiple factors have been recognized as major responsible behind this trend, including non-adherence to treatment guidelines, physician inertia, medication non-compliance, or limited health literacy (122), underlining the urgent need to integrate comprehensive hypertension screening and management strategies into the care protocols for MASLD patients.

6 Our novel findings regarding the prevalence of carotid plaques in HCC patients

In the attempt to provide further clinical evidence for the surveillance of HCC patients, we explored the relationship between the risk of hepatocarcinoma and cardiometabolic comorbidities in a large cohort of biopsied-proved MASLD patients (n=1538; Overall cohort). The Overall cohort was enrolled at the Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico of Milan, as previously described (11, 24).

The Overall cohort (n=1538) included n=1452 MASLD subjects with different stages of the disease (Hepatology service cohort) and n=86 MASLD-HCC individuals (Table 1). Histological evaluation was staged according to NAFLD activity score (NAS) by Kleiner et al. for steatosis, necroinflammation and ballooning degree.

TABLE 1 Demographic, anthropometric and clinical features of the Overall cohort (n=1538), stratified according to class enrollment criteria (n=1452, Hepatology service cohort and n=86, MASLD-HCC).

	Overall cohort (n=1538)	Hepatology service (n=1452)	MASLD-HCC (n=86)	*P* value*
Sex, M	808 (52.5)	742 (51)	66 (77)	**<0.0001**
Age, years	49 ± 0.34	48 ± 0.33	67 ± 1.42	**<0.0001**
BMI, kg/m2	34.5 ± 0.23	34.7 ± 0.23	28.8 ± 1.12	**<0.0001**
Obesity, yes	925 (60.1)	904 (62.3)	21 (24.4)	**<0.0001**
IFG/T2D, yes	402 (26)	351 (24.1)	51 (60)	**<0.0001**
Glucose, mg/dL	102.9 ± 0.8	101.6 ± 0.8	131.5 ± 3.8	**<0.0001**
Insulin, IU/ml	20.7 ± 0.8	20.6 ± 0.8	24.3 ± 5.9	0.35
HOMA-IR	5.4 ± 0.26	5.2 ± 0.25	11.04 ± 1.9	**<0.0001**
Total cholesterol, mmol/L	197.5 ± 1.14	198.8 ± 1.15	164.9 ± 5.7	**<0.0001**
LDL cholesterol, mmol/L	123.3 ± 1.03	124.3 ± 1.05	99.2 ± 5.2	**<0.0001**
HDL cholesterol, mmol/L	49.9 ± 0.4	49.9 ± 0.4	49.9 ± 1.99	0.98
Triglycerides, mmol/L	142 ± 2.4	143.5 ± 2.4	107.2 ± 12.1	**0.003**
Lp(a), nmol/L	32.5 ± 1.6	32.9 ± 1.7	24.7 ± 6.8	0.22
Carotid IMT, mm	0.83 ± 0.009	0.83 ± 0.009	0.93 ± 0.04	**0.01**
Statin, yes	148 (9.6)	137 (9.4)	11 (13.4)	0.39
ALT, IU/l	34 {21–57}	33 {20–57}	43 {27–54}	0.29
AST, IU/l	26 {19–39}	25 {19–38}	47 {27–72}	**<0.0001**
GGT, IU/l	41 {22–82}	39 {22–78}	84 {49–176}	**<0.0001**
LDH, U/l	188 {221–263}	184 {109–254}	247 {179–335}	**0.008**

Values are reported as mean ± SE, number (%) or median {IQR}, as appropriate. BMI, body mass index; IFG, impaired fasting glucose; T2D, type 2 diabetes; IMT, intima-media thickness. Variables with skewed distribution were logarithmically transformed before analyses. IFG defined as fasting glucose >110 mg/dL. Mean carotid artery intima-media thickness (IMT), an index of the early atherosclerotic process, was determined by high–resolution B-mode ultrasonography with a 7.5-MHz transducer. Values of IMT represent the mean IMT on the left and right sides. The presence of plaques was defined as a focal carotid thickening >1.2 mm. Systolic and diastolic blood pressures were measured twice on the same day, and the mean values were used for analysis. The presence of hypertension was defined when systolic blood pressure was over 140 mm Hg or diastolic blood pressure was over 90 mm Hg more than twice or in subjects treated with antihypertensive medication (80). *p<0.05 was considered statistically significant at two-way ANOVA (MASLD-HCC vs Hepatology service cohort). Bold values are those statistically significant.

MASH was diagnosed when (a) steatosis, (b) lobular inflammation and (c) ballooning were concomitantly present. Fibrosis stage was defined according to the recommendations of the NAFLD Clinical Research Network (123). Informed written consent was obtained from each patient and the study protocol was approved by the Ethical Committees of the Fondazione IRCCS Ca' Granda, Milan and it was in conformity with the ethical guidelines of the 1975 Declaration of Helsinki.

MASLD-HCC patients, were predominantly older men and overweight, exhibited higher circulating transaminase (AST and GGT) and lactate dehydrogenase (LDH) levels compared to those belonging to the Hepatology service cohort (p<0.001, Table 1).

Moreover, a reduced total cholesterol, LDL cholesterol and TGs together with higher fasting glucose concentration and HOMA-IR values were observed in MASLD-HCC patients compared to the Hepatology service cohort (p<0.001, Table 1). These findings were consistent with those of Cao J et al, who found a strong association between low-LDL cholesterol levels and HCC occurrence in a mendelian randomization analysis across 212,453 individuals. Similarly, this correlation was confirmed by Li M. et al. in a multicentric, prospective study including 137,884 participants, who even reported that patients with a poor glycemic control showed a higher risk of liver cancer (91, 92).

The incidence of cardiovascular complications was firstly analyzed in the Overall cohort, stratified according to histological liver damage. In the Overall cohort, n=389 cases (25.3%) had uncomplicated steatosis, n=280 (18.2%) presented MASH, n=783 patients (50.9%) had MASH plus fibrosis (referred to as fibrosis) and n=86 (5.6%) developed HCC. In keeping with the notable increase in carotid intima-media thickness (IMT) observed in MASLD-HCC patients compared to the Hepatology service cohort (p<0.05, Table 1), we showed that the frequency of carotid plaques was significantly higher MASLD-HCC subjects compared to those with a less severe disease (carotid plaques: 75% vs 30%, 33.9% and 43.5%; p=0.001 at Pearson correlation analysis; padj=0.01 after the adjustment for age Figure 1A), despite the lower circulating lipids detected in these patients (Table 1). Alongside this, a marked increase in the incidence of hypertension and T2D were found in MASLD-HCC individuals, and their frequency progressively increased with MASLD worsening. Specifically, hypertension was present in 76.5% of MASLD-HCC patients compared to 33.6%, 34.2%, and 46.8% in earlier stages of the disease (p<0.0001 at Pearson correlation analysis; padj=0.05 after the adjustment for age Figure 1B), while T2D reached the 58.8% in HCC cases *vs.* 13.6% in uncomplicated steatosis, 19.15% in MASH and 34.8% in fibrosis (p<0.0001 at Pearson correlation analysis; padj=0.001 after the adjustment for age Figure 1C), thereby suggesting that these comorbidities may track the course of MASLD, exerting a significant impact on carotid plaque formation.

It is well established that T2D, hypertension and cardiovascular diseases are closely interconnected together to the point that their coexistence in the same individual exacerbates endothelial dysfunction, vascular inflammation and fibrosis, arterial remodeling, and atherosclerosis (124). However, less is known regarding their link with the occurrence of liver cancer. Intriguingly, at nominal logistic regression analysis, adjusted for age, sex, BMI, T2D, and statin use, we revealed a strong correlation between the presence of carotid artery plaques and MASLD-HCC (OR:3.78, 95% CI: 1.16–13.84, p=0.033, Figure 2A). Additionally, hypertension (OR:2.79, 95% CI: 1.22–6.38, p=0.015, Figure 2B) resulted independently associated with MASLD-HCC risk at multivariate analysis after the adjustment for age, sex, BMI, T2D and active smoking. Again, T2D associated with more than six-fold increase of MASLD-HCC risk at nominal logistic regression analysis adjusted for age, sex and BMI (OR:3.01, 95% CI: 1.64–5.49, p=0.0003, Figure 2C), thus aligning with and strengthening previously reported studies (105, 125). Moreover, we further revealed that the combined prevalence of carotid plaques, hypertension, and T2D was substantially higher in MASLD-HCC patients (18.2% in MASLD-HCC *vs.* 4.2% in the Hepatology service cohort, Figure 2D). Therefore, in our cohort, the risk of liver cancer was significantly amplified by the co-occurrence of these comorbidities (OR:4.10, 95% CI: 1.56–10.68, p=0.004), compared to their presence alone.

In sum, our data identifies a paradox in MASLD-HCC patients who exhibited lower circulating lipid levels and higher incidence of plaque formation compared to individuals at different MASLD

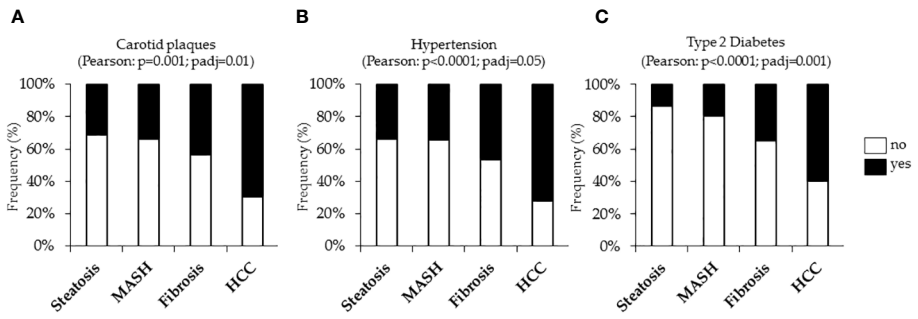

FIGURE 1
Cardiometabolic risk factors, including carotid plaques and hypertension and type 2 diabetes (T2D) were evaluated in the Overall cohort (n=1538) stratified according to the liver disease severity. Contingency analysis showed the frequency distribution of carotid plaques **(A)**, hypertension **(B)** and T2D **(C)** in the Overall cohort. p<0.05 was considered statistically significant. P values were further adjusted for age as confounding factor.

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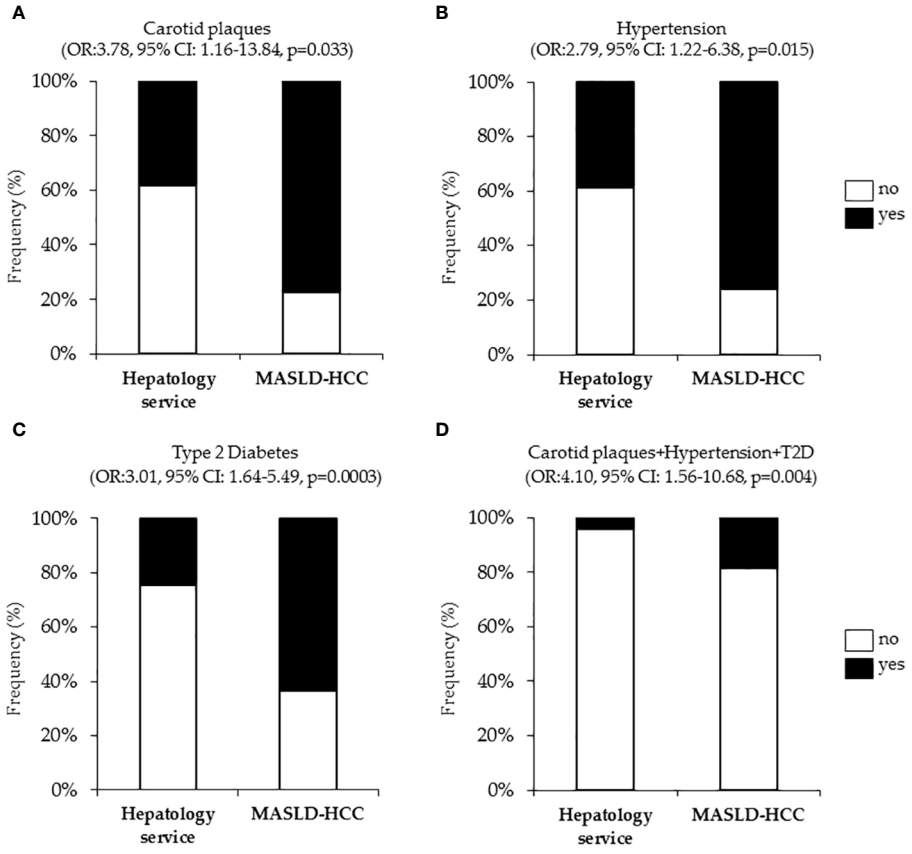

FIGURE 2
Association of cardiometabolic risk factors (carotid plaques and hypertension, T2D), with MASLD-HCC risk in the Overall cohort (n=1538). Nominal logistic regression models adjusted for confounding factors correlating MASLD-HCC occurrence with carotid plaques **(A)**, hypertension **(B)**, T2D **(C)** and cumulative presence of plaques, hypertension and T2D **(D)**. OR: odds ratio, p<0.05 was considered statistically significant.

histological stages. This discrepancy could stem from various factors. Firstly, among MASLD-HCC patients, those developing plaques exhibited higher serum total cholesterol (169 mmol/L *vs* 148 mmol/L), LDL (163 mmol/L *vs* 78 mmol/L), and TGs (117 mmol/L *vs* 99 mmol/L) concentrations compared to subjects with MASLD-HCC without plaques. This observation possibly suggests that MASLD-HCC patients developing plaques have a worsened lipid profile which may contribute to their heightened cardiovascular risk. Additionally, at the time of HCC diagnosis, many patients may be in a deteriorated state of health, often with significant weight loss, which inherently affects lipid levels. Moreover, the advanced liver dysfunction, characteristic of HCC, impacts the liver's capacity to produce lipoproteins and TGs, leading to altered circulating lipid profiles. Equally important is the prevalence of cardiometabolic conditions such as T2D, hypertension, insulin-resistance, and hyperglycemia in our MASLD-HCC cohort, which may predominantly contribute to plaque formation, possibly overshadowing the influence of lipids. Finally, we cannot rule out that genetic predispositions and the usage of cholesterol-lowering medications are likely to have significant effects on both the lipid metabolism and cardiovascular risk profiles. Collectively, our findings may contribute to the growing body of evidence linking cardiometabolic factors with HCC and highlight the necessity for comprehensive clinical evaluations in patients with MASLD.

7 Conclusion

Given the ever-increasing global incidence of HCC, effective strategies of prevention, surveillance and personalized therapeutic approaches are still mandatory. In particular, since around 40% of HCC cases are attributable to metabolic disorders, including MetS, T2D, obesity, and hyperlipidemia (126, 127), preventive approaches aimed to counteract the development of cardiometabolic risk factors may be useful in limiting the oncologic predisposing background. In patients with MASLD-driven HCC, we could postulate that the introduction of an integrated dietary regimen and more healthy lifestyles, including physical exercise may be useful to reduce the incidence of advanced stages of disease and HCC. However, conflicting results are currently available and further studies are required to better dissect the association between metabolic risk factors, cardiovascular diseases and HCC onset. To shed light on this steamy landscape, we carry out an observational study in our large biopsied MASLD cohort, including 86 MASLD-HCC. Our findings sustained the critical role of T2D

and hypertension in enhancing HCC risk and emphasized the pivotal contribution of cardiometabolic comorbidities, particularly when they coexist, on disease prognosis. Interestingly, while our data revealed that MASLD-HCC patients showed lower circulating lipid levels, a paradoxical increase in IMT thickness and plaque formation was observed. Nonetheless, within the MASLD-HCC subgroup, patients with plaques present a worse lipid profile compared to HCC subjects without plaques, potentially elevating their cardiovascular risk. Indeed, some population-based studies have supported our statements, pointing out that patients affected by HCC are more vulnerable to develop stroke/cardiovascular events than patients with other types of cancers (128–130). Therefore, these findings highlighted the complexity of lipid profiles in MASLD-HCC and the importance of a detailed assessment of cardiovascular risk, advocating for an integrated approach which considers the spectrum of both metabolic and cardiovascular factors.

Author contributions

MM: Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing. ML: Conceptualization, Investigation, Writing – original draft. PD: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by Italian Ministry of Health (Ricerca Corrente 2024 -Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico), by Italian Ministry of Health (Ricerca Finalizzata Ministero della Salute GR-2019-12370172; RF-2021-12374481) and by 5x1000 2020 -Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico (RC5100020B).

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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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 19 September 2024
DOI 10.3389/fendo.2024.1451383

OPEN ACCESS

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RECEIVED 19 June 2024
ACCEPTED 30 August 2024
PUBLISHED 19 September 2024

CITATION
Shen D, Cai X, Hu J, Song S, Zhu Q, Ma H, Zhang Y, Ma R, Zhou P, Yang W, Hong J, Zhang D and Li N (2024) Associating plasma aldosterone concentration with the prevalence of MAFLD in hypertensive patients: insights from a large-scale cross-sectional study.
Front. Endocrinol. 15:1451383.
doi: 10.3389/fendo.2024.1451383

Associating plasma aldosterone concentration with the prevalence of MAFLD in hypertensive patients: insights from a large-scale cross-sectional study

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Objective: To explore the link between plasma aldosterone concentration (PAC) and the prevalence of metabolic dysfunction-related fatty liver disease (MAFLD) in hypertensive patients.

Methods: We analyzed data from 41,131 hospitalized patients from January 1, 2014, to December 31, 2023. Multivariate logistic regression models tested associations, with threshold, subgroup, and sensitivity analyses conducted to validate findings.

Results: For each 5-unit increase in PAC, the risk of MAFLD rose by 1.57 times, consistent even in the fully adjusted model. The odds ratios for the Q2, Q3, and Q4 groups compared to Q1 were 1.21, 2.12, and 3.14, respectively. A threshold effect was observed at 14 ng/dL, with subgroup and sensitivity analyses supporting these results.

Conclusions: This study reveals a significant positive association between elevated PAC levels and the prevalence of MAFLD in hypertensive patients. These findings underscore the imperative for further large-scale, prospective studies to validate and expand upon this correlation.

KEYWORDS

plasma aldosterone concentration, metabolic-dysfunction-associated fatty liver disease, hypertension, cross-sectional study, risk factors

1 Introduction

Metabolic-dysfunction-associated fatty liver disease (MAFLD), a prevalent condition that affects approximately one-quarter of the global adult population, represents a significant health and economic burden across all societies, with a notable impact on the Asian demographic (1–4). In 2019, a consensus was reached by an international panel of experts who proposed the term "MAFLD" to more accurately encapsulate the condition, regardless of alcohol consumption or the presence of other concurrent liver pathologies. This nomenclature underscores the centrality of metabolic dysfunction in the etiology, clinical presentation, progression, and outcomes of hepatic steatosis (1, 5–7). MAFLD is recognized as the hepatic manifestation of a broader multisystem disorder, characterized by heterogeneity in its etiologies, manifestations, clinical course, and outcomes (8–10). Epidemiological data indicate that the prevalence of MAFLD in Asian countries varies from 10% to 30% and is on an ascending trend (11–15). Hypertension, a chronic condition with a substantial global incidence, is a well-established risk factor for cardiovascular diseases (CVD) (16–18). Moreover, MAFLD has been shown to intensify the progression of atherosclerosis and heighten the risk of cardiovascular events (19, 20). Emerging research has delineated a bidirectional relationship between MAFLD and hypertension, with evidence implicating MAFLD as both a consequence and a precipitant of hypertensive conditions (21). The co-occurrence of hypertension and MAFLD has been associated with more adverse cardiovascular outcomes than either condition in isolation (22). Therefore, early identification, management, and intervention for the combined burden of MAFLD and hypertension are of critical importance, with far-reaching implications for public health (23).

Previous research on MAFLD has primarily focused on factors such as insulin resistance, metabolic syndrome, genetic predisposition, excessive obesity, and lifestyle influences, while notably overlooking the impact of aldosterone (1, 5, 9, 24). Aldosterone, a steroid hormone produced by the adrenal zona glomerulosa, plays a vital role in regulating sodium and water balance in the body, which significantly affects blood pressure control (25–31). Numerous studies have identified the excessive secretion of aldosterone as a major risk factor for cardiovascular and kidney diseases, as well as metabolic disorders (26, 28, 29, 32, 33). Furthermore, recent research suggests that plasma aldosterone concentration (PAC) can influence liver metabolism (34, 35). For instance, studies have revealed a nonlinear relationship between elevated PAC levels and the incidence of non-alcoholic fatty liver disease (NAFLD) in patients with hypertension (36). Additionally, in animal models, aldosterone receptor blockers have been found to inhibit hepatic stellate cells and reduce liver fibrosis, indicating their potential effectiveness in treating fatty liver disease (37).

However, the relationship between PAC and MAFLD, a newer term for metabolic fatty liver disease, remains unexplored and unclear. This study aims to delineate the correlation between PAC and MAFLD, aspiring to provide innovative insights into the prevention and therapeutic management of metabolic fat deposition, particularly within the hypertensive patient population.

2 Material and methods

2.1 Study population

Between January 1, 2014, and December 31, 2023, a total of 41131 hospitalized patients were included in the study. Exclusion criteria were applied to participants under the age of 18 and those with incomplete data on PAC or insufficient information for the diagnosis of MAFLD. To mitigate the potential confounding effects of certain conditions and medications on study outcomes, we meticulously excluded individuals with positive serology for hepatitis B, C, or Delta viruses, autoimmune hepatitis, cirrhosis, history of liver resection, liver cancer, or gastrointestinal surgery. Furthermore, participants with a diagnosis of endocrine hypertension, severe thyroid disorders, chronic use of mineralocorticoid receptor antagonists, recent severe cardiovascular or cerebrovascular events, significant hepatic or renal impairment, or malignancies diagnosed within the preceding three months were also excluded. Individuals with a history of heavy alcohol consumption were additionally excluded to account for the impact on liver metabolism. After these exclusions, 35159 participants were included in the final analysis (Figure 1). Informed consent was obtained in writing from all patients or their legal guardians, and the study was approved by the hospital's ethics committee. Adherence to the STROBE guidelines was ensured in the reporting of this research (38).

2.2 Data collection and definitions

Data including clinical information, test findings, lifestyle variables, medical history, and medication history were obtained from the electronic medical record as baseline. Clinical data at admission included age, sex, height, weight, body mass index (BMI), systolic, diastolic, and waist circumference (WC). Smoking and alcohol drinking were classified as current or non-current. For specific measurement methods, please refer to the Supplementary Material.

Peripheral venous blood was collected after an 8–10 hour fast to measure serum potassium, serum sodium, platelets (PLT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gammaglutamyl transferase (GGT), serum creatinine (Scr), uric acid(UA), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol(LDL-C), HbA1c,high sensitivity C-reactive protein (hs-CRP), andtriglyceride–glucose (TyG) index. The above blood biochemical indicators were detected by Automatic Analyzer (7600-010, Hitachi, Tokyo, Japan) according to the manufactures instruction. Thehexokinase/glucose-6-phosphate dehydrogenase method was used to measure FBG levels, while TG levels were measured using the enzymatic colorimetric method. Before calculation, the units of TG and FBG were converted from mmol/L to mg/dL (For TG, 1 mmol/l = 88.57 mg/dl; For FBG, 1 mmol/l = 18 mg/dL), and the TyG index was then calculated as ln [TG (mg/dL) × FBG (mg/dL)/2]. Hormone measurements are based on current guidelines and our previous studies. The PAC was measured by radioimmunoassay (DSL-8600; DSL, Webster, TX)

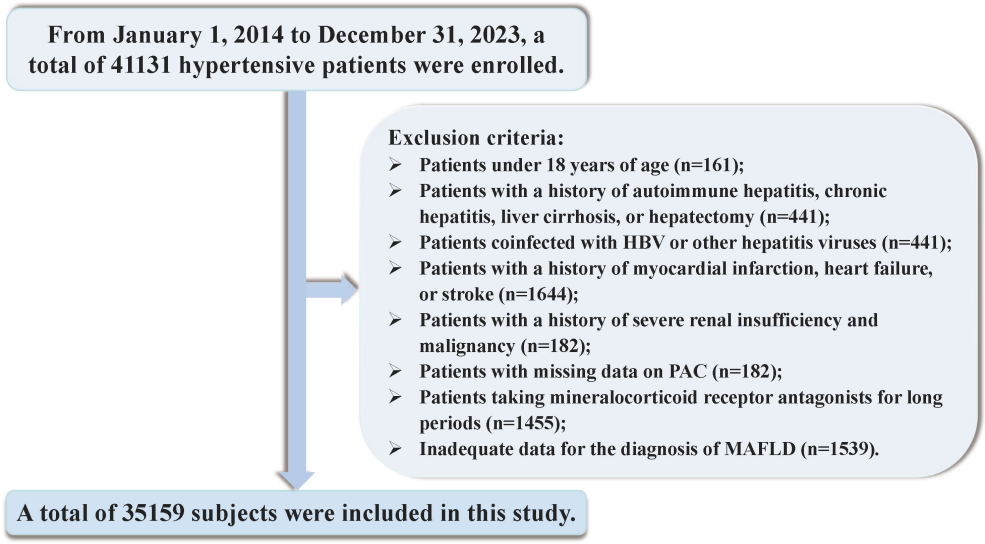

FIGURE 1
Flowchart of the study.

(29, 36, 39). Please refer to the Supplementary Material for detailed definitions of the diseases.

2.3 Outcome

Hepatic steatosis with evidence of metabolic dysfunction defines MAFLD (11). Metabolic dysfunction was defined as satisfying one of the following three conditions: overweight or obese (BMI ≥ 23 kg/m^2); type 2 diabetes mellitus (T2DM); metabolic abnormality score ≥ 2 (WC ≥ 90 cm in men and ≥ 80 cm in women; blood pressure ≥ 130/85 mmHg or use of antihypertensives; TG ≥ 150 mg/dL or use of antidyslipidemics; HDL-C < 40 mg/dL in men and < 50 mg/dL in women or use of antidyslipidemics; FBG 5.6-6.9 mmol/L; hs-CRP > 2 mg/L; homeostasis model assessment of insulin resistance score [HOMA-IR] ≥ 2.5). For lack of information on HOMA-IR, we used the TyG index over the 75th percentile as an alternative to the HOMA-IR diagnostic criteria (40, 41).

2.4 Statistical analysis

Multicollinearity was assessed using the variance inflation factortest (Supplementary Table S1). The relationship between PAC levels and MAFLD was analyzed using a multivariate logistic regression model, and the odds ratio (OR) was calculated. Additionally, we used restricted cubic splines (RCS) to evaluate the dose-response relationship and conducted a two-stage comparative analysis based on the inflection points of the RCS curve. Additionally, subgroup analyses were conducted to ascertain the influence of PAC on MAFLD across a spectrum of stratifying variables. Several extra sensitivity analyses were performed to assess the reliability of the results. Supplementary Materials provide details on the statistical analysis.

The statistical analysis was executed using R software, version 4.1.1. Significance was defined as a p-value of less than 0.05, employing a two-tailed test for statistical inference.

3 Results

3.1 Baseline characteristics

Based on the quartiles of PAC, baseline characteristics of each group are presented in Table 1. A total of 35159 patients were included, among which 20078 (57.11%) were male. The high PAC group was younger, comprised more females, and had a higher BMI. In terms of test indicators, Scr, WC, and TyG indexes were significantly higher in the high PAC group compared to the low PAC group. Moreover, the high PAC group were more likely to be taking diuretics and calcium channel blockers (CCB) (Table 1). The most significant discovery was that the prevalence of MAFLD appeared to rise as PAC increased (Figure 2). Furthermore, after dichotomizing according to the presence or absence of MAFLD, there were significant differences in PAC, age, TyG index, and smoking history between the two groups (Supplementary Table S2).

3.2 Relationship between PAC and the prevalence of MAFLD

Our research has found a close association between PAC and MAFLD. In the original model, for every 5-unit increase in PAC, the risk of developing MAFLD increases by 1.57 times. This relationship remains reliable in the fully adjusted model. Compared to the Q1 group, the OR values for the Q2, Q3, and

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TABLE 1 Baseline characteristics according to quartiles of PAC.

Variables	Q1 (<11.83ng/dL)	Q2 (11.83-14.08ng/dL)	Q3 (14.08-18.65ng/dL)	Q4 (>18.65ng/dL)	P-value
Sample size, n	8790	8790	8790	8789	
Demography					
Age, years	52.05 ± 12.58	51.40 ± 11.48	50.54 ± 12.07	49.50 ± 12.03	<0.001*
Sex, %					0.037*
Women	3705 (42.15%)	3790 (43.12%)	3714 (42.25%)	3872 (44.06%)	
Men	5085 (57.85%)	5000 (56.88%)	5076 (57.75%)	4917 (55.94%)	
BMI, kg/m^2	26.86 ± 3.68	26.93 ± 3.66	26.95 ± 3.61	26.98 ± 3.60	0.163
WC, cm	95.63 ± 11.34	95.84 ± 11.25	95.91 ± 11.19	96.02 ± 11.13	0.128
SBP, mmHg	146.13 ± 18.39	146.07 ± 18.20	145.70 ± 18.24	146.17 ± 18.34	0.31
DBP, mmHg	88.34 ± 13.67	88.14 ± 13.56	87.82 ± 13.48	88.14 ± 13.67	0.094
Current smoking, %	3115 (35.44%)	2991 (34.03%)	2959 (33.66%)	2647 (30.12%)	<0.001*
Current drinking, %	2769 (31.50%)	2762 (31.42%)	2764 (31.44%)	2553 (29.05%)	<0.001*
Biochemical indexes					
Serum potassium (mmol/L)	4.07 ± 0.29	3.98 ± 0.27	3.83 ± 0.31	3.63 ± 0.28	<0.001*
Serum sodium (mmol/L)	141.11 ± 2.55	141.11 ± 2.45	141.03 ± 2.49	141.04 ± 2.58	0.089
PLT, 10^9/L	242.29 ± 58.68	243.15 ± 58.15	242.90 ± 57.83	240.01 ± 57.66	0.001*
ALT, U/L	27.36 ± 17.54	27.17 ± 17.54	27.36 ± 17.60	27.25 ± 17.42	0.871
AST, U/L	21.10 ± 8.24	21.00 ± 8.16	21.08 ± 8.17	21.05 ± 8.31	0.877
GGT, U/L	36.25 ± 25.77	35.53 ± 24.80	35.86 ± 25.17	36.09 ± 25.30	0.261
Scr, μmol/L	64.93 ± 14.46	65.03 ± 14.32	65.05 ± 14.37	65.43 ± 14.48	0.101
BUN, mmol/L	5.05 ± 1.36	5.04 ± 1.35	5.05 ± 1.36	5.08 ± 1.36	0.325
UA, umol/L	343.61 ± 91.51	342.97 ± 90.57	343.16 ± 90.80	345.74 ± 91.23	0.161
Total cholesterol, mmol/L	4.55 ± 0.99	4.51 ± 0.97	4.55 ± 0.98	4.53 ± 0.97	0.032*
Triglyceride, mmol/L	1.82 ± 1.04	1.80 ± 1.01	1.81 ± 1.03	1.82 ± 1.06	0.439
HDL-C, mmol/L	1.06 ± 0.25	1.05 ± 0.24	1.06 ± 0.25	1.06 ± 0.25	0.039*
LDL-C, mmol/L	2.73 ± 0.83	2.74 ± 0.82	2.78 ± 0.82	2.77 ± 0.82	<0.001*
FBG, mmol/L	5.05 ± 1.07	5.03 ± 1.04	5.00 ± 1.02	5.03 ± 1.05	0.032*
HbA1c, %	5.99 ± 0.82	5.95 ± 0.79	5.91 ± 0.75	5.89 ± 0.79	<0.001*
hs-CRP, mg/dL	3.40 ± 3.01	3.58 ± 3.12	3.50 ± 3.03	3.54 ± 3.13	<0.001*
TyG index	6.53 ± 0.52	6.89 ± 0.11	7.27 ± 0.12	7.98 ± 0.47	<0.001*
Previous history					
T2DM, %	1524 (17.34%)	1384 (15.75%)	1324 (15.06%)	1406 (16.00%)	<0.001*
Dyslipidemia, %	1676 (19.07%)	1682 (19.14%)	1767 (20.10%)	1540 (17.52%)	<0.001*
CAD, %	944 (10.74%)	808 (9.19%)	746 (8.49%)	745 (8.48%)	<0.001*

(Continued)

TABLE 1 Continued

Variables	Q1 (<11.83ng/dL)	Q2 (11.83-14.08ng/dL)	Q3 (14.08-18.65ng/dL)	Q4 (>18.65ng/dL)	P-value
Medications use					
ACEI/ARB, %	4235 (48.18%)	4016 (45.69%)	3942 (44.85%)	4034 (45.90%)	<0.001*
Diuretic, %	905 (10.30%)	893 (10.16%)	937 (10.66%)	1052 (11.97%)	<0.001*
CCB, %	2021 (22.99%)	2069 (23.54%)	2228 (25.35%)	2665 (30.32%)	<0.001*
β-blockers, %	1688 (19.20%)	1542 (17.54%)	1489 (16.94%)	1505 (17.12%)	<0.001*
Antidiabetic agents, %	758 (8.62%)	676 (7.69%)	570 (6.48%)	612 (6.96%)	<0.001*
Lipid-lowering drugs, %	1139 (12.96%)	1050 (11.95%)	980 (11.15%)	928 (10.56%)	<0.001*

Data are mean (standard deviation), n (%), or median (interquartile range).
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; PLT, platelets; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; Scr, serumcreatinine; BUN, blood urea nitrogen; UA, uric acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasing blood glucose; HbA1c, glycosylated hemoglobin; hs-CRP, high sensitivity C-reactive protein; TyG index, triglyceride glucose index; DM, diabetes mellitus; CAD, coronary artery disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blockers.
*Significant P value ($P < 0.05$).

Q4 groups are 1.21, 2.12, and 3.14 respectively, showing an increasing trend (Table 2). We further utilized the RCS model to identify the nonlinear dose-response relationship between PAC levels and the prevalence of MAFLD (p for nonlinear < 0.001) (Figure 3). Furthermore, we conducted a two-stage comparative analysis based on the inflection point of RCS. The results indicate that the risk of developing MAFLD for individuals with a PAC level greater than 14ng/dL is 2.32 times higher than that of individuals with a level less than 14ng/dL (Table 3).

3.3 Subgroup analysis

After stratifying the data based on basic conditions and diseases, the results remained stable across the population, with no interactions observed (Figure 4). In addition, a subgroup analysis examined the potential impact of antihypertensive drugs on outcomes, which also showed no significant changes (Supplementary Figure S1). This indicates that our findings are not affected by these stratification factors and that PAC can predict the occurrence of MAFLD regardless of stratification. In order to eliminate any bias caused by missing data, we conducted sensitivity analyses after excluding patients with missing values and obtained essentially the same results (Supplementary Table S3). Furthermore, to mitigate the impact of alcohol abuse on the results, we excluded patients with a history of alcohol abuse and the results remained consistent (Supplementary Table S4). Additionally, to eliminate the influence of severe liver fibrosis on aldosterone inactivation, we excluded patients with severe liver fibrosis. The correlation between PAC and the prevalence of MAFLD remained largely unchanged (Supplementary Tables S5, S6). Additionally, to assess the influence of unmeasured confounders, we conducted an E-value analysis, which indicated that the impact of confounding factors was minimal and the likelihood of our results being overturned was low (Supplementary Table S7 and Supplementary Figure S2).

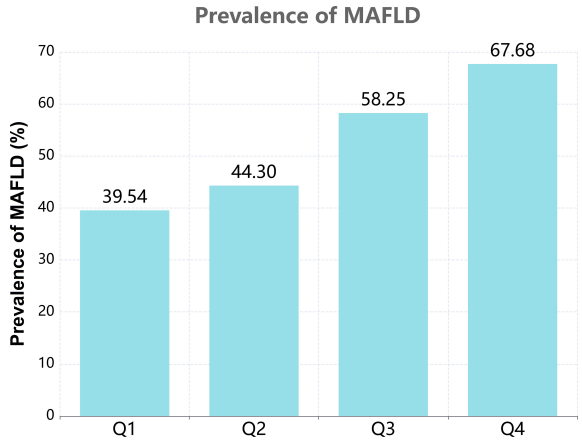

FIGURE 2
The prevalence of MAFLD according to the quartiles of PAC.

4 Discussion

Our study has, for the first time, uncovered the relationship between PAC levels and the prevalence of MAFLD in hypertensive patients, revealing an independent association between elevated PAC and an increased incidence of MAFLD. Our findings indicate that individuals with PAC levels exceeding 14 ng/dL exhibit a 2.32-fold heightened risk for the development of MAFLD compared to those with levels below this threshold. This suggests that maintaining PAC within a reasonable range may offer a new direction for preventing MAFLD in the future.

Aldosterone, a crucial mineralocorticoid hormone, plays an essential role in regulating the body's water and electrolyte balance (42–45). While its excess is known to indicate hypertension and organ damage, its connection to liver steatosis remains underexplored. Fallo and colleagues highlight that patients with primary aldosteronism are more prone to insulin resistance and have a higher prevalence of NAFLD, suggesting an increased risk of metabolic and liver diseases in this subgroup (46).

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TABLE 2 The relationship between PAC and the prevalence of MAFLD in hypertensive patients.

Exposure	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4 OR (95% CI)	Model 5 OR (95% CI)
PAC (per 5 ng/dl increase)	1.57 (1.54, 1.61)	1.57 (1.54, 1.60)	1.57 (1.54, 1.60)	1.56 (1.53, 1.59)	1.56 (1.53, 1.60)
PAC quartiles					
Q1 (<11.83)	Reference	Reference	Reference	Reference	Reference
Q2 (11.83-14.08)	1.21 (1.14, 1.29)	1.21 (1.14, 1.29)	1.21 (1.14, 1.29)	1.21 (1.14, 1.28)	1.21 (1.14, 1.29)
Q3 (14.08-18.65)	2.13 (2.00, 2.26)	2.13 (2.00, 2.26)	2.13 (2.00, 2.26)	2.11 (1.99, 2.25)	2.12 (1.99, 2.25)
Q4 (>18.65)	3.20 (3.01, 3.40)	3.19 (3.00, 3.39)	3.19 (2.99, 3.39)	3.12 (2.93, 3.32)	3.14 (2.95, 3.35)
P for trend	<0.001	<0.001	<0.001	<0.001	<0.001

Model 1: crude model.
Model 2: adjusted for age, sex, smoking status, alcohol consumption, BMI, SBP, and DBP.
Model 3: adjusted for variables in Model 2 plus DM, dyslipidemia, CAD.
Model 4: adjusted for variables in Model 3 plus Serum potassium, Serum sodium, PLT, ALT, AST, GGT, Scr, BUN, UA, Total cholesterol, Triglyceride, HDL-C, LDL-C, FBG, HbA1c, hs-CRP, TyG index.
Model 5: adjusted for variables in Model 4 plus ACEI/ARB, diuretic, CCB, β-blockers, antidiabetic agents, lipid-lowering drugs.
SD, standard deviation; OR, Odds ratio. Other abbreviations, see Table 1.

Additionally, a large cohort study observed that angiotensin-converting enzyme inhibitors are linked to a reduced risk of adverse liver events in liver steatosis patients (47). Spironolactone, an aldosterone receptor antagonist, has shown beneficial effects on serum insulin and HOMA-IR in NAFLD patients, according to animal studies (48). Further supporting this, the Jackson Heart Study, involving 2,507 participants, found a positive correlation between aldosterone levels and fatty liver in African American women (49). Research by Srinivasa et al. indicates that elevated aldosterone could be a risk factor for liver fat accumulation in HIV-infected individuals (50). A recent study shows that in hypertensive patients, the risk of developing new-onset NAFLD significantly increases when PAC levels are ≥13 ng/dL (36). This research overcomes previous studies' limitations, such as animal reliance, limited populations, small samples, and inadequate variable adjustments, providing a more comprehensive analysis.

The specific mechanisms by which PAC leads to the development of MAFLD remain unclear and may involve several potential pathways. First, an excess of aldosterone can enhance oxidative stress and inflammation, which could potentially lead to liver damage and the progression of fatty liver disease (34, 51–54). Second, an overabundance of aldosterone can also cause a decrease in adiponectin levels in the bloodstream, and a corresponding reduction in its expression in visceral adipose tissue. This hormone, known as adiponectin, plays a crucial regulatory role in fat storage and reducing insulin resistance (30, 55–57). Third, aldosterone can trigger a direct sequence of events leading to the activation of hepatic stellate cells (HSC) and eventually liver fibrosis, primarily by inducing the activation of the NLRP3 inflammasome (58). Finally, more recent research has shed light on the fact that aldosterone can be locally produced during the process of liver fibrinogenesis, thereby contributing to organ fibrosis (31, 35, 59). To counter these effects, the therapeutic efficacy of aldosterone antagonists has been recognized. Existing studies have reliably shown that certain aldosterone antagonists, notably spironolactone and eplerenone, can diminish the symptoms of fatty liver and liver fibrosis (33, 58, 60, 61). This significantly underlines the critical role that aldosterone plays in the pathophysiology of fatty liver disease.

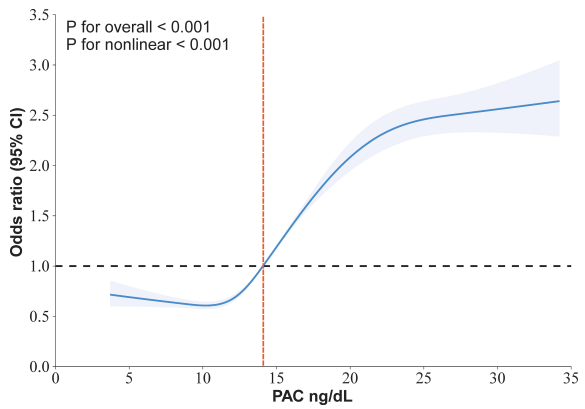

FIGURE 3
Restricted cubic spline for the association between PAC and the prevalence of MAFLD in in hypertensive patients.

Our study's strengths lie in the large sample size, strict exclusion criteria, and the first-time revelation of the relationship between PAC and MAFLD. We utilized multiple statistical methods to further validate the reliability of our research findings. These groundbreaking results may also offer new insights into the early identification and intervention of MAFLD in hypertensive patients. However, while considering these advantages, we must also acknowledge that our study may have some limitations. Firstly, our study is cross-sectional in design, which prevents us from establishing a causal relationship between PAC and MAFLD. Secondly, we did not take into account the potential influence of confounding factors such as dietary habits and level of physical activity, so we conducted an E-value analysis. The results indicate that the likelihood of our findings being overturned is very low. Thirdly, rather than using liver biopsy, abdominal ultrasound was

10.3389/fendo.2024.1451383

TABLE 3 Analysis of the prevalence of MAFLD Based on RCS Turning Point.

Exposure	Model 1 OR (95% CI),	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4 OR (95% CI)	Model 5 OR (95% CI)
PAC					
<14 ng/dL	Reference	Reference	Reference	Reference	Reference
≥ 14 ng/dL	2.35 (2.25, 2.45)	2.34 (2.24, 2.44)	2.34 (2.24, 2.44)	2.31 (2.22, 2.41)	2.32 (2.22, 2.42)

Model 1: crude model.
Model 2: adjusted for age, sex, smoking status, alcohol consumption, BMI, SBP, and DBP.
Model 3: adjusted for variables in Model 2 plus DM, dyslipidemia, CAD.
Model 4: adjusted for variables in Model 3 plus Serum potassium, Serum sodium, PLT, ALT, AST, GGT, Scr, BUN, UA, Total cholesterol, Triglyceride, HDL-C, LDL-C, FBG, HbA1c, hs-CRP, TyG index.
Model 5: adjusted for variables in Model 4 plus ACEI/ARB, diuretic, CCB, β-blockers, antidiabetic agents, lipid-lowering drugs.
SD, standard deviation; OR, odds ratio; CI, confidence interval. Other abbreviations, see Table 1.

used to diagnose fatty liver. Ultrasound, however, has good accuracy in non-invasive detection of fatty liver, making it widely used in clinical practice and epidemiological studies. Fourthly, our study was limited to hypertensive patients in China, and the generalizability of the conclusions may be affected, necessitating further research in a more diverse and broader patient population to validate our findings.

5 Conclusion

This study revealed a groundbreaking positive relationship between PAC and the prevalence of MAFLD, particularly with a significant increase in the risk of developing MAFLD when PAC exceeds 14ng/dL. This further suggests that maintaining PAC at a reasonable level may be beneficial in preventing the occurrence of

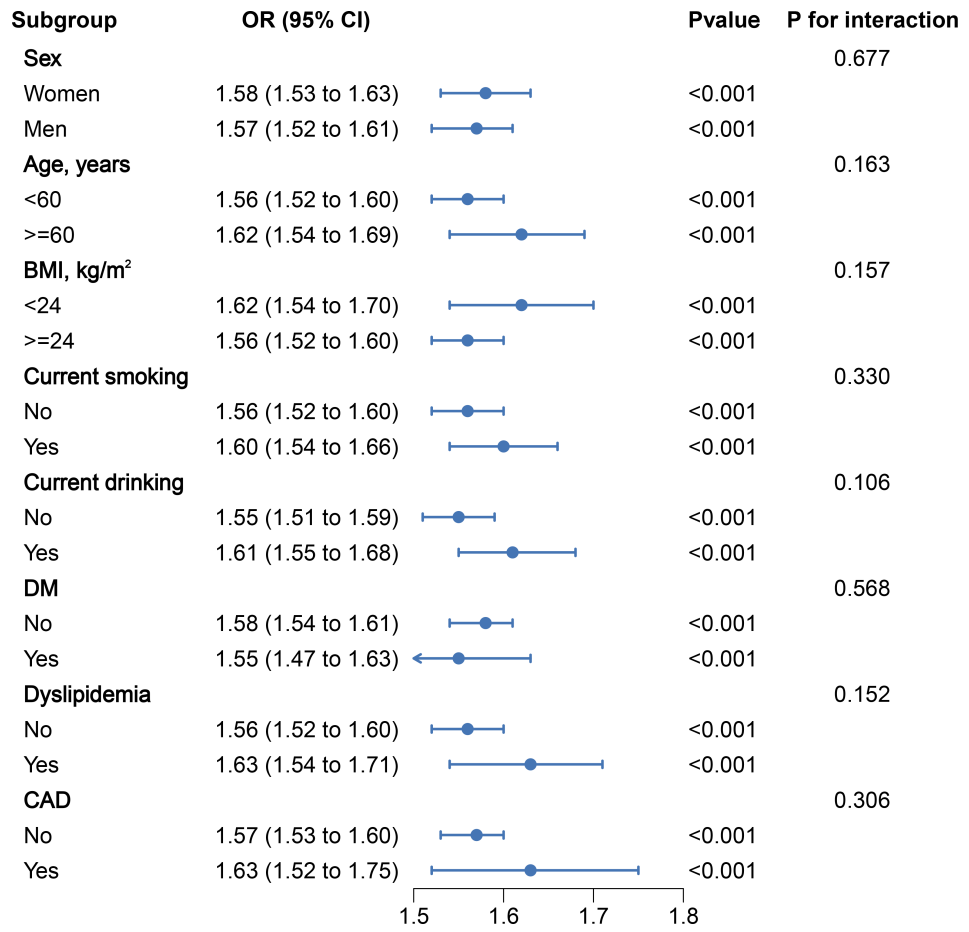

FIGURE 4
Stratified analyses of the association between PAC (per 5 ng/dL increment) and the prevalence of MAFLDin hypertensive patients.

MAFLD in hypertensive patients. However, to validate and confirm these findings, it is necessary to conduct more large-scale prospective studies in the future.

Data availability statement

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

Author contributions

DS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. XC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JLH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Validation, Writing – review & editing. SS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Writing – review & editing. QZ: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Software, Validation, Writing – review & editing. HM: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Validation, Writing – review & editing. YZ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – review & editing. RM: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing. PZ: Data curation, Investigation, Methodology, Project administration, Validation, Visualization, Writing – review & editing. WY: Data curation, Investigation, Methodology, Project administration, Validation, Visualization, Writing – review & editing. JH: Conceptualization, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing. DZ: Conceptualization, Data curation, Investigation, Project administration, Validation, Visualization, Writing – review & editing. NL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was provided by the Major Science and Technology Projects of the Xinjiang Uygur Autonomous Region (2022A03012-4).

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1451383/full#supplementary-material

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TYPE Original Research
PUBLISHED 25 September 2024
DOI 10.3389/fmed.2024.1425145

OPEN ACCESS

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RECEIVED 29 April 2024
ACCEPTED 04 September 2024
PUBLISHED 25 September 2024

CITATION
Alkaabi J, Afandi B, Alhaj O, Kanwal D and Agha A (2024) Identifying metabolic dysfunction-associated steatotic liver disease in patients with type 2 diabetes mellitus using clinic-based prediction tools.
Front. Med. 11:1425145.
doi: 10.3389/fmed.2024.1425145

Identifying metabolic dysfunction-associated steatotic liver disease in patients with type 2 diabetes mellitus using clinic-based prediction tools

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is a global cause of chronic liver disease. The prevalence of MASLD is high in patients with type 2 diabetes mellitus (T2DM). Various non-invasive tools such as the fibrosis-4 index (FIB-4) and NAFLD fibrosis score (NFS), liver ultrasound, and FibroScan can aid in the detection of liver fibrosis in MASLD, while the Hamaguchi ultrasound-based liver grading system has demonstrated high sensitivity and specificity comparable to liver biopsy.

Objective: We assessed the frequency of MASLD in patients with T2DM using the liver ultrasound Hamaguchi score and the accuracy of NFS and Fib-4 in identifying MASLD.

Patients and methods: We retrospectively collected data and reviewed the charts of all patients with T2DM who underwent liver ultrasound and laboratory tests during the past 5 years.

Results: A total of 6,214 medical records were screened, and only 153 patients (68.6% women; mean age, 59 ± 12.2 years) fulfilled the selection criteria. MASLD was diagnosed using the Hamaguchi grading criteria in 45.1% of patients. A high/intermediate NFS had a higher sensitivity (79.7%) for diagnosing MASLD with a specificity of 10.7%, while a high/intermediate Fib-4 score showed only 30.4% sensitivity but a higher specificity of 54.8%.

Conclusion: Our study indicates that MASLD is frequent in patients with T2DM, and clinical prediction tools such as NFS and Fib-4 can be applied in clinic/primary care settings with variable results.

KEYWORDS

metabolic dysfunction-associated steatotic liver disease, diabetes mellitus, Hamaguchi criteria, NAFLD fibrosis score, fibrosis-4 index

1 Introduction

NAFLD is described as an infiltration of the liver by fat deposits, which is not related to commonly identifiable causes of hepatic steatosis (e.g., alcohol, viral, secondary to drugs, or autoimmune processes) and comprises a wide array of presentations ranging from fat accumulation (steatosis), fat accumulation with inflammation (steatohepatitis), also called non-alcoholic steatohepatitis (NASH), and architectural changes (fibrosis), which may lead to liver damage and shrinkage (cirrhosis); and is currently the commonest cause of chronic liver disease globally with a prevalence of 30% (1). Though most of the patients having NAFLD may not fully progress to NASH cirrhosis, the sheer volume of patients with NAFLD who still end up developing cirrhosis is still quite high and has now become the leading indication for patients undergoing liver transplantation in the West (2). The prevalence data for NAFLD worldwide suggest that some regions, such as the Middle-East region, have one of the highest rates of NAFLD being an indication for liver transplantation, up to 32%; however, there have been no large-scale specific studies conducted in the United Arab Emirates (3). T2DM is one of the most important risk factors associated with NAFLD, with a recent systematic review indicating the worldwide prevalence of NAFLD is 50–70% in patients having T2DM, with the highest prevalence seen in European countries, but again, there are no data available for our local Emirati population (4). In 2023, Delphi consensus redefined NAFLD and NASH and introduced an overarching term of steatotic liver disease that includes metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as NAFLD, metabolic dysfunction-associated steatohepatitis (MASH), formerly known as NASH, and metabolic and alcohol-related/associated liver disease (MetALD) to represent a separate group of patients with MASLD that consumes alcohol (140–350 g/week for women and 210–420 g/week for men) (5). MASLD was redefined and defined as hepatic steatosis identified by imaging or biopsy along with the presence of at least one cardiometabolic criteria, i.e., either overweight (raised body mass index (BMI) or increased waist circumference), glucose intolerance (T2DM or pre-diabetes or impaired fasting glucose), hypertension, low high-density lipoprotein, or raised triglycerides (5). Recent Delphi consensus on the nomenclature of steatotic liver disease suggested only the new terms, such as metabolic dysfunction-associated steatotic liver disease (MASLD) and MASH, be used to describe the current International Classification of Diseases Manual-10 codes of NAFLD and NASH, respectively (6). The gold standard for diagnosing MASLD and MASH is the histological evaluation after performing a liver biopsy (gold standard). However, this is an invasive procedure with a subsequent risk of complications (7). Other non-invasive methodologies for identifying MASLD include vibration-controlled transient-elastography (VCTE), which uses FibroScan to provide the measurement of the liver stiffness (LSM) expressed as kilopascals (kPa), and this can correlate accurately with advanced stages of liver fibrosis such as stage F3 (bridging fibrosis) and stage F4 (advanced scarring or cirrhosis), making it an alternative to the liver biopsy (8). Further non-invasive means to identify the MASLD include clinical/biochemical scoring systems such as FIB-4, NFS, enhanced liver fibrosis panel, Hepascore, alanine aminotransferase (ALT), aspartate aminotransferase (AST) to platelet ratio index (APRI), and imaging techniques such as VCTE, liver CT scan, and liver magnetic resonance elastography (MRE) (9). There are no current unanimously agreed-upon screening guidelines for MASLD in all patients with diabetes mellitus or risk factors. The American Association for the Study of Liver Disease recommends the Fib-4 score, whereas the European Association for the Study of Liver Disease recommends screening with blood tests, including ALT, NFS, and/or Fib-4, among others, for high-risk patients (10, 11).

Imaging modalities such as ultrasonography of the liver may be used for the evaluation of hepatic fat infiltration, and although it is a safe, low-cost, and non-invasive procedure that is most frequently utilized in clinical settings for other indications, its accuracy for liver fat quantification has not been established consistently (12). Hamaguchi et al. proposed an ultrasound liver grading system that scores based on hepatorenal echogenicity and liver brightness (score of 0–3), deep attenuation (score 0–2), and liver vessel blurring (score 0–1). This system enhances sensitivity and specificity for identifying MASLD (with liver steatosis >10%) to 97 and 100%, respectively, at a score ≥ 2, compared to liver biopsy (13). The Hamaguchi grading system also reduced the operator-dependent inter-observer variation with accuracy similar to liver computed tomography (13, 14).

NFS has been advocated as a non-invasive predictor of NAFLD fibrosis, which utilizes six clinical/biochemical variables, namely age, BMI, impaired fasting glucose or diabetes, platelet count, AST/ALT ratio, and albumin. NFS of 0.676 or more has been shown to have 67% sensitivity and 97% specificity to predict MASLD with an area under receiver operating curve (AUROC) of 0.85 in a recent large meta-analysis (15). A recent study compared various non-imaging predictive scores and imaging tools, such as MRE and VCTE, to liver histology for accurately diagnosing MASLD fibrosis. The results showed that NFS outperformed APRI, BARD, and the AST/ALT ratio, and was equally effective as Fib-4 and MRE in identifying liver fibrosis in patients with liver biopsy-proven MASLD (16). Current worldwide guidelines mostly recommend either Fib-4 or NFS as prediction tools for identifying patients with MASLD, but there is no consensus on which one is better (17).

Our study aimed to assess the frequency of metabolic dysfunction-associated steatotic disease (MASLD) in patients with T2DM in our local population using a liver ultrasound Hamaguchi score of ≥ 2 (which has comparable accuracy to liver biopsy), as well as to compare the performance of clinic-based non-invasive predictors, NFS and Fib-4, in identifying MASLD.

2 Patients and methods

This was a retrospective study design, approved by the local ethical committee (Approval Number. MF2058-2022-855), and it included all adult (aged 18 and above) patients of either gender, with T2DM, who had attended the diabetes clinic at Tawam Hospital from January 2017 to December 2021 and had undergone liver ultrasound and liver function tests (within 3 months of each other) during this period for any clinical indication other than MASLD. The patients with alcohol intake history (past or present), any evidence of existing hepatobiliary disease (biliary tract obstruction; hepatoma, liver cirrhosis secondary to infection or immune, or congenital), or having secondary diabetes (e.g., diabetes following pancreatitis) or type 1 diabetes mellitus were excluded. After reviewing over 6,214 medical records, 153 patients were identified, and their demographic details, clinical parameters, and biochemical test results, including liver

function tests, albumin, bilirubin, glycated hemoglobin (HbA1c), prothrombin time, urea, and creatinine, were collected and recorded on a Microsoft Excel™ sheet. The ultrasound reports and images were reviewed for the visual estimation of fatty liver/MASLD, and these images were then reviewed by an additional expert ultrasound specialist radiologist. The Hamaguchi score for MASLD was calculated. A score of 2 or higher was considered diagnostic for the presence of MASLD. The Hamaguchi ultrasound liver grading system is based on the sum of points scored on three separate subsets (13). The first subset evaluates hepatorenal echogenicity and liver brightness: a score of 0 indicates the absence of bright liver and hepatorenal echo contrast, a score of 1 indicates the presence of either bright liver or hepatorenal echo contrast, a score of 2 indicates mild bright liver and positive hepatorenal echo contrast, and a score of 3 indicates severe bright liver and positive hepatorenal echo contrast. The second subset evaluates deep attenuation: a score of 0 is given for negative deep attenuation, a score of 1 for obscure diaphragm visualization, and a score of 2 for indistinguishable diaphragm. The third subset evaluates liver vessel blurring: a score of 0 is given for negative vessel blurring and a score of 1 is given when intrahepatic vessels are unclear and/or have a narrowed lumen.

The NFS was calculated based on the available formula from the website (http://gihep.com/calculators/hepatology/nafld-fibrosis-score/). Fib-4 and NFS were calculated, and the ability of these screening tests (low risk versus indeterminate or intermediate/high risk of significant fibrosis) to diagnose fatty liver disease was compared to that of the Hamaguchi scoring criteria. The cutoff values for Fib-4 and NFS as being abnormal were taken as ≥ 1.45 and ≥ -1.455, respectively. The sensitivity and specificity of Fib-4 and NFS for predicting a low risk of MASLD in patients with no fatty liver observed on ultrasound were also calculated. The data were analyzed using the SPSS package of Windows version 22. Pearson's chi-square test was used to determine the effectiveness of non-invasive predictors, such as Fib-4 and NFS, in identifying MASLD based on ultrasound findings using the Hamaguchi criteria.

TABLE 1 Characteristics of the patients with and without advanced metabolic dysfunction-associated steatotic liver disease identified via Hamaguchi grading (score ≥ 2) ($n = 153$).

Characteristics	Patient with advanced metabolic dysfunction-associated fatty liver disease ($n = 69$)	Patients without advanced metabolic dysfunction-associated fatty liver disease ($n = 84$)
Age	57.5 + 13.1	60.7 ± 10.8
Gender	51 (73.9%) women	54 (64.3%) women
Ethnicity	46 (66.7%) Emirati	61 (72.6%)
Presence of hypertension	39 (56.5%)	50 (59.5%)
Duration of diabetes in years	12.1 ± 5.2	12.3 ± 5.1
BMI on the last clinic visit	31.4 ± 9.8	28.3 ± 6.6
Weight in kg	78.7 ± 20.5	72.5 ± 15.1
Microalbumin (mg/mmol)	184.7 ± 498.48	164.2 ± 368.7
Serum sodium mmol/L	138.6 ± 3.1	137.7 ± 4.3
Creatinine umol/L	109.0 ± 140.0	158.9 ± 182.7
Albumin g/dL	33.7 ± 5.2	31.9 ± 6.0
INR	1.1 ± 0.4	1.1 ± 0.3
Bilirubin µmol/L	7.9 ± 7.2	8.6 ± 10.0
ALT IU/L	30.1 ± 25.4	22.6 ± 21.5
AST IU/L	27.3 ± 17.1	24.1 ± 16.5
Platelets count × 10^9 /L	270.6 ± 101.3	244.7 ± 92.5
Average of the last 3 HbA1c (in %)	7.8% ± 1.9%	7.9% ± 2.2
Last HbA1c prior to ultrasound	7.7% ± 2.1	7.8% ± 2.5
Hamaguchi grading score	3.19 ± 1.20	0.53 ± 0.5

3 Results

A total of 6,214 patient medical records, including laboratory data and ultrasound imaging results, were reviewed; 5,926 patients were found to be ineligible due to incomplete biochemical tests (especially prothrombin time), and only 288 were found to be eligible as per our inclusion criteria. A total of 135 patients were excluded due to either the presence of type 1 or secondary diabetes and/or pre-existing liver disease (e.g., chronic active viral hepatitis) or a history of alcohol intake. For these selected 153 patients with available ultrasound images, a new expert radiologist reviewed all liver ultrasound scan images and applied the Hamaguchi criteria, identifying and confirming fatty liver disease in 69 patients (45.1%). Of these patients, 68.6% (105/153) were female, with a mean age of 59 ± SD 12.2 years. Additionally, 69.9% (107/153) were from the local Emirati population. The average duration of diabetes in all these patients was 12.2 ± SD 5.1 years, while 58.1% (89/153) had coexisting hypertension. Logistic regression was used to determine the correlation between quantitative variables, such as age and biochemical tests (platelet count, bilirubin, and HbA1c%) with the final outcome of MASLD. The chi-square test was used to assess the correlation of qualitative variables such as history of hypertension, gender, and the final outcome. No significant difference was observed. The details of the clinical and biochemical characteristics of patients with and without MASLD are shown in Table 1. A multi-logistic regression of all factors could not be performed due to the multicollinearity of factors such as the Hamaguchi score and various liver function tests, which are part of the NFS or Fib-4 scoring systems.

Meanwhile, the ultrasound revealed that 45.1% (69/153) of the patients had MASLD according to the Hamaguchi criteria. An intermediate/high NFS was 79.7% sensitive for identifying MASLD on ultrasound, but its specificity was only 10.7%. Meanwhile, an intermediate/high Fib-4 had low sensitivity (30.4%) but higher specificity (54.8%) for identifying MASLD. However, both NFS and Fib-4 failed to achieve statistical significance on Pearson's chi-square test for predicting MASLD (Table 2). Serum sodium <134 mmol/L was also predictive of MASLD with a specificity of 79.8%, but its sensitivity was only 10.1%.

4 Discussion

The prevalence of MASLD has surged globally, becoming a major public health concern due to its association with increasing morbidity

TABLE 2 Comparison of the non-alcoholic fatty liver disease fibrosis score (NFS) and fibrosis-4 index (FIB-4) for predicting advanced metabolic dysfunction-associated steatotic liver disease (diagnosed via Hamaguchi grading on liver ultrasound).

Clinical prediction tool	Fatty liver*	No Fatty liver*	Sensitivity/specificity	*p*-value[3]
NFS[1] low risk	14	9	79.7% sensitive and 10.7% specific	0.09
NFS[1] intermediate/high risk	55	75		
FIB-4[2] low risk	55	75	30.4% sensitive and 54.8% specific	0.06
FIB-4[2] indeterminate/high risk	69	84		

*Identified as per liver ultrasound via Hamaguchi grading. 1. NFS, Non-alcoholic fatty liver disease fibrosis score. 2. FIB-4 = Fibrosis 4 index. 3. Significance is calculated via Pearson's chi-square with a *p*-value of < 0.05 as significant.

and mortality (1). In our study, we wanted to assess the predictive value of non-invasive clinic-based tools, namely NFS and Fib-4, in identifying MASLD among patients with T2DM within our local Emirati population. Additionally, we compared these tools with the Hamaguchi ultrasound scoring system, which is known for its accuracy in identifying MASLD relative to liver biopsy (13, 14).

Our study deliberately concentrated on the utilization of cost-effective and readily available clinical tools, excluding more advanced imaging modalities such as MRI and FibroScan. While advanced techniques, such as MRI and FibroScan, are valuable in assessing liver fibrosis and steatosis, their availability and affordability can be limiting factors, particularly in resource-constrained healthcare settings (18, 19). By employing tools such as ultrasound and clinically derived scores like NFS and Fib-4, we aimed to align our study with the practical realities of clinical practice, especially in regions where access to high-end imaging technologies may be limited. The Hamaguchi ultrasound liver grading system, in particular, offers a feasible alternative with proven efficacy in identifying MASLD, boasting both sensitivity and specificity compared to more invasive procedures such as liver biopsy (14). By focusing on these clinically accessible tools, our study enhances the relevance of our findings to a broader spectrum of healthcare settings, facilitating the potential for wider implementation and impact on routine patient care.

Our findings underscore the substantial burden of MASLD in patients with T2DM, with 45.1% of patients identified as having MASLD according to the Hamaguchi criteria. This aligns with the global trends of increasingly emerging MASLD prevalence, as well as emphasizing the close association between T2DM and MASLD (4). The high frequency of MASLD in this cohort necessitates efficient yet economical screening tools to identify individuals at risk of advanced fibrosis or cirrhosis. Our study used and compared both NFS and Fib-4 as laboratory-based tools in the outpatient clinic setting, and it showed that NFS performed as a much more sensitive tool for predicting MASLD in our local population, exhibiting a sensitivity of 79.7%; however, its specificity was limited at 10.7%, suggesting a higher rate of false positives. On the other hand, the Fib-4 index demonstrated much lower sensitivity (30.4%) but higher specificity (54.8%). These results highlight the trade-off between sensitivity and specificity in non-invasive prediction tools for MASLD in T2DM patients. We wanted to see if having adding Fib-4 (indeterminate or high) score to NFS for screening MASLD could help improve the sensitivity/specificity of the prediction tool, but it only improved the sensitivity of NFS marginally from 79.7 to 81.7% with no change in specificity.

Comparing our results with the existing literature, NFS demonstrated favorable performance in identifying MASLD, which is something also seen in previous meta-analyses (20). However, the limitations of NFS, particularly its low specificity, should be considered in clinical practice. Although the Fib-4 index is currently recommended as a first-line screening test in the United States as part of the MASLD investigation pathway (21), our study found that it missed a substantial proportion of patients with MASLD. These observations emphasize the need for a multifaceted approach to MASLD screening, integrating various non-invasive tools and clinical parameters, including NFS, which can be used as a useful tool in primary care/outpatient setup to initially screen for MASLD.

The Hamaguchi ultrasound scoring system, used as a reference in our study, has proven valuable in identifying MASLD with high sensitivity and specificity (13, 14). The simplicity and non-invasive nature of this grading system make it an attractive option for routine clinical use. The feasibility and reliability of this system warrant further investigation, particularly in diverse populations. Furthermore, our study revealed a correlation between MASLD and serum sodium levels below 134 mmol/L; however, this did not achieve any statistical significance as the total number of patients having hyponatremia were low. This finding, however, introduces an intriguing avenue for further investigation.

4.1 Limitations

Despite contributing valuable insights, our study has limitations. First, the retrospective design may introduce selection bias, as patients with specific indications for liver-related concerns may have undergone liver ultrasound and laboratory evaluations. Second, the relatively small sample size from a single center limits the applicability/generalisability of our findings to a broader Emirati population, and further large-scale prospective studies are required to confirm these results. The absence of liver biopsy data, which is the gold standard for MASLD diagnosis, poses a challenge in accurately characterizing the disease severity. Furthermore, the study only focused on NFS and Fib-4, and we could not calculate the fatty liver index, another clinical tool to predict steatosis, due to a lack of clinical data on waist circumference measurements. Although the current technological advances have allowed the use of newer imaging modalities such as MRI and FibroScan to accurately diagnose MASLD, as these are not easily accessible in primary care settings compared to ultrasound liver, we did not include them in our study. Furthermore, our study lacks longitudinal data, hindering the assessment of disease progression and the impact of interventions.

5 Conclusion

Our study describes the frequency of MASLD in patients with T2DM in our local population and evaluates the performance of

non-invasive clinic-based prediction tools. MASLD was identified in approximately half of our patients with T2DM, which necessitates the need to use MASLD screening in clinical practice effectively. Using ultrasound with the Hamaguchi criteria offers an inexpensive and readily available tool, in most regional healthcare setups, for diagnosing MASLD and for referring to a specialist for further care and management. NFS and Fib-4 show distinct sensitivities and specificities, emphasizing the importance of considering these factors in clinical decision-making, especially in outpatient diabetes clinic settings/primary care. Our study indicated that NFS seemed to outperform Fib-4 in terms of its sensitivity in identifying patients with MASLD and thus could be used as a screening test in our population. However, future prospective studies with larger, diverse cohorts and longitudinal follow-up are crucial to validate these findings and develop evidence-based clinical guidelines for prospective clinic-based MASLD screening in patients with T2DM.

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 Tawam Hospital Ethical Review Committee Approval Number MF2058-2022-855. 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 Retrospective data collection only with no Patient identifiable information collected.

Author contributions

JA: Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. BA: Data curation, Investigation, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. OA: Data curation, Investigation, Resources, Software, Validation, Writing – original draft, Writing – review & editing. DK: Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. AA: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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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frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 26 September 2024
DOI 10.3389/fmed.2024.1431935

OPEN ACCESS

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RECEIVED 13 May 2024
ACCEPTED 10 September 2024
PUBLISHED 26 September 2024

CITATION
Sharma C, Hashim MJ, Yasin J, Alnaqbi MRS, Alkaabi ASA, Aldhaheri MSM, Alkaabi J and Agha A (2024) Predicting cardiometabolic disease in medical students using FibroScan and 30-year Framingham risk scores. *Front. Med.* 11:1431935. doi: 10.3389/fmed.2024.1431935

Predicting cardiometabolic disease in medical students using FibroScan and 30-year Framingham risk scores

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Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) has become a major cause of end-stage hepatic disease worldwide requiring liver transplantation, whereas cardiovascular disease (CVD) remains the leading cause of morbidity and mortality globally. Development of MASLD and CVD among young adults is understudied. This study aimed to assess CVD risk in healthy young medical university students using lipid-based and body mass index (BMI)-based 30-year Framingham risk scores (FS30) and to evaluate disease burden for asymptomatic patients with MASLD by performing FibroScan.

Methods: We included medical university students aged 18–30 years without any known medical conditions. All participants underwent physical and anthropometric measurements, and completed a questionnaire. Blood samples were collected for the analysis of glycosylated haemoglobin levels, renal and liver function, biomarker analysis to calculate liver fibrosis risk, and subclinical atherosclerosis biomarkers. Liver stiffness measurements (LSM) and controlled attenuation parameter (CAP) values were measured using FibroScan 430 mini to calculate liver fibrosis and steatosis, respectively. FS30 based on body mass index (FS30-BMI) and lipid levels (FS30-Lipid) were also calculated.

Results: Overall, 138 medical students participated in this study after providing informed consent. Using FS30-Lipid and FS30-BMI, CVD risk was identified in two (1.5%; $n = 138$) and 23 (17.6%; $n = 132$) individuals, respectively. MASLD fibrosis was identified based on FibroScan LSMs >7.0 kPa in 12 medical students (9.4%, $n = 128$; 95% CI, 4.7–14.8%). Consumption of coffee and sugary soft drinks were predictive of liver fibrosis. In total, 36 students (28.6%; $n = 128$) were found to have hepatic steatosis based on FibroScan CAP values >236 dB, and the predictive factors included increased body fat percentage, male sex, and lack of physical activity. Levels of inflammatory biomarkers, such as C-reactive protein and lipids were not elevated in participants with MASLD.

Discussion: CVD risk was identified in >17% of young medical students. The frequency of liver fibrosis and steatosis was also high among the participants, indicating that liver damage starts at a relatively early age. Early intervention is needed among young adults via health promotion and lifestyle changes.

KEYWORDS

cardiovascular disease, cardiovascular risk prediction, metabolic dysfunction-associated steatotic liver disease, Framingham risk score, FibroScan

1 Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide and almost 90% of deaths are secondary to ischemic heart disease and stroke, with the commonest risk factors being diabetes mellitus, obesity, dyslipidaemia, and high-blood pressure (1). The global prevalence of diabetes is only 9.3%. In the United Arab Emirates (UAE), it is present in nearly twice as much (15.4%) in the adult population and another significant proportion of patients with diabetes remain undiagnosed (2). These CVD risk factors can accumulate, starting in adolescence and continuing into middle- and old-age, with increased risk of atherosclerosis and CVD development over time (3, 4). Other than being a significant cardiovascular risk factor, both diabetes and obesity can also cause metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as nonalcoholic fatty liver disease (NAFLD), which has become the leading reason for liver transplantation in the West (5). Various non-invasive methods that can detect the presence of advanced fibrosis in MASLD cases are available, including clinical or biochemical scoring systems, such as NAFLD fibrosis scores (NFS), fibrosis 4 index (FIB-4), and imaging techniques, including magnetic resonance elastography (6).

The global prevalence of MASLD is reported to be 32% among adults, and higher among men (40%), compared with women (26%). The prevalence of MASLD varies substantially by region across the world, contributed by differing rates of obesity, and genetic and socioeconomic factors (5). The prevalence of MASLD in Asia is 30% (7), whereas that in Europe is 30.9% (7, 8) and in UAE is 25% as in 2017; this is projected to increase to 46% by 2030 (9). A small number of studies have reported on cardiometabolic risk and MASLD among lean and non-lean individuals (10), and on metabolically healthy to metabolically unhealthy participants from baseline to 10 years later (11); however, there is no evidence reported in the literature which can predict the 30-year cardiometabolic risk as well as presence or risk of MASLD and diabetes in healthy young university students. In the UAE, the recently concluded healthy future study showed that cardiometabolic risk factors are highly prevalent in the population of Emirati ethnicity and can contribute to increasing the burden of CVD risk (12). Cardiometabolic risk factors can be assessed using measurement of physical and anthropometric indices, which can also serve as a useful tool to identify future risk of developing obesity (13). However, recent studies have shown that subclinical atherosclerosis may exist even without these traditional cardiometabolic risk factors and new biomarkers, such as apolipoprotein A (ApoA), Apolipoprotein B (Apo B), polymeric immunoglobulin receptor (PIGR), immunoglobulin heavy constant alpha 2 (IGHA2), and heparin cofactor 2 (HEP2) can predict subclinical atherosclerosis independently and offer potential for future disease prediction (14).

Longer-term risk assessments are known to be better predictors of subclinical and clinical CVD than are shorter-term risk predictions. They account for competing causes of death, thereby providing a more realistic assessment of the overall CVD burden (15). The currently used evidence-based traditional risk score usually predicts 10-year risk of cardiovascular events and includes tools such as systematic coronary risk evaluation, Framingham risk score (10 years), assign risk score, Reynold risk score, and prospective cardiovascular Münster risk score, among others (16). A long term cardiovascular risk score, 30-year Framingham heart study score (FS30), based on Framingham cardiovascular study cohort of individuals between the ages of 20–59 years has been proposed, and this model has shown excellent performance with cross-validated discrimination of C = 0.803 and calibration of $\chi^2 = 4.25$ ($p = 0.894$) (17). The FS30 is the only longer-term risk prediction function designed to be used with young adults because it accurately estimates the extent of CVD risk and future disease burden in this population. There is CVD risk assessment studies based on FS30 in the region.

In this study, we sought to fill this knowledge gap by primarily assessing the 30-year risk for future cardiovascular events using available predictive scores, such as FS30, and assessing the presence of subclinical atherosclerosis using available serum biomarkers in young university students. We also aimed to estimate the presence of undiagnosed diabetes, or other cardiovascular risk factors based on biomarkers, anthropometric and physical measurements, as well as identify MASLD cases using FibroScan, a non-invasive bedside tool. Finally, we aimed to assess whether non-invasive clinic-based tools could accurately predict the presence of MASLD.

2 Materials and methods

2.1 Study population

We conducted a prospective cross-sectional study with participating students (after informed consent) at the UAE University Al-Ain. We planned to include a minimum of 112 participants as per study power calculations with an estimated prevalence of 37% of hepatic steatosis (power 80%, and alpha level 5%). The study included university students aged 18 to 30 years without any medical condition that caused them to have a disability that limited their activities of daily living and those who agreed to participate via informed consent. The exclusion criteria were students with mental health issues on undergoing treatment, established cardiovascular disease or previous cardiovascular events, or having any history of alcohol use.

This study was conducted in accordance with the Declaration of Helsinki and was reviewed by our institutional ethics committee. The study was approved by UAE University Human Medical Research Ethics Committee (UAEU.HREC) with approval no# ERH_2023_2353. Informed consent were obtained from all participants involved in this study.

2.2 Clinical parameters

The participants completed a questionnaire which included questions regarding their lifestyle, diet, exercise, and other pertinent

history, including family history of CVD, smoking, and stress. All participants underwent various physical and anthropometric measurements, including weight, height, blood pressure and body mass index (BMI). A tape measure was used for the determination of body indices. The three common measurements obtained were waist, hip, and neck circumferences, and the standard unit used was the SI unit of centimetre (cm).

2.3 Blood sampling

All students participating in the study fasted for at least 4 h, and blood samples were collected via venipuncture. Plasma was separated and stored at −20°C until analysis. The plasma samples were processed for biomarker analysis including low-density lipoprotein, high-density lipoprotein, triglycerides, total cholesterol, urea, creatinine, total protein, albumin, glycosylated haemoglobin (HbA1c), and renal and liver function tests for FIB-4 calculation. Currently recognised subclinical atherosclerotic biomarkers such as ApoA, ApoB, PIGR; IGHA2, and haptoglobin and HEP2 were also determined. Haptoglobin, blood glucose and HbA1c were analysed using an automated analyser, Integra 400 Plus (Roche Diagnostics, Mannheim, Germany). To measure human immunoglobulin A2 and human PIGR levels, we used a commercially available enzyme-linked immunosorbent assay kit from Elabscience Biotechnology Inc., United States.

2.4 Body fat measurement

Body fat thickness measurements were performed using the Harpenden Skinfold Calliper-RH15 (Baty International, England), which is a precise tool used to determine the thickness of skin fat in specific areas, such as the triceps and waist. The Calliper provided measurements in centimetre and captured increments of 0.2 mm. Participants also underwent body fat (BF) composition analysis, and electrical impedance or BF percentage using a TBF-300 Tanita Body composition analyser (Meano-Cho, Tokyo Japan). Tanita was used to determine body composition, including BF, BF mass (kg), fat-free mass, body muscle mass, and total body water.

2.5 MASLD assessment using FibroScan

The LSMs were conducted using FibroScan 430 Mini (Fibro Scan mini+430, France) with both M and XL probes. Measurements were performed following a 3–4 h of fasting by the medical students. The tip of the transducer was covered with a drop of gel and placed perpendicularly in the intercostal space of the participant, who was required to lie in the dorsal decubitus position with the right arm at maximal abduction. Scanning was conducted in a region encompassing the sixth, seventh, and eighth intercostal spaces between anterior axillary and mid-axillary lines. Vibration-controlled transient elastography utilising FibroScan provided the LSMs, which were expressed in kilopascals (kPa), whereas liver steatosis measurements were performed via controlled attenuation parameter (CAP) values expressed in decibels per m (dB/m). A cut-off value of 7 kPa was used to define elevated liver stiffness in the FibroScan results.

2.6 30-year cardiovascular risk assessment

For all participants, the FS30 based on BMI (FS30-BMI) and lipid levels (FS30-Lipid) were calculated using an online tool available[1] cardiovascular-disease-30-year-risk/ developed by Pencina et al. (17). Although a 12% risk is generally considered elevated, with >40% considered as high risk, we used FS30 ≥ 6% as a cut-off for above-normal or elevated CVD risk (with <6% considered as no risk for CVD) as almost all students had values below the 12% threshold.

2.7 Markers for predicting MASLD

Liver function tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin and prothrombin clotting time, and other clinical or biochemical scoring systems, such as FIB-4 and NFS, were calculated and used to predict risk of MASLD. The abnormal cut-off scores for FIB-4 and NFS were taken as ≥1.45 and ≥ −1.455, respectively (18).

2.8 Statistical analysis

Statistical analysis was performed using SPSS v.22.0 (IBM SPSS Inc., Armonk NY). Data were collected using paper forms, entered into an Excel spreadsheet, and subsequently imported into SPSS for statistical analysis. After obtaining descriptive measures, bivariate analysis was conducted using Pearson correlations and one-way analysis of variance tests. Multivariate analysis was carried out with stepwise linear regression. An alpha level of 0.05 was considered statistically significant.

3 Results

A total of 138 medical students participated in the study. The participants were young adults with a mean age of 20.0 years (standard deviation [SD], 1.6; range: 17–24 years). A majority of the students were female (86 of 138 [62.3%]), reflecting the overall sex distribution in the medical college. Demographic characteristics of the participants are summarised in Table 1, which includes breakdown by sex. Among the 138 participants, 10 did not undergo the liver FibroScan. For an additional two participants, the LSM measurements were obtained; however, the CAP values could not be assessed due to technical issues. Six students did not complete their Tanita BF or body weight/BMI measurements; however, all of them underwent complete blood work-up. None of the students had any history of cardiovascular disease or any alcohol intake.

There were significant sex differences in the daily consumption of take-away food ($p < 0.001$), and sugary soft drinks ($p = 0.002$). Stress ($p = 0.019$) and familial history of CVD ($p = 0.022$) were significantly elevated among the female participants. We did not observe any differences in BMI among the participants.

1 https://www.framinghamheartstudy.org/fhs-risk-functions/

TABLE 1 Clinical characteristics of study participants ($n = 138$ except where indicated).

	Females N (%)	Males N (%)	All N (%)	*p*-value
Age				
≤20 years	52 (66.7)	26 (33.3)	78 (56.5)	NS
21+ years	34 (56.7)	26 (43.3)	60 (43.5)	
BMI (kg/m^2; $N = 132$)				
Underweight <20	17 (21.3)	6 (11.5)	23 (17.4)	NS
Normal 20–24	31 (38.8)	19 (36.5)	50 (37.9)	
Overweight 25–29	23 (28.8)	14 (26.9)	37 (28.0)	
Obese 30–34	6 (7.5)	8 (15.4)	14 (10.6)	
Morbidly obese 35+	3 (3.8)	5 (9.6)	8 (6.1)	
Diabetes	2 (2.4)	0 (0)	2 (1.4)	NS
Hypertension	0 (0)	1 (1.9)	1 (0.7)	NS
Dyslipidemia	3 (3.5)	1 (1.9)	4 (2.9)	NS
Asthma	6 (7.1)	4 (7.7)	10 (7.2)	NS
Inhaler use	1 (1.2)	2 (3.8)	3 (2.2)	NS
Outside foods daily	22 (25.6)	29 (55.8)	51 (37.0)	< 0.001
Sugared drinks daily	23 (27.1)	28 (53.8)	51 (37.2)	0.002
Coffee 2 or more cups daily	26 (31.0)	14 (27.5)	40 (29.6)	NS
Sedentary	45 (52.9)	15 (28.8)	60 (43.8)	0.006
Stress/anxiety	14 (16.5)	2 (3.8)	16 (11.7)	0.026
Smoking	1 (1.2)	5 (9.6)	6 (4.4)	0.019
Family history of CVD	17 (20.0)	3 (5.8)	20 (14.6)	0.022

BMI, Body mass index; CVD, Cardiovascular disease; NS, Non-significant.

3.1 CVD: FS30 risk assessment

As we wanted to assess the CVD risk, FS30-Lipid and FS30-BMI were calculated in terms of having a risk of hard cardiovascular (myocardial infarction, and fatal or non-fatal stroke) and full cardiovascular events (which included coronary insufficiency, angina pectoris, stroke plus transient ischemic attack, intermittent claudication, and congestive heart failure in addition to hard cardiovascular events). Based on the FS30-Lipid results, only 2 (1.5%) of participants were found to be at risk for full cardiovascular events. On calculating FS30-BMI, 23 (17.6%) participants were identified to be at risk for full cardiovascular events (CVE), with five (3.8%) of them having a risk of hard CVE (Table 2).

3.2 FibroScan results

To assess the liver fibrosis, LSMs were measured using FibroScan ranged from 2.50 to 13.7 kPa (mean 5.02; SD 1.75; $n = 128$; 10 missing values). Using an LSM of >7.0 kPa as a cut-off, 12 of 128 (9.4%; 95% CI, 4.7–14.8%) students had elevated readings (Table 2).

The LSMs were weakly correlated with steatosis ($r = 0.20$, $p = 0.02$) and age ($r = 0.22$, $p = 0.01$) but not with the levels of liver biomarkers, such as ALT, AST, and albumin.

The CAP values measured using FibroScan (which assesses hepatic fatty infiltration) were moderately correlated with BMI ($r = 0.50$, $p < 0.001$) and to a lesser extent with waist circumference ($r = 0.48$, $p < 0.001$) and body fat mass ($r = 0.47$, $p < 0.001$). The CAP values were also associated with higher risk of full CVE ($r = 0.42$, $p < 0.001$) using FS30-BMI and higher risk of hard CVE ($r = 0.43$, $p < 0.001$) using FS30-BMI (Table 3).

TABLE 2 FibroScan and Framingham risk scores of participants.

	Females N (%)	Males N (%)	All N (%)	*p*-value
Liver stiffness >7 kPa	5 (6.5)	7 (13.7)	12 (9.4)	NS
Liver Steatosis (dB/m)				
Absent (S0) <236	61 (81.3)	29 (56.9)	90 (71.4)	0.003
Mild (S1) 236–269	11 (14.7)	10 (19.6)	21 (16.7)	
Moderate (S2) 270–301	3 (4.0)	6 (11.8)	9 (7.1)	
Severe (S3) ≥302	0 (0)	6 (11.8)	6 (4.8)	
Fib-4 score > 1.45	0 (0)	1 (1.9)	1(0.7)	NS
FS No risk of Full CVD (FS30-lipid)	79 (60.3%)	50 (38.2%)	129 (98.5%)	
FS Risk of Full CVD (FS30-lipid)	0 (0%)	2 (1.5%)	2 (1.5%)	
FS No risk of hard CVD (FS30-lipid)	79 (60.3%)	52 (39.7%)	131 (100%)	
FS Risk of hard CVD (FS30-lipid)	0 (0%)	0 (0%)	0 (0%)	
FS No risk of Full CVD (FS30-BMI)	77 (58.8%)	31 (23.7%)	108 (82.4%)	
FS Risk of Full CVD (FS30-BMI)	2 (1.5%)	21 (16.0%)	23 (17.6%)	
FS No risk of hard CVD (FS30-BMI)	79 (60.3%)	47 (35.9%)	126 (96.2%)	
FS Risk of hard CVD (FS30-BMI)	0 (0%)	5 (3.8%)	5 (3.8%)	
FibroScan liver stiffness (kPa)	5 (6.5%)	7 (13.7)	12 (9.4%)	

There were 86 (62.3%) females and 52 (37.7%) males.

TABLE 3 Comparison of participants with and without elevated liver stiffness.

	Elevated liver stiffness Mean ± SD	Control Mean ± SD	*p*-value
FS Risk of Full CVD (FS30-lipid)	1.3 ± 1.4	1.1 ± 1.0	
FS Risk of Hard CVD (FS30-lipid)	0.1 ± 0.3	0.0 ± 0.4	
FS Risk of Full CVD (FS30-BMI)	4.1 ± 3.8	2.9 ± 2.6	
FS Risk of Hard CVD (FS30-BMI)	1.8 ± 2.1	1.3 ± 1.4	
FibroScan liver stiffness (kPa)	9.0 ± 2.2	4.6 ± 1.0	
WBC (10^3/mm^3)	7.7 ± 2.6	6.4 ± 2.2	0.048
RBC (10^6/mm^3)	6.1 ± 0.7	5.7 ± 0.9	
Haemoglobin (g/dl)	17.3 ± 2.2	15.6 ± 2.5	0.021
Haematocrit (%)	49.4 ± 6.1	45.2 ± 7.1	0.050
Platelets (10^3/mm^3)	226.4 ± 59.6	250.7 ± 76.8	
MCV (fl)	80.6 ± 6.7	79.3 ± 8.2	
MCH (pg)	28.3 ± 3.0	27.4 ± 3.6	
MCHC (g/dl)	35.1 ± 1.2	34.4 ± 1.2	
HbA1c (%)	5.1 ± 0.4	5.2 ± 0.5	
Plasma glucose (mmol/L)	5.3 ± 0.6	5.6 ± 1.0	
Albumin (g/L)	52.2 ± 2.9	52.0 ± 3.8	
Total protein (g/L)	70.8 ± 5.0	70.7 ± 5.1	
ALT (U/L)	19.8 ± 8.1	17.7 ± 5.9	
AST (U/L)	27.0 ± 8.6	22.6 ± 7.7	
Haptoglobin (g/L)	1.1 ± 0.5	1.3 ± 0.5	
Apo-A (g/L)	1.7 ± 0.3	1.8 ± 0.3	
Apo-B (g/L)	1.0 ± 0.3	1.0 ± 0.2	
TC (mmol/L)	4.5 ± 0.8	4.7 ± 0.8	
HDL (mmol/L)	1.5 ± 0.4	1.6 ± 0.4	
LDL (mmol/L)	2.9 ± 0.7	2.9 ± 0.7	
TG (mmol/L)	0.8 ± 0.3	0.9 ± 0.5	
BUN (mg/dl)	8.5 ± 2.9	7.7 ± 2.0	
Creatinine (umol/l)	65.3 ± 14.2	64.8 ± 12.5	
Urea (mmol/L)	3.0 ± 1.1	2.7 ± 0.7	
hs-CRP (mg/l)	1.5 ± 2.2	2.4 ± 3.7	
pIgR (pg/ml)	704.4 ± 418.9	755.9 ± 423.2	
IgA2 (g/L)	0.3 ± 0.1	0.3 ± 0.1	

FS, Framingham Score; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; HbA1c, Haemoglobin A1c; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Apo-A, Apolipoprotein-A; Apo-B, Apolipoprotein-B; TC, total cholesterol; HDL, high-density lipoprotein, LDL, low-density lipoprotein, TG, triglycerides; BUN, blood urea nitrogen; hs-CRP, high sensitivity C-Reactive protein; pIgR, polymeric immunoglobulin receptor, IgA2, Immunoglobulins A2.

3.3 Fib-4

FIB-4 was used biochemical predictor for liver fibrosis. There was only one participant (male) with an elevated FIB-4 score of 1.59, while all female participants did not have an elevated FIB-4 score of >1.45. FIB-4 correlated with AST ($r = 0.57$, $p < 0.001$) and weakly correlated with age ($r = 0.27$, $p = 0.001$) and ALT ($r = 0.27$, $p = 0.001$) but not with other parameters such as LSMs and CAP values, and FS for major (hard) and all (full) cardiovascular events obtained using either lipid or BMI metrics, and anthropometric measurements.

A FIB-4 cut-off value of 1.45 was not useful in discriminating between high and low liver stiffness (mean FibroScan values of 5.03 and 5.40 kPa, respectively; $p = 0.833$).

3.4 Cardiovascular risk

CVD risk was assessed by assessing Framingham risk scores and these were analysed for correlations with MASLD parameters.

The risk for all or [or full cardiovascular events (CVE)] by FS30-Lipid was associated with higher CAP value ($r = 0.28$, $p = 0.001$), age ($r = 0.33$, $p < 0.001$), and body measurements, such as BMI ($r = 0.41$, $p < 0.001$), waist circumference ($r = 0.46$, $p < 0.001$), BF mass ($r = 0.37$, $p < 0.001$), and ALT ($r = 0.33$, $p < 0$ 0.001) but not with LSM in kPa, or FIB-4.

The risk for hard CVE using lipid metrics (FS30-Lipid) did not correlate with any MASLD parameters, such as the LSM or CAP value, age, anthropometric measurements (BMI, BF percentage, and BF mass), or liver markers (ALT, AST, albumin; $r < 0.5$ for all correlations).

The risk for full CVE obtained using FS30-BMI was associated with CAP values ($r = 0.42$, $p < 0.001$) but not with LSM or FIB-4. This was also the case for the risk of hard CVE obtained using FS30-BMI and CAP values ($r = 0.43$, $p < 0.001$). As expected, the cardiovascular risk scores obtained using BMI-based calculations showed stronger correlations with BMI and waist circumference, compared with those obtained using lipid-based formulas.

There were strong correlations ($r > 0.8$, $p < 0.001$) among FS30 obtained using lipid- and BMI-based formulae (Table 4).

3.5 Anthropometric measurements

We wanted assess the relationship of waist circumference, body fat and other anthropometric measurements with presence of MASLD. Waist circumference was strongly correlated with BMI ($r = 0.81$, $p < 0.001$). However, BF analysis metrics were not correlated with MASLD measurements, such as LSM or CAP values, or FIB-4. BF percentage was associated with BMI ($r = 0.78$, $p < 0.001$) and waist circumference ($r = 0.54$, $p < 0.001$).

3.6 Multivariate analyses

FibroScan liver stiffness (measured in kPa; a marker for MASLD) was predicted by ALT only predicted by ALT only, whereas the other parameters did not have any predictive value (adjusted R-squared = 0.03). A machine automated modelling procedure showed that AST, asthma, coffee intake, and the frequency of sugary soft drink consumption were predictive of LSM.

A higher CAP value was predicted by male sex, high BF percent, and a lack of regular physical activity but not by dyslipidaemia, diabetes, hypertension, smoking, inflammatory biomarkers, or liver biomarkers (124 cases included, adjusted R-squared = 0.38).

TABLE 4 Comparison of participants with and without liver steatosis ($n = 138$).

	Liver steatosis N (%)	Normal N (%)	All N (%)	*p*-value
Age				
≤20 years	12 (40.0)	56 (58.3)	68 (54.0)	0.079
21+ years	18 (60.0)	40 (41.7)	58 (46.0)	
Gender Female	10 (33.3)	65 (67.7)	75 (59.5)	< 0.001
Male	20 (66.7)	31 (32.3)	20 (66.7)	
BMI (kg/m^2)				
Underweight <20	3 (10.0)	18 (18.8)	21 (16.7)	< 0.001
Normal 20–24	4 (13.3)	44 (45.8)	48 (38.1)	
Overweight 25–29	9 (30.0)	27 (28.1)	36 (28.6)	
Obese 30–34	9 (30.0)	5 (5.2)	14 (11.1)	
Morbidly obese 35+	5 (16.7)	2 (2.1)	7 (5.6)	
Diabetes	0 (0)	2 (2.1)	2 (1.6)	0.423
Hypertension	0 (0)	1 (1.1)	1 (1.8)	0.573
Dyslipidemia	1 (3.3)	3 (3.2)	4 (3.2)	0.962
Asthma	2 (6.7)	6 (6.3)	8 (6.4)	0.945
Inhaler use	1 (3.3)	2 (2.1)	3 (2.4)	0.702
Outside foods daily	11 (36.7)	35 (36.5)	46 (36.5)	0.983
Sugared drinks daily	12 (40.0)	32 (33.7)	44 (35.2)	0.528
Coffee 2 or more cups daily	7 (25.0)	30 (31.6)	37 (30.1)	0.505
Sedentary	15 (50.0)	39 (41.1)	54 (43.2)	0.388
Stress/anxiety	3 (10.0)	10 (10.5)	13 (10.4)	0.934
Smoking	3 (10.0)	3 (3.2)	6 (4.8)	0.126
Family history of CVD	5 (16.7)	14 (14.7)	19 (15.2)	0.797

FIB-4 was predicted by male sex, higher age, and frequent consumption (daily intake) of take-away food but not by other parameters (adjusted R-squared = 0.20).

Multivariate models for FS showed better predictive power.

The risk of full cardiovascular events obtained using FS30-Lipid was predicted by hypertension, Apo B, sex, smoking, age, Apo A, BF percent (in order of decreasing influence, as indicated by standardised beta coefficients) but not by other parameters, such as MASLD markers including LSM, CAP and FIB-4 (adjusted R-squared = 0.83; Table 5).

The risk of hard cardiovascular events obtained using FS30-Lipid was predicted by hypertension, FibroScan kPa, and smoking (in order of decreasing influence, as indicated by standardised beta coefficients) but not by other parameters, such as FibroScan CAP and FIB-4 (adjusted R-squared = 0.66).

The risk of full cardiovascular events obtained using FS30-BMI was predicted by hypertension, sex, BMI, smoking, age, FibroScan kPa, diabetes, and ApoB (in order of decreasing influence, as indicated by standardised beta coefficients) but not by other parameters, such as FibroScan CAP and FIB-4 (adjusted R-squared = 0.89).

The risk of hard cardiovascular events obtained using FS30-BMI was predicted by hypertension, sex, BMI, smoking, age, diabetes, and ApoB (in order of decreasing influence, as indicated by standardised beta coefficients) but not by other parameters, such as MASLD measures, for example, CAP value or LSM in kPa, and FIB-4 (adjusted R-squared = 0.85; Figure 1).

4 Discussion

In this study of over 130 medical students, there was evidence of early liver damage and disease due to metabolic stress and obesity in almost 10% of subjects. There were two main findings of importance. Firstly, there was evidence of early onset of metabolic liver disease among young adults. Secondly, there were no reliable clinical or biomarker predictors of liver steatosis (apart from body fat and sedentariness). Other findings from our study include the following. Novel inflammatory biomarkers were not associated or predictive of liver damage. Fib-4 score seem to have diagnostic accuracy in this group of young adults.

To the best of our knowledge, this is the first study on the prevalence of MASLD and Framingham score among UAE medical students. This study was also the first that has established the association of CVD risk categories and MASLD in UAE. A study on CVD risk assessment in the gulf region has been performed in Saudi Arabia among university students and found the relationship of long-term cardiometabolic risk calculated via FS30-BMI to be strongly associated with central obesity indices such as visceral adiposity index and mid-arm muscular area in men and body mass index and waist circumference in women (13). Our study did not show any significant in these predictors of CVD risk but it did show that male participants were at disproportionately higher risk for developing full CVD on FS30-BMI with 40.4% (21/52) of males identified as compared to

TABLE 5 Predictors of liver disease and cardiovascular risk based on multivariate analyses.

Outcome	Predictors	Explanatory power
Liver stiffness	AST	Machine automated modelling
	Asthma	
	Coffee intake	
	Sugared drinks intake	
Liver steatosis	Male gender	0.38
	Body fat percent	
	Sedentariness	
FS30-lipid Full CVD risk	Hypertension	0.83
	APO-B	
	Male gender	
	Smoking	
	Age	
	APO-A	
	Body fat percent	
FS30-lipid major CVD risk	Hypertension	0.66
	Liver stiffness	
	Smoking	
FS30-BMI Full CVD risk	Hypertension	0.89
	Male gender	
	Smoking	
	Age	
	Liver stiffness	
	Diabetes	
	APO-B	
FS30-BMI major CVD risk	Hypertension	0.85
	Male gender	
	Smoking	
	Age	
	Diabetes	
	APO-B	

BMI, body mass index; CVD, cardiovascular disease; NS, not statistically significant. Importance is based on standardised beta coefficients in each model. Explanatory power is based on adjusted R-squared which ranged from 0 to 1, higher values indicating greater explanatory power of the predictors.

only 2.5% (2/79) of females and this difference was statistically significant with p value of <0.001. This 10-fold increased risk among males could be associated with factors such smoking, increased sugary intake, and consuming food from outside (Table 2), and this warrants further investigations.

In this study we assessed medical students for the evidence of liver stiffness and cardiovascular risk among UAE nationals using various diagnostic scores. We observed that theses scores are useful indicators of CVD risk and that liver stiffness is an important independent risk factor for CVD. The study showed evidence of early onset of liver stiffness and steatosis were associated with increased risk of CVD among young adults. The estimated prevalence of MASLD varies worldwide, ranging from 6 to 35% (19, 20). In a recent meta-analysis on the global epidemiology of MASLD, Younossi et al. (20) reported its prevalence to be highest (32%) in the Middle East. In our study, 10% students, who were apparently healthy, had liver stiffness, and 26.5% had steatosis (≥S1). In addition, we have demonstrated that male gender, body fat percent, sedentary life style were the main risk factors associated with hepatic fat accumulation. This is in contrast to study done in the Europe among Romanian medical students who had a prevalence of liver steatosis in only 17.4% while fibrosis (F2 and above) on FibroScan in only 3.1% of the participants while our medical students seems to have much higher rates of fibrosis and steatosis (21). A similar study in young Korean population showed prevalence of liver fibrosis among apparently healthy participants being 7% but it is still less than the one reported in our population (22). In light of the reported high frequency of liver steatosis in our population, we suggest a counselling tool (Figure 2) which visualises the level of liver fat damage in relation to BMI with a take home message that reducing weight may decrease the risk of MASLD.

Several previous studies have assessed the association between MASLD and CVD risk and have demonstrated that MASLD is an independent predictor of cardiovascular risk. A previous study argued that the mechanisms by which MASLD increases cardiovascular risk are very complex and involve multiple pathways, but that insulin resistance is the main determinant of MASLD pathogenesis (23). Another study reported evidence that MASLD is strongly associated with increased CVD risk, and that MASLD may not only be a marker, but also an early mediator, of atherosclerosis (24). Aside from insulin resistance, MASLD and atherosclerosis share unifying mechanisms involving pro-inflammatory, thrombogenic factors, and adipokines (25). Systemic diffusion of cytokines and chemokines due to hepatic necro-inflammation triggers vascular damage and coagulation system abnormalities; along with dysregulation of hepatokines can lead to MASLD (26). Expanded and inflamed visceral adipose tissue is the key mechanism linking MASLD with increased CVD risk with release of pro-inflammatory and pro-atherogenic factors, which may lead to the development of insulin resistance and atherogenic dyslipidemia, however due to genetic heterogenicity in MASLD pathophysiology CVD and insulin resistance is not always present (27).

Framingham Risk model has been used to predict 10-year CHD risk in adult UAE nationals without diabetes aged 30–79 without a baseline history of cardiovascular disease and diabetes (28). However, it has not been evaluated in young adults. In this study we correlated the liver steatosis/fibrosis and CVD risk among the young adults. Hypertension, male gender, smoking, age, were common predictors for Framingham Risk scoring.

5 Conclusion

MASLD with liver fibrosis and steatosis are both common in our young healthy asymptomatic population. This suggests that liver damage may appear at an early age; therefore, screening strategies and early intervention via lifestyle changes may be required in our young at-risk population. Our medical students seem to have higher rates of MASLD or liver steatosis when compared with similarly aged participants in other studies involving the West and far East regions. We also observed that the estimated CVD risk seems to be high in

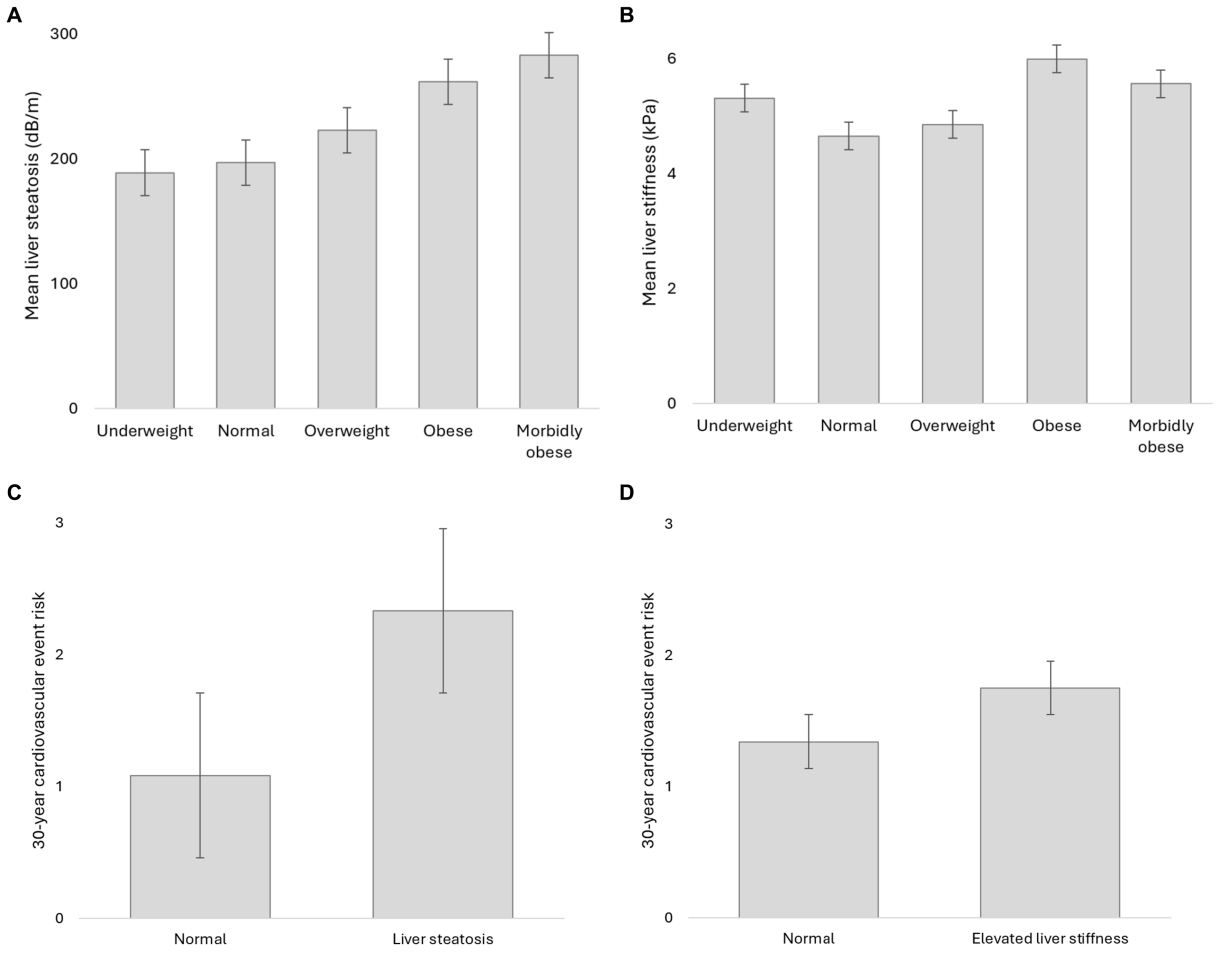

FIGURE 1
Hepatic and cardiovascular risk among young adults. **(A)** Liver steatosis. **(B)** Liver stiffness. **(C)** Liver steatosis; **(D)** Liver stiffness. 30-year cardiovascular risk estimates based on Framingham model for major ('hard') cardiovascular events based on BMI schema.

this group of participants, especially in young men. MASLD may pose cardiovascular risk beyond that conferred by traditional factors, such as dyslipidaemia, diabetes, and smoking. Healthcare providers managing individuals with MASLD should recognise this increased cardiovascular risk and should undertake early, aggressive risk-factor modification. Overall, the current body of evidence strongly suggests that MASLD is likely to be associated with increased CVD risk, and raises the possibility that MASLD may represent not only a marker but also an early mediator of atherosclerosis.

6 Limitations

Our study did not have sufficient numbers of participants for generalisability of the results to the broader population and further large-scale multi-centred prospective studies are required to confirm our findings. Our study used full CVD risk assessment using FS30-BMI and FS30-Lipid risk calculations and used a < 6% score as a cut-off for normal risk instead of classifying it into low or intermediate risk (6–12%), and high risk >12% scores. Also, the research subclinical biomarkers used in our study lacks established recommendations for use in clinical practice.

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 UAE University Human Medical Research Ethics Committee (UAEU HREC) Approval # ERH_2023_2353. 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

CS: Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MH: Data curation, Formal analysis, Investigation, Methodology, Software, Supervision,

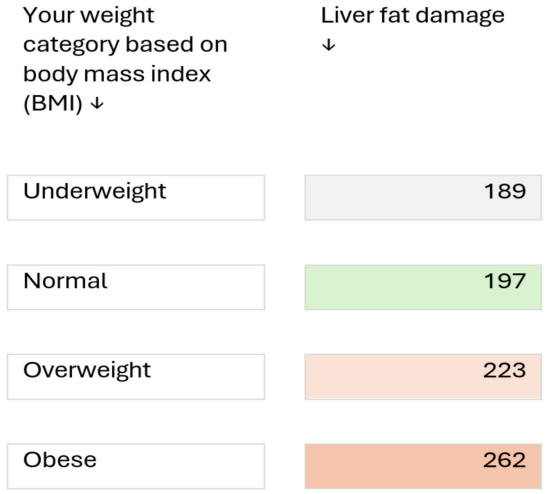

FIGURE 2
Clinical risk discussion tool for counselling healthy young adults at risk of metabolic liver disease (based on a study of 138 medical students, aged 17–24 years). Numbers indicate the level of liver fat damage (based on ultrasound measurements; values >236 dB/m indicate abnormal fat deposition in liver cells). Risk may be increased by other factors such as consumption of alcoholic beverages, certain medicines, genetic predisposition, and certain liver diseases, such as hepatitis B and C. Reducing weight may decrease the risk of metabolic liver disease.

Validation, Visualization, Writing – original draft, Writing – review & editing. JY: Data curation, Investigation, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. MRA: Data curation, Investigation, Validation, Writing – original draft, Writing – review & editing. ASA: Data curation, Investigation, Validation, Writing – original draft, Writing – review & editing. MSA: Data curation, Investigation, Validation, Writing – original draft, Writing – review & editing. JA: Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. United Arab Emirates University summer Undergraduate research grant SURE+ 2023 was used for purchasing biochemical testing kits and disposables in this study.

Acknowledgments

We would like to thank the participants of the study and research assistants and support staff.

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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Glossary

MASLD	metabolic dysfunction-associated steatotic liver disease
CVD	cardiovascular disease
BMI	body mass index
FS	Framingham risk score
LSM	liver stiffness measurements
CAP	controlled attenuation parameter
UAE	United Arab Emirates
ApoA	apolipoprotein A
ApoB	apolipoprotein B
PIGR	polymeric immunoglobulin receptor
IGHA2	immunoglobulin heavy constant alpha 2
HEP2	heparin cofactor 2
BF	body fat
ALT	alanine aminotransferase
AST	aspartate aminotransferase
SD	standard deviation
NFS	NAFLD fibrosis scores
FIB-4	fibrosis 4 index
HbA1c	glycosylated haemoglobin

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 21 November 2024
DOI 10.3389/fmed.2024.1383112

OPEN ACCESS

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RECEIVED 06 February 2024
ACCEPTED 04 November 2024
PUBLISHED 21 November 2024

CITATION
Anastasaki M, Papadakis S, Gergianaki IN, Papastamatiou L, Aligizakis E, Grillaki N, Boutzoukaki E, Sivaropoulos N, Anastasiou F, Mendive J, de Juan-Asenjo C, Hernández-Ibáñez R, Martínez-Escudé A, Garcia-Retortillo M, Koek G, Heyens L, Muris J and Lionis CD (2024) Development and pilot evaluation of an evidence-based algorithm for MASLD (formerly NAFLD) management in primary care in Europe. *Front. Med.* 11:1383112. doi: 10.3389/fmed.2024.1383112

Development and pilot evaluation of an evidence-based algorithm for MASLD (formerly NAFLD) management in primary care in Europe

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD, formerly NAFLD), emerges as major cause of morbidity and mortality globally, with chronic patients facing increased risk. Guidelines on MASLD management in primary care (PC) are limited. This study aimed to develop and evaluate a clinical care pathway for use in PC to improve MASLD screening and management, including early detection, communication and treatment, in three European countries (Greece, Spain, the Netherlands).

Methods: An international multidisciplinary panel of experts oversaw pathway development, which was designed as a two-step algorithm with defined and sequenced tasks. To evaluate algorithm implementation, a controlled pilot study was conducted. Patients at risk of MASLD were assigned to general practitioners (GPs) trained in algorithm implementation (active group) or usual care (control group) and followed for 4–8 weeks. Primary outcomes were the number of patients screened for MASLD, managed in PC and referred to specialists.

Results: In this algorithm, patients with metabolic or liver dysfunction, confirmed MASLD or cardiovascular disease are screened with FIB-4 and classified as having risk of low-level (FIB-4 < 1.3), intermediate-level (1.3 ≤ FIB-4 < 2.67) or high-level MASLD (FIB-4 ≥ 2.67). The algorithm provides evidence-based tools to support GPs manage patients with risk of low-level MASLD in PC, coordinate linkage of patients with risk of high-level MASLD to specialists and refer patients

with risk of intermediate-level MASLD for elastography (low-risk if <7.9 kPa or intermediate/high-risk if ≥7.9 kPa). During pilot evaluation, $N = 37$ participants were recruited in Spain (54.1% women, median age: 63 years). Significantly higher rates of patients in the active group ($n = 17$) than the control group ($n = 20$) were screened with FIB-4 (94.1% vs. 5.5%, $p = 0.004$). Patients in the active group received significantly more frequently a PC intervention for weight loss (70.6% vs. 10.0%, $p < 0.001$), alcohol regulation (52.9% vs. 0%, $p < 0.001$) and smoking cessation (29.4% vs. 0%, $p = 0.005$). In Greece no algorithm implementation was observed in either the active or control group, while the evaluation was not conducted in the Netherlands for logistic reasons.

Conclusion: This study provides evidence on the development and implementation of a new PC algorithm for MASLD screening and management. Variations among participating settings in algorithm implementation are indicative of context-specific particularities. Further research is necessary for integrating such pathways in tailored interventions to tackle this emerging public health issue.

KEYWORDS

metabolic dysfunction-associated steatotic liver disease (MASLD), primary care, clinical care pathways, risk classification, non-alcoholic fatty liver disease (NAFLD), screening, management

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder. Metabolic dysfunction-associated steatohepatitis (MASH), formerly non-alcoholic steatohepatitis (NASH) is its most aggressive manifestation and is characterised by cell damage and inflammation which can further progress to fibrosis, cirrhosis and hepatocellular carcinoma (1). It is expected that MASH will become the leading cause of liver transplantation within the next years (2), while it is currently the main risk factor of hepatocellular carcinoma (3). Despite the significant burden on public health, appropriate suspicion, screening, identification, and linkage to care of patients with signs of advanced fibrosis remain an unmet need.

Since obesity, metabolic syndrome and diabetes are the most frequent co-morbidities in chronic liver disease, patients at high risk for MASLD are often managed in primary care (PC) and followed up by general practitioners (GPs). Although liver fibrosis staging is critical for diagnosing MASLD (4, 5), it is difficult to identify patients with significant fibrosis in primary care due to limited access to fibrosis tests. Without comprehensive guidance and awareness, proper referral to specialty care for high-risk patients is also challenging for GPs (6). Patients with mild disease are often referred when the appropriate preventative interventions of lifestyle changes can be delivered effectively in PC (7). In contrast, advanced fibrosis or cirrhosis is often under-estimated, remaining undetected and leading to late diagnosis of progressed disease. In the absence of comprehensive pharmacological treatment for advanced fibrosis (8), the use of readily available non-invasive tests, standardized referral and treatment algorithms, as well as multi-disciplinary collaboration between GP, endocrinology, diabetology, hepatology, cardiovascular and obesity specialists are key factors for optimal care delivery.

Evidence on non-invasive liver fibrosis tests and innovative pathways for the earlier identification of patients with chronic liver disease and subsequent access to specialist care indicates promising results. A study evaluating a clinical care pathway for patients identified with MASLD using non-invasive fibrosis assessment to stratify patients suggested that the pathway detected five times more cases of advanced fibrosis and cirrhosis while reducing unnecessary referrals from primary to secondary care by 81% (9). Still, comprehensive guidance on such diagnostics and processes remains needed (10), while there is little development and evaluation of similar clinical care pathways for MASLD in PC internationally (11). Such pathways can help address bottlenecks and can be used as part of a comprehensive action plan for screening individuals at risk and providing appropriate referral, intervention, and follow-up. Evaluation and validation of such models is, however, necessary for establishing their effectiveness, including factors related to process, outcomes and feasibility and guide necessary adjustments for achieving optimal adaptation, impact and integration.

The overall aim of this study was to develop an evidence-based pathway to enhance the screening and management of MASLD in primary care, including detection, communication and treatment. We also sought to adapt the pathway to the local cultural and clinical practice contexts of three European countries with diverse health care systems (Greece, Spain, the Netherlands) and evaluate its implementation in a pilot observational study.

Methods

Pathway development

Design

The pathway was designed as a standardized clinical care algorithm with defined, optimized and sequenced tasks developed through an expert panel consensus.

Target population

The algorithm was designed for use by GPs in Greece, Spain and the Netherlands.

Outcomes

The primary outcome of this activity was the documentation of the MASLD algorithm in terms of best practices, guidelines, theoretical framework, patient journey, care pathway, barriers/solutions, quality improvement, implementation procedures/tools and evaluation tools. A secondary outcome was the documentation of local adaptations performed per model domain in each country.

Theoretical framework

The Chronic Care Model was used to guide pathway development as it provides the background to shift from acute, episodic and reactive care towards care that embraces longitudinal, preventative, community-based and integrated approaches (12).

Expert panel eligibility, mandate and activities

Local and international experts ranging from GPs, specialists, academicians and health officers with documented experience in the field of MASLD and PC were eligible to join the multidisciplinary panel. Experts were identified by consortium members from local networks in Greece, Spain and the Netherlands, the European Society of Primary Care Gastroenterology (ESPCG) and other relevant scientific societies, including the European Association for the Study of the Liver (EASL). A minimum of 10 experts were expected to participate in the panel.

Experts provided scientific and clinical expertise to support the development of the MASLD algorithm and were invited to:

- Conduct an assessment of evidence base and needs related to MASLD screening, detection, and management;
- Support the creation of pathway objectives;
- Support the development in terms of clinical content and practical modalities;
- Review and provide feedback on the draft pathway synthesis;
- Provide consensus and final approval of the pathway;
- Overview adaptation of the pathway for use in the targeted countries.

Algorithm development procedures

Development activities followed the Plan-Do-Study-Act framework (13) and the pathway was designed as a standardized care algorithm where different tasks were defined, optimized and sequenced. It aimed to systematically identify and follow patients at risk for low-intermediate or high-level MASLD, beginning from PC and aiming to improve care quality and efficiency, professional coordination/cooperation and patient satisfaction. Using Continuous Quality Improvement elements (14), the algorithm's framework, content and procedures were addressed, focusing on available guidelines and evidence regarding the use of serum markers, non-invasive and imagining techniques to assess advanced fibrosis. The expert panel consolidated local assessments and drafted the algorithm during the following phases:

Preparation phase

Local stakeholder meetings were held in each country prior to the international panel meetings to identify needs and priorities from each setting. An initial synthesis of algorithm elements and a guide to its implementation was produced based on individual country reports and a literature review. These were disseminated to experts before the panel meeting. The experts were asked to review the draft and suggest modifications via e-mail. Individual responses were collected and processed. Emerging questions were drafted and sent to experts for discussion in the main panel meeting.

Main phase

This included the meeting of the expert panel, which, due to COVID-19 restrictions, was held online (March 2022). During the meeting, experts were asked to reflect on the questions drafted in the preparation phase, which related to algorithm content and were organised into topics addressing:

1 Evidence-base, best-practices and pathway framework;
2 Model objectives and prioritization criteria;
3 Mapping the patient journey;
4 Clinical algorithm (decision nodes and process needing standardization).
5 Pathway implementation tools and supportive materials;
6 Assessment of risk level;
7 Patient education, behaviour change and self-management.

During the meeting the panel refined the draft pathway in terms of supporting background (evidence-base, best-practice criteria, and guidelines), implementation and evaluation. The meeting started with the agenda presentation and included small group discussions and plenary sessions moderated by a consortium member.

Consensus phase

Elements of the Rand/UCLA method (RAM) were used to reach consensus (15). The overarching themes, topics and conclusions produced by the expert panel meeting were summarized in a report that was circulated among all experts. Components identified by the expert panel meeting were then triangulated with information from other sources, including literature. All information was fitted into the pathway draft (algorithm and guide to implementation) which was finalized and approved by all experts through a final consensus.

Local adaptation phase

The pathway was developed in English and translated in Greek, Spanish and Dutch. Individual country meetings were held to address the potential necessity of further adaptations. Local GPs were also invited to comment on algorithm content, comprehensiveness and feasibility before evaluation.

Analysis/reporting

Description and outcomes of each process step were summarized in a final report.

Pilot evaluation

Design

A controlled trial pilot study was conducted to evaluate the implementation of the proposed MASLD pathway compared to standard care. Eligible patients were assigned to either an 'active' or a

'control' GP practice. Patients were blinded to the type of practice they were assigned.

Setting and participants

This pilot was conducted in PC settings in Crete (Greece) and Barcelona (Spain). The pilot evaluation could not be conducted in the Netherlands due to logistic reasons, including inability of GPs to facilitate the study reporting post-COVID workload and limited MASLD interest. In each country, four GP practices served as study sites. As such, a total of eight GPs representing a range in gender, age, years of experience and area of practice were purposively selected to facilitate the study based on the following criteria:

1 Holder of specialty degree in GP and/or PC serving in public or private sector;
2 Service in a practice of a well-defined health area;
3 Minimum of 15 patients seen per day.

Patients consecutively visiting the selected GPs were considered eligible for participation based on the following criteria:

1 Metabolic dysfunction: presence of either overweight/obesity, type 2 diabetes, metabolic syndrome OR.
2 Hypertransaminasemia: raised ALT OR raised AST OR.
3 Confirmed MASLD: ultrasound or Fatty Liver Index (FLI) > 60 AND no other causes of liver disease AND no alcohol excess OR.
4 Presence of CVD: any diagnosis or on medication for CVD.

Eligibility criteria were assessed by research assistants through electronic/paper based medical records and based on the specific definitions provided in the pathway guide of Appendix 1. Patients unwilling or unable to provide signed informed consent and complete the procedures for any reason were excluded.

The intervention

Prior to study initiation, GPs caring for patients of the active group received training in pathway implementation and attended a MASLD eLearning developed by our research team and described elsewhere (16). GPs of the control group received no training and provided usual care. GPs of both groups were then allowed to perform their clinical practice as preferred. We hypothesized, however, that trained GPs would screen eligible patients for MASLD and would carry out the pathway procedures regarding referral and management of patients with risk of high-level MASLD in higher rates than GPs of the control group. According to pathway, screening included calculation of FIB-4 (next-to-patient; Appendix 1). Patients with FIB-4 < 1.30 were considered as having no sufficient evidence of liver fibrosis, thus not requiring referral. However, they were supported to modify their lifestyle and further managed in PC. For indeterminate FIB-4 ($1.3 \leq$ FIB-4 < 2.67), patients were referred for elastography and were further classified, with patients having risk of low-level MASLD (<7.9 kPa) retained for PC management. Patients with risk of high-level MASLD (FIB-4 ≥ 2.67 or elastography $\geq$7.9 kPa) were directly linked to specialists.

Sampling and sample size

Patient sampling was consecutive from participating GP practices and not stratified. Rough sample size estimations, assuming that the number of the patients screened will be 4 times higher in the intervention group and that the number of patients diagnosed with advanced fibrosis will be 6 times higher in the intervention group than in the control (based on two-sided test, 80% power and alpha level of 0.05), suggested that 50 patients would need to be recruited per practice.

Study outcomes

Outcomes were assessed in both study groups at patient's first visit in the practice (baseline) and at 4–8 weeks follow-up (September–December 2022). Primary outcomes were the number of patients screened, found with fibrosis, and referred to specialty care as measured at follow-up. Other variables assessed via patients' self-report, medical records or physical examination, respectively, included demographic characteristics (age, gender, education), health habits (smoking, alcohol, diet), biomedical indexes (weight, height, blood pressure), existing and new laboratory tests (in particular for metabolic dysfunction and liver enzymes), existing and new diagnoses (particularly for liver diseases), existing and new medications, existing and new diagnostic tests (particularly FIB-4, elastography, liver ultrasound, liver biopsy).

Data collection tools and procedures

Baseline assessment

Data collection was parallel and same in the participating countries. In both study groups, research assistants assessed eligibility criteria for all patients consecutively visiting the GPs over a period of 2 weeks and invited them to participate using a detailed information sheet. Patients who provided signed informed consent completed the first part of a case report form (CRF), which was administered by research assistants and assessed sociodemographic characteristics and health habits. Using patients' electronic medical records, research assistants also completed the second part of the CRF, which assessed medical history, including tests, examinations, diagnoses, and medications. Research assistants finally observed participants' consultations with the study GPs and completed the third part of the CRF, assessing GPs' practice regarding pathway implementation.

Follow-up assessment

Follow-up was performed 4–8 weeks after the baseline assessment in both study groups. Using patients' medical records, research assistants completed the final part of the CRFs, which tracked patient outcomes, progress through the care system, and follow-up by recording referrals, decisive diagnoses, and new treatments.

Data analysis

Data were presented using descriptive statistics. Mann–Whitney U tests were performed to examine between-group differences in continuous variables. Fishers' exact tests were performed in small samples to explore between-group differences in categorical variables, while X^2 tests were used in larger samples. Statistical significance was set at $p < 0.05$ and analyses were performed using SPSS (Version 25.0. Armonk, NY: IBM Corp).

Results

Pathway development

Expert panel synthesis

The established expert panel included 10 international experts from three countries and multiple disciplines, namely general practice ($n = 4$), hepatology/gastroenterology ($n = 2$), public health ($n = 2$) and academia ($n = 3$). The panel exchanged several e-mail communications and conducted an expert meeting until consensus (March 2022).

Synthesis of the evidence base

The following clinical guidance and resources were used, among others, as the basis for expert panel discussions and pathway formation:

- EASL–EASD–EASO Clinical Practice Guidelines on the management of NAFLD (17);
- NICE. Non-alcoholic fatty liver disease (NAFLD) assessment and management (18);
- The Lancet Live Campaign (19);
- The Camden and Islington NAFLD pathway (9);
- Screening for NAFLD in PC (20, 21).

The following acknowledgments were also made by the expert panel before algorithm development, based on the synthesis of the evidence:

- PC is vital in preventing the development and progression of MASLD;
- Systematic response to abnormal liver blood tests and screening high-risk patients with referral to secondary care is necessary;
- A focus on managing metabolic comorbidities to reduce CVD risk and prevent MASLD complications is required;
- There is an unmet need for integrated interface between primary and secondary care with robust pathways for screening, fibrosis testing and subsequent referrals;
- Lack of such pathways results in missing a significant proportion of the risk population;

Pathway priority areas

Table 1 presents the priority topics and questions addressed by the expert panel during the preparation phase and based on which decisions on model content were taken. In summary these address the level of care to which the pathway should be implemented, expected implementation barriers, patient population to be screened by the pathway (including eligibility criteria), MASLD screening tools and MASLD management in PC.

TABLE 1 Questions addressed by the international expert panel on MASLD pathway priorities.

1. Who screens for MASLD?
a. Which level of care?
- Primary or secondary care?
b. What barriers are expected?
- Providers' knowledge
- Availability of screening tools
- Costs
2. Who/when to screen for MASLD?
a. Which patients to target?
- Population screening or high risk only?
b. When to screen?
- On regular visits or upon abnormal liver tests only?
3. How to screen?
a. Which tests to use?
- type of available tests
- risk classification thresholds
- patient preferences
4. How to manage MASLD?
a. What should MASLD management in primary care include?
- consolidate with evidence and previous work
- synthesize available guidelines
- reinforce doctor-patient communication

Final MASLD algorithm

In accordance with EASL recommendations and international evidence suggesting that almost 90% of unnecessary referrals for MASLD can be avoided by structured screening in PC, the expert panel consented that PC physicians are particularly suited to identify MASLD risk factors and determine respective risk of MASLD level (17, 20). Screening the general patient population was not considered as, of those, about 20–30% will have MASLD and 7–10% will develop complications. Instead, literature and EASL guidelines suggest that screening patients with risk factors, including obesity, type 2 diabetes and metabolic syndrome, is of particular importance, as over 75% of them will be identified with MASLD (17, 20). Taking into consideration the growing evidence on the association of MASLD with cardiovascular disease (CVD) morbidity and mortality, the expert panel included CVD diagnosis among the algorithm's eligibility criteria for MASLD screening. In terms of screening tests to be employed by the algorithm, the decision was made based on availability in PC of partnering countries, with FIB-4 score and elastography primarily used for the detection of risk of fibrosis level. Apart from pharmacotherapy, focusing on lifestyle modification was deemed important based on literature (17, 21). Thus, the pathway further provided resources and guidelines for behavioural interventions, along with the specific MASLD training for PC providers that was developed by our research group and has been reported elsewhere (16).

As such, in a two-step clinical care pathway, patients with metabolic dysfunction, hypertransaminasemia, confirmed MASLD or cardiovascular disease are considered eligible for MASLD screening based on FIB-4. The algorithm classifies screened patients at risk of low-level (FIB-4 < 1.3), intermediate-level (1.3 ≤ FIB-4 < 2.67) or high-level MASLD (FIB-4 ≥ 2.67). Patients at risk of low-level MASLD are managed in PC, with the pathway providing the evidence-base, training resources and guidelines to support GPs perform behaviour/lifestyle modification interventions, treatment and follow-up. Patients with risk of high-level MASLD based on FIB-4 are directly referred to specialty care, with the pathway providing all the resources for care coordination and subsequent primary care monitoring. Patients with intermediate-level MASLD are referred for further examination with elastography and are subsequently classified as low-risk (<7.9 kPa or fibrosis stages F0/F1) or intermediate/high risk (≥7.9 kPa or fibrosis stages F2/F3/F4). Low-risk patients based on elastography are managed in primary care, whilst patients at intermediate/high risk are

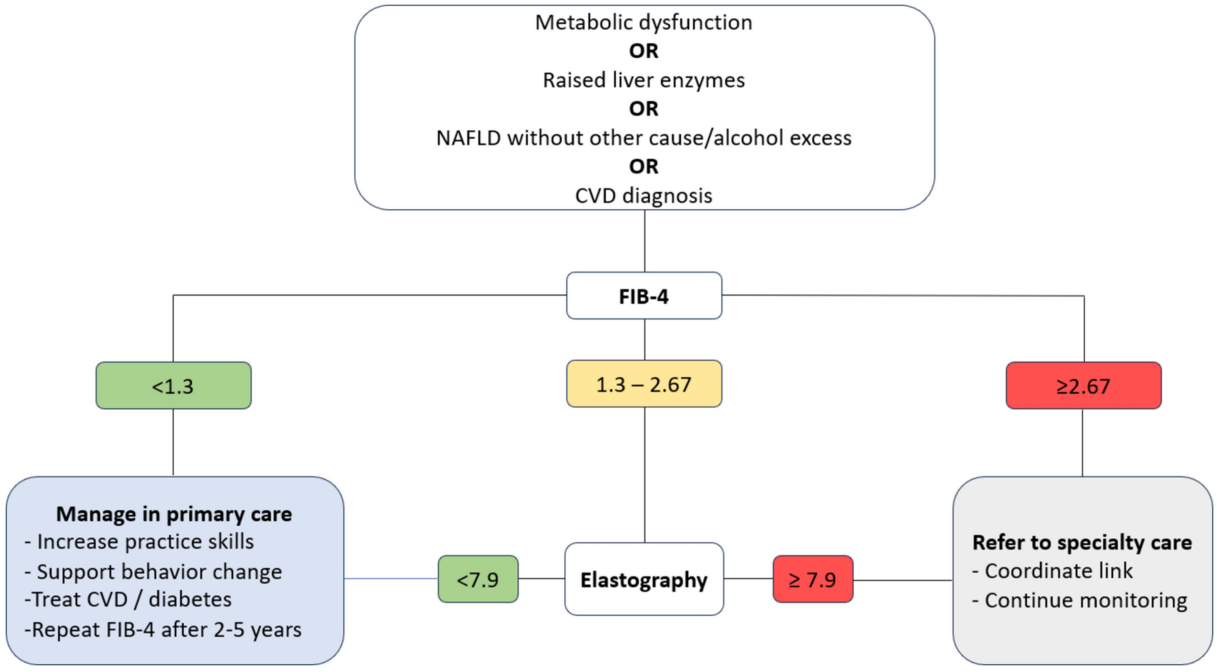

FIGURE 1
The final MASLD primary care pathway (short version).

directly referred to specialty care with specific guidance on care coordination and subsequent primary care monitoring.

The comprehensive version of the final clinical care pathway produced by the expert panel processes is illustrated in Figure 1, while its detailed version and associated implementation guide is provided in Appendix 1. All experts and all three countries endorsed the model without further adaptations apart from translation.

Pilot evaluation

MASLD risk profile

In Spain, $N = 37$ participants were recruited at baseline (54.1% female, median age: 63 years). In terms of risk profile, 21.6% were smoking, 73.0% had BMI > 30, 37.8 and 45.9% had abnormal triglycerides and HDL respectively, 54.1% had increased fasting glucose, 83.8% had increased systolic blood pressure, while 56.8 and 62.2% had abnormal AST and ALT, respectively (Table 2).

In Greece, $N = 182$ patients were recruited at baseline (51.1% female, median age: 64 years). In terms of MASLD risk profile, 51.6% of Greek participants were found with metabolic dysfunction, 3.3% with hypertransaminasemia and confirmed NAFD respectively, while 68.1% had a confirmed CVD diagnosis (data not shown).

Existing tests and diagnoses

In Spain (Table 3), a confirmed MASLD diagnosis was found in the records of 18 patients (48.6%). Twenty-one patients (56.6%) already had a FIB-4 score, with 13 (61.9%) of them classified as having risk of low-level MASLD and 8 (38.1%) as having risk of intermediate-level MASLD. An ultrasound examination was present for 12 (32.4%) patients, indicating hepatic steatosis for 10 (83.3%) of them. Nine (24.3%) patients had an existing elastography, with eight (88.9%) identified at low risk and one (2.7%) at high risk. Finally, two (5.4%) patients had a liver biopsy in their records.

In Greece, no confirmed MASLD diagnoses were found in patients' records. However, two (1.5%) diagnoses of alcoholic liver disease (ALD) were documented (ALD was not part of MASLD at the time when these diagnoses were recorded). None of the patients had ever had a FIB-4 score, an elastography or a liver biopsy recorded. Still, an ultrasound result was available for 138 (75.8%) patients, indicating MASLD for 71.4% of them (data not shown).

Pathway implementation: screening

As presented in Table 4, in Spain, patients in the active group ($n = 17$) received a FIB-4 score more frequently than patients in the control group ($n = 20$) and this difference was statistically significant ($n = 16$ or 94.1% vs. $n = 10$ or 50.0%, $p = 0.004$). From patients having risk of intermediate-level MASLD based on FIB-4 ($n = 1$ or 31.3% active vs. $n = 4$ or 20.0% control), one (5.9%) and three (15.0%) were referred for elastography in the active and control group, respectively, ($p = 0.609$). One-month follow-up data suggest that, from the four elastographies ordered in total, only one had been performed within the study time frame. This concerned a control patient and indicated a low risk of fibrosis (5.2 kPa, results not shown).

In Greece, no FIB-4 scores were performed and no elastographies were ordered by GPs of either the active or the control group.

Pathway implementation: management

As shown in Table 5, in terms of PC management, GPs in the active group of Spain intervened significantly more frequently compared to the control group in terms of weight loss (70.6% vs. 10.0%, $p < 0.001$), alcohol regulation (52.9% vs. 0%, $p < 0.001$) and smoking cessation (29.4% vs. 0%, $p = 0.005$). They also communicated a MASLD diagnosis at higher rates (88.9% vs. 30.0%) and kept

TABLE 2 MASLD risk profile of *N* = 37 patients recruited at baseline in Barcelona, Spain.

Variable	*n*	%
Smoking (yes)	8	21.6
Body mass index		
25–29.9	9	24.3
≥30	27	73.0
Triglycerides (>150)	14	37.8
HDL (<50)	17	45.9
Fasting glucose (>100)	20	54.1
Systolic blood pressure (>130)	31	83.8
AST (9-32)	21	56.8
ALT (7-30)	23	62.2

TABLE 3 Existing MASLD assessments and diagnoses for *N* = 37 patients in Barcelona, Spain.

Variable	*n*	%
Alcoholic Liver Disease	2	5.4
MASLD	18	48.6
MASH	2	5.4
Fibrosis	1 (stage 4)	2.7
Type 2 diabetes	20	54.1
CVD	29	78.4
Anti-HCV, HBsAg, anti-HBc	5	13.5
Positive	0	0
FIB-4	21	56.8
Risk of low-level MASLD	13	61.9
Risk of intermediate-level MASLD	8	38.1
Risk of high-level MASLD	0	0
Ultrasound	12	32.4
Hepatic steatosis	10	83.3
Elastography	9	24.3
Low-risk	8	88.9
Intermediate-/high-risk	1	11.1
Biopsy	2	5.4

TABLE 4 Primary care MASLD screening based on pathway implementation for *N* = 37 patients in Barcelona, Spain.

Variable	Active group (*n* = 17)	Control group (*n* = 20)	*p*-value
FIB-4 performed	16 (94.1%)	10 (50.0%)	0.004
Risk of low-level MASLD	11 (68.8%)	6 (60.0%)	
Risk of intermediate-level	5 (31.3%)	4 (20.0%)	
Risk of high-level MASLD	0 (0%)	0 (0%)	
Elastography referral	1 (5.9%)	3 (15.0%)	0.609

patients in PC for monitoring and management (17.6% vs. 20.0%), however these differences were not statistically significant. One-month follow-up data suggest that the FIB-4 score was repeated within the study time frame for two control group patients (results not shown).

TABLE 5 Primary care MASLD management based on pathway implementation for *N* = 37 patients in Barcelona, Spain.

	Active group (*n* = 17)	Control group (*n* = 20)	*p*-value
Weight loss			
Recommended	4 (23.5%)	16 (80.0%)	<0.001
Intervened	12 (70.6%)	2 (10.0%)	
None	1 (5.9%)	2 (10.0%)	
Alcohol regulation			
Recommended	1 (5.9%)	6 (30.0%)	<0.001
Intervened	9 (52.9%)	0 (0%)	
None	7 (41.2%)	14 (70%)	
Smoking cessation			
Recommended	0 (0%)	4 (20.0%)	0.005
Intervened	5 (29.4%)	0 (0%)	
None	0 (0%)	0 (0%)	
Treatment			
Prescription	9 (52.9)	6 (30.0%)	0.193
No prescription	8 (47.1%)	14 (70.0%)	
Diagnosis communicated	9 (52.9%)	10 (50.0%)	0.858
MASLD	8 (88.9%)	3 (30.0%)	
Metabolic disorder	1 (11.1%)	2 (20.0%)	
Other	0 (0%)	5 (50.0%)	
Follow-up arranged			
Yes	17 (100%)	20 (100%)	-
No	0 (0%)	0 (0%)	
Referral performed	3 (17.6%)	4 (20.0%)	0.855
Hepatologist	3 (100%)	2 (50%)	

Discussion

Summary of findings

This study provides insights on the development and pilot implementation of a MASLD clinical care pathway for use in PC of three European countries. Pathway development was based on expert opinion, while its pilot evaluation was conducted in a controlled study in Spain and Greece. In Spain, despite the small study sizes, GPs exposed to the MASLD pathway screened significantly higher proportions of patients using the FIB-4 score compared to GPs who followed usual care procedures. Given that our algorithm provided a detailed framework with explicit guidance and resources for MASLD management in PC (including tools for behavioural change interventions), exposed GPs indeed documented significantly higher rates of performance of such interventions, compared to GPs of the control group. Contrary to Spain, no implementation of the MASLD was observed among both exposed and not exposed GPs, which is indicative of local context particularities and warrants further

investigation. Logistic issues precluded evaluation in the Netherlands. This variability in implementation success across participating countries is indicative of the challenges related to tailoring and integrating such pathways in diverse and complex clinical systems across Europe and warrants further investigation.

Comparison with literature

Despite large differences in study designs, our findings align with published studies assessing the effectiveness of clinical care algorithms for MASLD management in PC. A prospective study from the UK examining the implementation of a similar pathway among PC patients with screening based on FIB-4, suggested significant improvements in the detection of advanced fibrosis and cirrhosis, while reducing unnecessary referrals in patients with MASLD, highlighting the importance of such strategies for improving resource use and patient outcomes (9). In another study estimating the proportion of patients with type 2 diabetes that should be referred to hepatologists, it was found that the use of age-adjusted FIB-4 cut-offs can lead to more sustainable referrals to specialists (22). Similarly, a study assessing the diagnostic performance of nine clinical non-invasive fibrosis models in MASLD, indicated that the combination of these models performed best for diagnosing advanced fibrosis, providing valuable reference tools for clinical practice (23). Finally, several other studies and individual actions provide algorithms to support PC professionals screen patients with MASLD using liver enzymes, assess advanced fibrosis using prediction rules and determine when to refer patients to specialists (21, 24).

The results observed for Greece are indicative of the context within which the study was performed. The prevalence of MASLD in Greece is largely unknown, however, it is estimated that it exceeds 30% of the general population (25). Moreover, evidence suggests that MASLD is increasing in parallel with risk factors including obesity and diabetes (26, 27). Despite this growing burden, previous work of our group shows that factors driving health behaviour, such as MASLD health literacy and illness perception, are limited among Greek PC patients (28). At the same time, a recently published report of our group, also highlights the low levels of MASLD-related knowledge, confidence and clinical practices among Greek GPs, which however present statistically significant increases after exposure to a newly developed professional training intervention (16). As such, it is not surprising to observe these low levels of pathway implementation and the absence of differences between the active and control GP groups of this study.

Strengths and limitations

To our knowledge, this is the first study that mobilizes the expertise of an international multidisciplinary panel in an attempt to develop and evaluate an integrated clinical care pathway for MASLD screening, diagnosis and referral in Greek, Spanish and Dutch PC. It is also among the few that provides model implementation data on the outcomes of a PC algorithm for MASLD using FIB-4 for risk stratification in Europe. Although there has been some discussion about the accuracy of FIB-4 and its value for the comprehensive management of MASLD patients considering the complexity of the disease (29), it is a practical tool suggested by international clinical practice guidelines (17) and, often the only available option in certain PC settings, like Greece.

However, our study has several limitations. First, the small sample sizes at both the GP and the patient levels, together with the lack of robust sample size estimation and proper statistical power calculation, do not allow for robust conclusions and generalizability of the results. Moreover, the design of this study precludes assessment of the prospective and long-term impact of our pathway to properly determine its effectiveness. Although an external research assistant conducted the data collection in most cases, it is possible that GPs of the active group may have been more motivated to implement the clinical pathway due to their exposure to the training. Finally, the particularities of each study setting and the variability in implementation success across participating settings must be taken into account when making cross-country comparisons and interpreting overall results.

Implications for research, policy and practice

This study was the pre-final part of a larger international collaborative project on MASLD/MASH models in primary care.[1] According to the highlights of the EASL liver commission (30), in a model of care process, this project compiled straightforward algorithms for MASLD screening and referral, new modes of collaborative care and explicit tools for PC management, including behavioural interventions, with the goal of achieving meaningful changes in clinical practice standards. Countries in southern Europe generally lack such multidisciplinary partnerships in PC, while a focus on early disease identification and management of risk factors is not regularly part of clinical practice priorities (31, 32).

Particularly for MASLD, despite the availability of practice guidelines on its clinical management, including the joint guidance from EASL, EASD and EASO, in many healthcare settings no pathways exist or, if they do, they are frequently empirical and not evidence-based (17). Furthermore, under systems' fragmentation and lack of integration and coordination, insufficient services are provided to patients along the MASLD continuum, negatively impacting patient outcomes (33). To improve care for patients with MASLD, it is necessary for health policies and strategies to build on multidisciplinary, context-driven, patient-centred frameworks that provide explicit guidance on MASLD care, an action that has been proven effective in improving care for other diseases (34). Aiming to contribute to bridging the gap between guidance and practice and address the increasing need for best-practice care for patients with MASLD, our pathway assets, along with existing evidence and expert recommendations (35), can be used by stakeholders in the development of high-level models of care to improve the future management of this condition.

Further prospective and longitudinal research is required to confirm the (cost)effectiveness of our proposed PC pathway and the best methods to further screen for advanced fibrosis. In particular, it is imperative that subsequent studies address the limitations through larger, more diverse study populations and methodologies that allow for a comprehensive assessment of the algorithm's effectiveness,

1 http://www.nash.med.uoc.gr/

sustainability, and adaptability across different healthcare contexts. However, with the growing burden of MASLD as a global public health issue, primary care has an important role to play in terms of screening patients and preventing the development and progression of MASLD (36). Along with building robust pathways to support the interface between primary and secondary care, raising public and professional MASLD awareness and education and increasing skills on the active management of cardiovascular risk factors can result in better identification of high-risk patients who will benefit the most from early intervention (37). Given the ongoing PC reforms in settings like Greece, with positive results that include the establishment of community-based multidisciplinary health teams (38), the time to act for MASLD is now.

Conclusion

This study points to better performance in MASLD screening and management for GPs exposed to a MASLD PC pathway compared to GPs attending routine practice, although further research is required to overcome limitations and confirm results. Cross-country variations indicate the different levels of preparedness for MASLD actions and highlight the need for context-driven approaches to increase MASLD screening, management and referral among all settings. Prospective and longitudinal studies are necessary to assess the long-term effects of our pathway and determine its potential for scaling up and integration.

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 Research Ethics Committee of the University of Crete (protocol number 144/23.06.2020). 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

MA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SP: Conceptualization, Funding acquisition, Methodology, Validation, Writing – review & editing. IG: Funding acquisition, Project administration, Validation, Writing – review & editing. LP: Data curation, Investigation, Project administration, Validation, Writing – review & editing. EA: Investigation, Project administration, Validation, Writing – review & editing. NG: Investigation, Project administration, Validation, Writing – review & editing. EB: Investigation, Project administration, Validation, Writing – review & editing. NS: Investigation, Project administration, Validation, Writing – review & editing. FA: Investigation, Methodology, Resources, Validation, Writing – review & editing, Funding acquisition. JMe: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. CJ_A: Investigation, Project administration, Validation, Writing – review & editing. RH-I: Investigation, Project administration, Validation, Writing – review & editing. AM-E: Investigation, Project administration, Validation, Writing – review & editing. MG-R: Investigation, Methodology, Resources, Validation, Writing – review & editing. GK: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. LH: Investigation, Methodology, Project administration, Validation, Writing – review & editing. JMu: Resources, Supervision, Validation, Writing – review & editing, Conceptualization, Funding acquisition, Investigation, Methodology, Project administration. CL: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was funded by an unrestricted research grant from Gilead Science Inc. (NASH Models of Care grant number IN-EU-989-5753).

Acknowledgments

The co-authors would like to thank Myron Galenianos for his support in the administrative aspects of the project and Llorenç Caballeria for his assistance in algorithm development.

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

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1383112/full#supplementary-material

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 05 December 2024
DOI 10.3389/fendo.2024.1426103

OPEN ACCESS

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RECEIVED 30 April 2024
ACCEPTED 11 November 2024
PUBLISHED 05 December 2024

CITATION
Zhu J, Liao X, Du L, Lv P and Deng J (2024) Associations of serum folate and vitamin B_{12} levels with all-cause mortality among patients with metabolic dysfunction associated steatotic liver disease: a prospective cohort study.
Front. Endocrinol. 15:1426103.
doi: 10.3389/fendo.2024.1426103

Associations of serum folate and vitamin B_{12} levels with all-cause mortality among patients with metabolic dysfunction associated steatotic liver disease: a prospective cohort study

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Introduction: Serum folate and vitamin B_{12} levels correlate with the prevalence of fatty liver disease, but it is not clear how they affect mortality. Therefore, this study aimed to investigate the association of serum folate and vitamin B_{12} concentrations with all-cause mortality in individuals with metabolic dysfunction-associated steatotic liver disease (MASLD).

Methods: MASLD subjects were from the Third National Health and Nutrition Examination Survey (NHANES III) in the United States, and mortality follow-up data were obtained by linkage to death records from the National Death Index. Multivariable Cox proportional regression models and restricted cubic spline (RCS) models were used to evaluate the association of serum folate/vitamin B_{12} with all-cause mortality in the MASLD population.

Results: 3,636 and 2,125 MASLD individuals were included in the analyses related to serum folate and vitamin B_{12}, respectively. During a follow-up period of more than 20 years, the RCS models demonstrated significant nonlinear associations of both serum folate (P <0.001) and vitamin B_{12} (P =0.016) with all-cause mortality in MASLD. When their serum concentrations were below the median level, the risk of all-cause mortality decreased with increasing concentration, reaching a lowest risk around the median level, and then leveled off. In the multivariable cox regression model, for vitamin B_{12}, the risk of all-cause mortality was reduced by 42% and 28% in the third and fourth quartile groups, respectively, compared with the lowest quartile group (hazard ratio [HR]=0.58, 95% CI: 0.39-0.86, P =0.008; HR =0.72, 95% CI: 0.54-0.96, P=0.026, respectively). For folate, the risk of all-cause mortality was reduced by 28% in the third quartile compared with the lowest quartile (HR =0.72, 95% CI: 0.57-0.91, P =0.005).

Conclusion: This longitudinal cohort study suggests that low serum folate and vitamin B_{12} levels in patients with MASLD are significantly associated with an elevated risk of all-cause mortality.

KEYWORDS

metabolic dysfunction-associated steatotic liver disease, folate, vitamin B12, all-cause mortality, cohort study

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a syndrome of liver disease associated with cardiometabolic dysregulation, previously known as non-alcoholic fatty liver disease (NAFLD) (1). Because of its potential stigmatization and ambiguity in determining etiology, the term NAFLD has been widely criticized (1, 2). Therefore, a new nomenclature framework for fatty liver disease has recently been proposed, in which MASLD is introduced as a new term to replace NAFLD and accompanied by modified diagnostic criteria (1). As the predominant type of steatotic liver disease, the worldwide prevalence of MASLD has been estimated to be more than 30% (3). Alarmingly, MASLD is the leading cause of hepatocellular carcinoma and cirrhosis and is associated with a significant increase in all-cause mortality (4–6). With the rising prevalence of obesity, the incidence of MASLD continues to increase and has resulted in a huge global disease burden (7). The mechanisms underlying the pathogenesis and progression of MASLD are not fully understood; as a multifactorial disease, it is closely related to insulin resistance, oxidative stress, lipid metabolism, and lifestyle environmental factors (8). Although hundreds of drugs are being developed for fatty liver disease, only resmetirom has recently been approved for the treatment of non-alcoholic steatohepatitis, a subtype of NAFLD characterized by hepatitis and liver damage (9). Lifestyle interventions based on exercise and diet modification remain the cornerstone of MASLD management (8, 10). Therefore, it is particularly important to explore new targets for intervention and to identify biomarkers that can be used for risk management in this chronic disease.

Folate, as an important B vitamin, mediates one carbon (1C) metabolic reactions that play a key role in a variety of physiological processes in the body, including nucleic acid and protein synthesis, amino acid homeostasis, redox defense, methylation modification, and immune response (11, 12). Folate deficiency has been found to be strongly associated with a variety of systemic conditions such as cancer, cardiovascular disease, and psychiatric disorders (13–16). In addition, previous studies have demonstrated that folate affects oxidative stress, chronic inflammation, and lipid metabolism in the liver, all of which are pathogenic mechanisms of fatty liver (17–19). The liver is the primary processing and storage site for folate and is critical in the maintenance of folate homeostasis throughout the body (20, 21). Similar to folate, vitamin B12 (also known as cobalamin) is an essential water-soluble vitamin for the maintenance of 1C metabolism, and plays an important role in human health and disease (11, 12). As an integral component of 1C metabolism, vitamin B12 has the ability to support the translocation and storage of folate within the cell (11, 12). Deficiencies of both are often known as the cause of megaloblastic anemia (22). In addition, vitamin B12 is a cofactor for methyl malonyl coenzyme A mutase, which regulates the transfer of long-chain fatty acyl-CoA to the mitochondria and influences lipid metabolic pathways (23). The liver is the major storage organ for vitamin B12, and previous studies have shown that vitamin B12 is associated with hepatocellular carcinoma, cirrhosis, and hepatitis and can independently predict the histologic severity of non-alcoholic steatohepatitis (24–26).

In fact, the relationship between serum folate/vitamin B12 and NAFLD has been explored in several cross-sectional studies. Previous studies have found that serum folate and vitamin B12 are inversely associated with the prevalence of NAFLD and that their levels are lower in patients with NAFLD than in healthy controls (27–30). These studies suggest that folate and vitamin B12 may be involved in the onset and progression of NAFLD. However, no studies have examined the effect of these two essential B vitamins on mortality in patients with NAFLD. Furthermore, for MASLD, this recently proposed concept, its association with serum folate and vitamin B12 has not been reported. To address this research gap, we therefore analyzed the association of serum folate and vitamin B12 levels with long-term all-cause mortality among patients with MASLD in a nationally representative prospective cohort of U.S. adults.

Method

Study population

The third National Health and Nutrition Examination Survey (NHANES III) was a major project conducted by the U.S. National Center for Health Statistics from 1988 to 1994 aimed at providing national estimates of the nutritional and health status of children

and adults in the United States (31, 32). To enable the survey population to be nationally representative, NHANES III utilized a stratified multistage complex sampling design (31, 32). NHANES III has been frequently used as an unbiased and high-quality dataset for studies in the field of fatty liver disease (33–38). There are other cycles of NHANES that were not included because hepatic imaging data were not available or the linked follow-up period was insufficient. The study population included in the current study was participants aged 20-74 years who underwent hepatic ultrasound. NHANES III has been approved by the NCHS Institutional Review Board. Documented consent was obtained from all participants (https://www.cdc.gov/nchs/nhanes/irba98.htm). The data that we used were completely de-identified for participants, thus exempting the institutional review board.

Definition of MASLD

The procedure for detecting hepatic steatosis based on hepatic/gallbladder ultrasound is described in detail in the Hepatic Steatosis Ultrasound Images Assessment Procedures Manual of NHANES III (39, 40). Briefly, gallbladder ultrasound was performed on all adults aged 20-74 years who received examinations at the mobile examination center. Hepatic steatosis was assessed based on the following five criteria: (a) liver to kidney contrast; (b) parenchymal brightness; (c) vessel walls definition; (d) deep beam attenuation; and (e) gallbladder wall definition. The original ultrasound video images were reviewed by three ultrasound readers who received standardized training from a board-certified radiologist specializing in liver imaging. Rigorous quality control and quality assurance procedures were used to standardize reading manner among the readers. In the assessment of hepatic steatosis, percentage agreement for intra-rater reliability and inter-rater reliability reached 91.3% and 88.7%, respectively, with kappa coefficients both >0.6 (39). In our study, any degree (mild-severe) of steatosis detected was defined as steatotic liver disease (41–43).

According to the MASLD diagnostic criteria, individuals with steatotic liver disease who had any one of the following five cardiometabolic risk factors were identified as MASLD: (a) body mass index (BMI) ≥25 kg/m^2, or waist circumference ≥94 cm (male) or ≥80 cm (female); (b) fasting glucose ≥100 mg/dL, or 2-hour post load glucose levels ≥ 140 mg/dl, or hemoglobin A1c ≥ 5.7%, or type 2 diabetes mellitus, or receiving treatment for type 2 diabetes mellitus; (c) blood pressure ≥ 130/85 mmHg or receiving antihypertensive medication; (d) plasma triglycerides ≥ 150 mg/dL or taking lipid-lowering medications; and (e) plasma HDL-cholesterol ≤40 mg/dl (male) or ≤50 mg/dl (female), or taking lipid-lowering medications (1). Steatosis due to underlying etiologies other than cardiometabolic criteria were excluded, including excessive alcohol consumption (alcohol intake ≥30 g/day for males and ≥20 g/day for females), HBV/HCV infection (serum hepatitis B surface antigen-positive or hepatitis C antibody-positive), and iron overload (transferrin saturation ≥50%). Participants who could not be diagnosed with MASLD because of missing data related to the above cardiometabolic risk factors were excluded.

Measurement of serum folate and victim B12

Both serum folate and vitamin B12 measurements were done by the National Center for Environmental Health using the Bio-Rad Laboratories "Quantaphase II Folate or Folate/B12" radioassay kit (44). The assay was conducted by combining serum samples with ^{57}Co- vitamin B12 and ^{125}I- folate in a solution that contained dithiothreitol and cyanide. All field-collected specimens were frozen and then transported on dry ice and stored at ≤ -20°C after receipt until analysis. Standard procedures for sample collection, storage, processing, analysis, and quality control are described in detail in Laboratory Procedures Used for the NHANES III (44). The coefficients of variation for the long-term accuracy of the NHANES III assays for serum folate and vitamin B12 were 3-6% (at 3-15 ng/mL) and 5-7% (at 300-1500 pg/mL), respectively (44). Folate or vitamin B12 concentrations below 1% or greater than 99% of the overall distribution were considered outliers and these participants were excluded.

Clinical and laboratory data

The following socio-demographic data were included: age, sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American, other), educational level (≤high school, >high school degree), marital status (married, unmarried), family income to poverty ratio (<1, 1-5, >5), smoking status (current smoker, ex-smoker, never smoker), physical activity (active, median, inactive), Healthy Eating Index, and self-reported general health (excellent, very good, good, fair, poor). These data were derived from the baseline questionnaire interviews. For physical activity, leisure-time activities (such as jogging, swimming, riding, calisthenics, and dancing) were categorized into moderate (MET 3-6) and vigorous (MET > 6) types based on intensity ratings (45). The active physical activity level group was defined as engaging in moderate activities at least five times or vigorous activities at least three times per week; the inactive group was defined as no leisure time physical activity; and the moderate group was participants whose physical activity level fell between the active and inactive groups (46). The Healthy Eating Index is an indicator developed by the U.S. Department of Agriculture to measure the overall quality of an individual's diet, with scores ranging from 0 to 100 (47). In addition to folate and vitamin B12, laboratory tests included as covariates included FIB-4 index, serum triglycerides, and C-reactive protein. FIB-4 was calculated as "(age (years) × AST (U/L))/((PLT [10^9/L]) × (ALT (U/L))$^{1/2}$)". Body measurements body mass index (BMI) and waist circumference were included. Common chronic diseases hypertension, diabetes, and history of heart attack were included. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or taking antihypertensive medication, or having ever been told a diagnosis of hypertension by a physician. Diabetes mellitus was defined as fasting plasma glucose concentration ≥126 mg/dL, or random/casual plasma glucose concentration ≥200 mg/dL, or Oral Glucose Tolerance Test ≥200 mg/dL, or HbA1c ≥6.5%, or taking antidiabetic medication, or ever been informed of a diagnosis of diabetes by a physician.

All-cause mortality

Mortality follow-up data were obtained by linking the unique identifiers of participants in NHANES III with death records from the National Death Index. The follow-up period was from the date that the NHANES interview was performed to the occurrence of a death or December 30, 2019, which is the latest data currently available.

Statistical analysis

Folate and vitamin B12 levels were categorized into four intervals based on quartiles to compare baseline characteristics and mortality status of participants in their respective groups. In comparisons of baseline characteristics, the Rao-Scott chi-squared test was used for dichotomous variables, and the Wilcoxon rank-sum test was used for continuous variables. Cox proportional regression models were applied to examine differences in all-cause mortality among MASLD patients in different serum folate and vitamin B12 quartile groups, with participants in the lowest serum folate and vitamin B12 quartile intervals being used as the reference group, respectively. Since the serum folate and vitamin B12 levels of most MASLD participants fell within the normal ranges suggested by some clinical guidelines for healthy adults (serum folate: 3–20 ng/mL; vitamin B12: 160–950 pg/mL) (48), we did not use the normal ranges for both metrics as a reference. In our analysis, the vast majority of participants in the lowest quartile for serum folate and vitamin B12 were still within the normal range for both markers, with fewer than 10% classified as deficient. Given the differences in sources of folate and vitamin B12, we conducted an analysis based on the combined status of both. Specifically, we classified the levels of each indicator into high-level and low-level groups based on their median values, resulting in the following four combinations: low folate & low vitamin B12 group, low folate & high vitamin B12 group, high folate & low vitamin B12 group, and high folate & high vitamin B12 group. The low folate & low vitamin B12 group was used as the reference group. Age, sex, and race-adjusted models considered age, sex, and race as confounders. Moreover, we developed multivariable Cox models further adjusting for demographic characteristics (educational level, marital status, family income level), lifestyle factors (smoking status, physical activity, Healthy Eating Index, vitamin C intake, and vitamin D intake), body measurements (body mass index, waist circumference), laboratory tests (FIB-4 index, serum triglycerides, C-reactive protein), and health status (self-reported general health, diabetes mellitus, hypertension, history of heart attack). To examine whether the effects of folate and vitamin B12 on mortality in MASLD were age-, sex-, or race-specific, we conducted stratified analyses according to them and analyzed interaction effects. To investigate the dose-response effects of vitamin B12 and folate levels on mortality in patients with MASLD, we used restricted cubic spline (RCS) models adjusted for baseline age, sex, and race/ethnicity, educational level, marital status, family income level, smoking status, physical activity, Healthy Eating Index, FIB-4 index, triglycerides, C-reactive protein, body mass index, waist circumference, self-reported general health, diabetes mellitus, hypertension, and history of heart attack. The respective median values of folate and vitamin B12 were used as reference points.

Sensitivity analyses were performed by excluding participants who died within two years of follow-up to rule out a reverse causal association between folate/vitamin B12 levels and all-cause mortality in patients with MASLD. Primary analyses were repeated (age-, sex-, and race-adjusted Cox regression models, multivariable Cox regression models, and RCS dose-response effect analyses) to examine whether the findings were robust.

We considered the complex survey design of NHANES III and used appropriate sample weights in all statistical analyses to make the results nationally representative. All tests were two-sided and $P<0.05$ was considered statistically significant. R version 4.3.1 (https://www.r-project.org/) was used to perform all statistical analyses.

Results

A total of 14,797 participants underwent an ultrasound examination, of which 941 were excluded because of missing or un-gradable image data. Of the 13,856 participants with available hepatic/gallbladder ultrasound data, hepatic steatosis was detected in 5016 individuals. After excluding participants with non-cardiometabolic etiologies and missing data on cardiometabolism, folate/vitamin B12 levels, and mortality status, 3636 participants with serum folate data and 2125 participants with serum vitamin B12 data were ultimately included in the formal analysis. The detailed study screening process is displayed in Figure 1.

For serum folate levels, the concentration ranges of the four groups according to the quartile method were: quartile 1 (<3.3 ng/mL), quartile 2 (3.3-4.7 ng/mL), quartile 3 (4.8-7.3 ng/mL), and quartile 4 (>7.3 ng/mL); for serum vitamin B12 levels, the concentration ranges of the four groups were: quartile 1 (<352 pg/mL), quartile 2 (352-457 pg/mL), quartile 3 (458-588 pg/mL), and quartile 4 (>588 pg/mL). In the serum folate analysis, the mean age of participants was 46.94 (SE: 0.43), and in the serum vitamin B12 analysis, the mean age of participants was 46.34 (SE: 0.71). Interestingly, the age tended to be older in the higher quartile serum folate group (P<0.001), whereas there was no significant difference in age among the different vitamin B12 groups (P=0.943). There was a difference in the gender ratio among the different serum vitamin B12 groups, with the high quartile groups (quartile 3 and quartile 4) tending to have a greater proportion of females (P=0.033), whereas the gender ratio was relatively balanced among the different folate groups (P=0.382). When complex sampling was considered, non-Hispanic whites were the most numerous, and there were differences in the ethnic distributions across the different quartile groups for both serum folate and vitamin B12. Detailed study characteristics of the included population are displayed in Table 1.

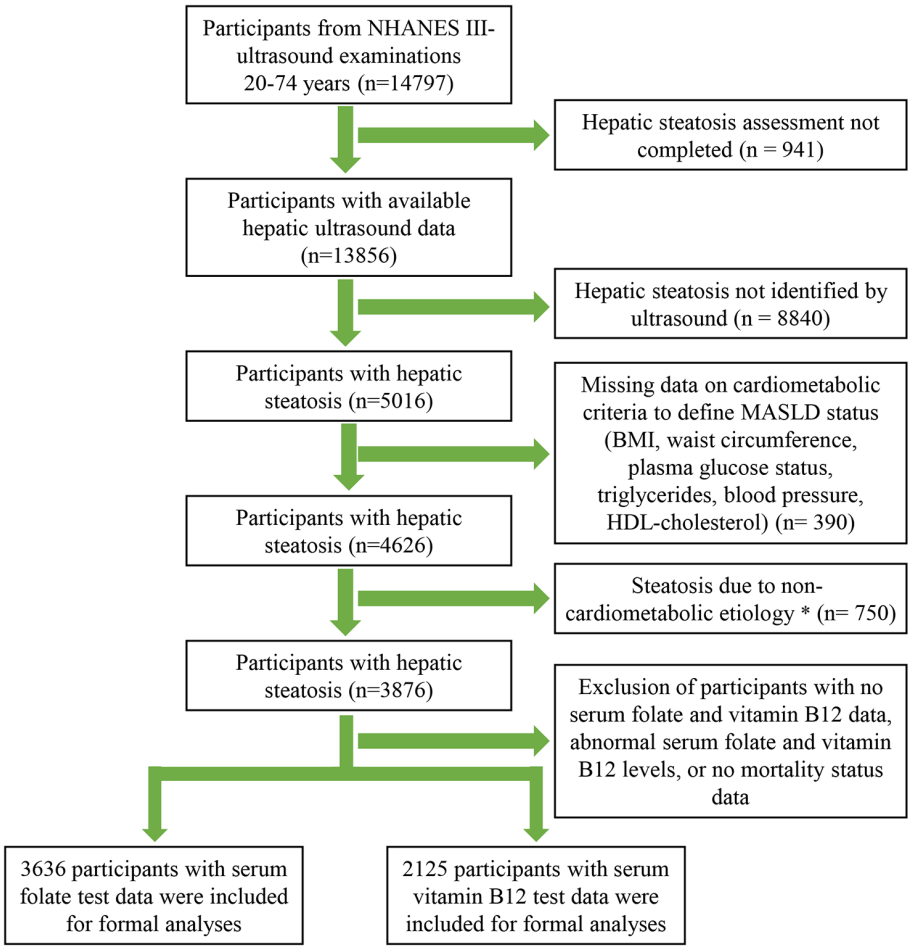

FIGURE 1
Flow Diagram of Participants Inclusion in the Study. *Alcohol consumption >30 g/day for male and >20 g/day for female, HBV/HCV infection, or serum transferrin saturation >50%.

Serum folate and mortality

During a median 26.08 (IQR:18.08-28) years of follow-up for 3636 participants, 1571 deaths occurred. In age, sex, race-adjusted Cox proportional regression models, the higher folate quartile had a significantly lower risk of mortality compared with the lowest quartile group, with HRs of 0.77 (95% CI: 0.60-0.97, P=0.026) for quartile 2, 0.68 for quartile 3 (95% CI: 0.56-0.82, P<0.001), and 0.74 (95% CI: 0.59-0.94, P=0.011) for quartile 4 (Table 2). In multivariable models further adjusted for other potential confounders, only the quartile 3 group showed a significant reduction in mortality (HR=0.72, 95% CI: 0.57-0.91, P=0.005) (Table 2). The multivariable RCS dose-response analysis showed a significant nonlinear association between serum folate levels and all-cause mortality in patients with MASLD ($P_{\text{for nonlinear}}$ <0.001) (Figure 2A). At serum folate levels less than the median value, the risk of all-cause mortality decreased with increasing folate levels, reaching a lowest risk at around 4.7 ng/mL and then slowly increasing.

Serum vitamin B_{12} and mortality

2125 participants incurred 835 deaths during a median 25.75 (IQR:19.25-26.83) years of follow-up. The age, sex, race-adjusted Cox proportional regression models indicated that higher vitamin B12 quartiles tended to have a lower risk of mortality compared with the lowest quartile group, but did not reach statistical significance (Table 2). After further adjustment for potential confounders, the multivariable Cox model indicated that the quartile 3 (HR=0.58, 95% CI: 0.39-0.86, P=0.008) and quartile 4 (HR=0.72, 95% CI: 0.54-0.96, P=0.026) groups had a significantly lower risk of mortality compared with the lowest quartile group (Table 2). Similar to folate, multivariable RCS models showed a significant nonlinear correlation between serum vitamin B12 concentrations and all-cause mortality ($P_{\text{for nonlinear}}$ =0.016), with the risk of mortality decreasing with increasing vitamin B12 levels when serum vitamin B12 concentrations were below the median value, reaching a nadir risk at around 457 pg/mL. In contrast, the change in risk of all-cause mortality was relatively smooth after serum vitamin B12 concentrations were greater than the median value (Figure 2B).

TABLE 1 Baseline characteristics of participants with MASLD according to the serum folate and vitamin B_{12} levels.

Characteristic	Serum folate level, ng/mL						Serum vitamin B_{12} level, pg/mL					
	Overall	Quartile 1 (<3.3)	Quartile 2 (3.3-4.7)	Quartile 3 (4.8-7.3)	Quartile 4 (>7.3)	*p*-value[a]	Overall	Quartile 1 (<352)	Quartile 2 (352–457)	Quartile 3 (458-588)	Quartile 4 (>588)	*p*-value[a]
Participants, no	3636	917	909	926	884		2125	533	532	530	530	
Age (years)	46.94 (0.43)	41.28 (0.69)	45.23 (0.68)	47.57 (0.85)	52.23 (0.78)	<0.001	46.34 (0.71)	46.31 (1.32)	46.65 (0.82)	46.13 (1.11)	46.25 (1.11)	0.943
Gender						0.382						0.033
Male	1,659 (50.9%)	435 (53.7%)	408 (48.8%)	423 (52.7%)	393 (48.4%)		903 (51.1%)	246 (54.5%)	246 (57.9%)	228 (47.7%)	183 (41.5%)	
Female	1,977 (49.1%)	482 (46.3%)	501 (51.2%)	503 (47.3%)	491 (51.6%)		1,222 (48.9%)	287 (45.5%)	286 (42.1%)	302 (52.3%)	347 (58.5%)	
Race/ethnicity						<0.001						<0.001
Non-Hispanic white	1,323 (76.0%)	269 (71.4%)	254 (71.1%)	331 (74.0%)	469 (84.7%)		724 (74.7%)	238 (81.5%)	194 (79.0%)	166 (73.0%)	126 (61.1%)	
Non-Hispanic black	837 (9.0%)	273 (12.8%)	224 (11.8%)	204 (8.4%)	136 (4.6%)		520 (9.0%)	83 (4.3%)	109 (7.4%)	126 (9.0%)	202 (18.0%)	
Mexican-American	1,341 (6.9%)	345 (7.4%)	401 (9.4%)	353 (7.4%)	242 (4.1%)		785 (7.0%)	187 (6.1%)	207 (7.5%)	216 (7.6%)	175 (6.9%)	
Other	135 (8.1%)	30.0 (8.4%)	30 (7.6%)	38 (10.2%)	37 (6.5%)		96 (9.3%)	25 (8.1%)	22 (6.1%)	22 (10.4%)	27 (14.0%)	
Marital status						0.499						0.417
Married	2,390 (69.1%)	575 (69.2%)	600 (70.4%)	621 (70.9%)	594 (66.6%)		1,352 (67.8%)	356 (67.7%)	339 (69.5%)	351 (70.3%)	306 (62.6%)	
Unmarried	1,238 (30.9%)	341 (30.8%)	307 (29.6%)	302 (29.1%)	288 (33.4%)		767 (32.2%)	176 (32.3%)	191 (30.5%)	178 (29.7%)	222 (37.4%)	
PIR						0.022						0.286
<1	827 (12.2%)	253 (16%)	228 (13.2%)	207 (12.8%)	139 (7.9%)		522 (11.7%)	110 (10.0%)	130 (11.0%)	141 (11.4%)	141 (15.4%)	
1-5	2,204 (72%)	524 (72.1%)	541 (73.0%)	571 (72.6%)	568 (70.7%)		1,261 (70.9%)	340 (71.8%)	308 (69.0%)	306 (69.9%)	307 (73.3%)	
>5	293 (15.8%)	46 (11.8%)	65 (13.9%)	72 (14.6%)	110 (21.4%)		182 (17.4%)	49 (18.2%)	53 (20.1%)	43 (18.6%)	37 (11.2%)	
Education						<0.001						0.252
≤High school degree	2,933 (71.0%)	785 (78.5%)	737 (74.1%)	757 (71.0%)	654 (62.5%)		1,705 (69.6%)	423 (65.6%)	424 (69.3%)	429 (72.3%)	429 (72.7%)	
>High school degree	689 (29.0%)	128 (21.5%)	169 (25.9%)	165 (29.0%)	227 (37.5%)		410 (30.4%)	108 (34.4%)	105 (30.7%)	98 (27.7%)	99 (27.3%)	
Physical activity						<0.001						0.889
Active	1,175 (38.1%)	231 (29.1%)	283 (35.1%)	312 (41.2%)	349 (44.8%)		691 (39.6%)	166 (39.4%)	176 (38.5%)	176 (42.9%)	173 (37.2%)	
Median	1,555 (44.9%)	411 (50.1%)	410 (49.7%)	393 (41.3%)	341 (40.5%)		889 (43.8%)	226 (43.8%)	223 (45.7%)	218 (41.2%)	222 (44.7%)	
Inactive	906 (17.0%)	275 (20.7%)	216 (15.2%)	221 (17.5%)	194 (14.7%)		545 (16.6%)	141 (16.9%)	133 (15.8%)	136 (15.9%)	135 (18.0%)	
HEI	63.93 (0.42)	58.86 (0.61)	60.50 (0.57)	64.54 (0.70)	69.85 (0.67)	<0.001	64.19 (0.67)	63.74 (0.79)	64.26 (0.97)	62.94 (1.37)	66.31 (0.96)	0.179

(Continued)

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TABLE 1 Continued

Characteristic	Serum folate level, ng/mL						Serum vitamin B_{12} level, pg/mL					
	Overall	Quartile 1 (<3.3)	Quartile 2 (3.3-4.7)	Quartile 3 (4.8-7.3)	Quartile 4 (>7.3)	*p*-value[a]	Overall	Quartile 1 (<352)	Quartile 2 (352–457)	Quartile 3 (458-588)	Quartile 4 (>588)	*p*-value[a]
Smoking status						<0.001						0.029
Current smoker	738 (21.9%)	301 (36.2%)	180 (21.6%)	164 (20.7%)	93 (11.7%)		414 (21.2%)	101 (21.1%)	119 (25.3%)	102 (22.4%)	92 (14.6%)	
Ex-smoker	1,056 (32.3%)	197 (25.5%)	260 (32.2%)	275 (30.8%)	324 (39.2%)		573 (31.9%)	142 (26.5%)	153 (36.8%)	135 (33.0%)	143 (32.4%)	
Never smoker	1,841 (45.8%)	419 (38.3%)	468 (46.2%)	487 (48.5%)	467 (49.2%)		1,138 (46.9%)	290 (52.4%)	260 (37.9%)	293 (44.6%)	295 (53.0%)	
BMI	30.01 (0.26)	30.90 (0.47)	30.16 (0.31)	30.11 (0.43)	29.08 (0.39)	0.003	30.03 (0.35)	29.61 (0.47)	29.82 (0.36)	30.72 (0.65)	30.08 (0.68)	0.532
Waist circumference	100.96 (0.61)	102.35 (0.96)	101.37 (0.75)	101.70 (1.08)	98.90 (0.76)	0.004	100.53 (0.80)	101.07 (1.12)	101.52 (0.89)	100.47 (1.67)	98.51 (1.12)	0.104
Diabetes	975 (20.1%)	185 (13.6%)	225 (18.6%)	269 (21.0%)	296 (25.6%)	<0.001	563 (19.4%)	123 (17.8%)	134 (18.0%)	133 (19.2%)	173 (23.6%)	0.487
Hypertension	1,587 (42.3%)	364 (38.9%)	367 (39.1%)	433 (45.9%)	423 (44.0%)	0.116	895 (39.8%)	235 (46.2%)	199 (32.6%)	222 (38.1%)	239 (41.9%)	0.050
Heart attack	185 (4.5%)	38 (3.4%)	38 (3.3%)	47 (4.2%)	62 (6.6%)	0.055	90 (3.8%)	24 (3.5%)	19 (4.4%)	21 (3.3%)	26 (3.8%)	0.828
Self-reported general health						0.163						0.642
Excellent	429 (15.6%)	96 (14.2%)	96 (15.1%)	106 (12.7%)	131 (19.6%)		246 (16.0%)	69 (19.4%)	53 (12.6%)	66 (15.6%)	58 (15.8%)	
Very good	774 (29.6%)	200 (30.2%)	177 (28.3%)	195 (31.2%)	202 (28.8%)		468 (31.1%)	128 (30.7%)	122 (34.2%)	110 (30.3%)	108 (28.8%)	
Good	1,389 (36.4%)	356 (38.0%)	364 (34.5%)	356 (38.5%)	313 (34.6%)		815 (36.2%)	195 (33.9%)	223 (38.8%)	203 (37.2%)	194 (34.8%)	
Fair	850 (15.1%)	216 (14.5%)	228 (19.5%)	216 (13.4%)	190 (14.1%)		491 (14.2%)	122 (13.9%)	109 (12.8%)	127 (13.4%)	133 (17.2%)	
Poor	193 (3.2%)	49 (3.0%)	43 (2.6%)	53 (4.2%)	48 (2.9%)		105 (2.6%)	19 (2.1%)	25 (1.6%)	24 (3.5%)	37 (3.4%)	
FIB-4	0.95 (0.01)	0.78 (0.02)	0.88 (0.02)	0.96 (0.02)	1.14 (0.03)	<0.001	0.94 (0.02)	0.97 (0.03)	0.92 (0.03)	0.92 (0.04)	0.94 (0.05)	0.815
TGP	194.72 (3.89)	192.05 (7.67)	181.68 (6.39)	196.41 (11.55)	204.69 (9.08)	0.167	200.12 (5.54)	199.17 (9.60)	188.32 (10.88)	224.18 (20.28)	186.30 (9.46)	0.730
CRP	0.47 (0.02)	0.48 (0.02)	0.46 (0.02)	0.47 (0.04)	0.47 (0.03)	0.237	0.49 (0.02)	0.43 (0.02)	0.50 (0.05)	0.50 (0.04)	0.52 (0.04)	0.414
Vitamin C intake (mg)	37.57 (1.68)	26.55 (1.81)	34.28 (3.61)	38.82 (2.71)	47.30 (2.71)	<0.001	35.32 (2.24)	31.47 (2.91)	38.72 (3.02)	37.31 (4.07)	33.94 (3.62)	0.488
Vitamin E intake (mg)	5.18 (0.26)	3.75 (0.32)	4.80 (0.42)	4.34 (0.24)	7.24 (0.62)	0.001	5.52 (0.36)	5.39 (0.42)	4.70 (0.42)	6.42 (0.95)	5.66 (0.66)	0.619

[a]For categorical variables, *p*-value was calculated by Rao-Scott chi-squared test; for continuous variables, Wilcoxon rank-sum test for complex survey samples was used.

MASLD, Metabolic dysfunction associated steatotic liver disease; HEI, Healthy eating index; PIR, Family income to poverty ratio; BMI, body mass index; CRP, C-reactive protein; TGP, serum triglycerides.

FIB-4 was calculated from "(age (years) × AST (U/L))/((PLT [10^9/L]) × (ALT (U/L))$^{1/2}$)".

All estimates account for complex survey designs, and data are shown as weighted means (standard errors) or percentages as appropriate.

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Serum folate and vitamin B_{12} combination status and mortality

Considering the significant differences in the sources of serum folate and vitamin B12, we next evaluated the combined effects of serum folate and vitamin B12 on MASLD population. In multivariable models adjusted for full potential confounders, compared to the low folate & low vitamin B12 group, both the low folate & high vitamin B12 group and the high folate & low vitamin B12 group tended to have lower all-cause mortality (HR=0.79, 95% CI: 0.51-1.22, and HR=0.84, 95% CI: 0.60-1.18, respectively), although these differences were not statistically significant (Table 2). Interestingly, the high folate & high vitamin B12 group showed a significantly reduced mortality (HR=0.64, 95% CI: 0.45-0.89, *P*=0.009) (Table 2).

Stratified analysis

In stratified analyses according to age, sex, and race, no significant interaction effects were found for them on the correlation between folate/vitamin b12 and all-cause mortality (Tables 3, 4). However, there were some scenarios of marginal statistical significance. Specifically, the association between folate and mortality in patients with MASLD appeared to be more significant in females (*P*=0.098). Serum folate was statistically correlated with reduced all-cause mortality in the fourth and third quartiles of the young and middle-aged groups, respectively (Table 3). In the analysis of vitamin b12, the association between elevated serum vitamin b12 concentrations and reduced mortality appeared to be more significant in middle-aged and older adults (*P* =0.071) (Table 4). In the combined analysis of vitamin B12 and folate, we found that Non-Hispanic Black individuals did not seem to benefit from the simultaneous elevation of both (Table 5).

Sensitivity analysis

We repeated the analyses for the primary findings after excluding participants whose deaths occurred within two years of follow-up. Sensitivity analyses showed that participants who died within a short period of time had little effect on the results of the Cox proportional regression model and the multivariable RCS model, suggesting that the correlation between folate/vitamin b12 and mortality was not confounded by reverse causal effects (Supplementary Table S1, Supplementary Figure S1).

TABLE 2 Hazard ratios of all-cause mortality by serum folate and vitamin B_{12} levels among adults with MASLD.

	Age, sex, race-adjusted model[a]		Multivariate model[b]	
	HR (95% CIs)	*P-value*	HR (95% CIs)	*P-value*
Serum Folate				
Quartile 1	1 (reference)		1 (reference)	
Quartile 2	0.77 (0.60-0.97)	0.026	0.81 (0.61-1.06)	0.128
Quartile 3	0.68 (0.56-0.82)	<0.001	0.72 (0.57-0.91)	0.005
Quartile 4	0.74 (0.59-0.94)	0.011	0.89 (0.69-1.15)	0.369
Serum Vitamin B12				
Quartile 1	1 (reference)		1 (reference)	
Quartile 2	0.83 (0.59-1.17)	0.293	0.73 (0.52- 1.03)	0.074
Quartile 3	0.77 (0.54-1.08)	0.128	0.58 (0.39-0.86)	0.008
Quartile 4	0.90 (0.68-1.20)	0.476	0.72 (0.54-0.96)	0.026
Serum Folate & Vitamin B12*				
Low folate & low vitamin B12	1 (reference)		1 (reference)	
Low folate & high vitamin B12	0.95 (0.65-1.39)	0.795	0.79 (0.51-1.22)	0.295
High folate & low vitamin B12	0.78 (0.56-1.09)	0.152	0.84 (0.60-1.18)	0.321
High folate & high vitamin B12	0.71 (0.52-0.97)	0.031	0.64 (0.45-0.89)	0.009

[a]Multivariable Cox proportional regression analysis adjusted for age, sex, and race/ethnicity;
[b]Multivariable Cox proportional regression analysis adjusted for age, sex, race/ethnicity, educational level, marital status, family income level, smoking status, physical activity, Healthy Eating Index, FIB-4 index, serum triglycerides, C-reactive protein, body mass index, waist circumference, self-reported general health, diabetes mellitus, hypertension, history of heart attack, vitamin C intake, and vitamin E intake.
*Serum folate and serum vitamin B12 levels were considered simultaneously. Values below the median were classified as low levels, and values above the median were classified as high levels. Based on these two serum indicators, patients were divided into four groups: low folate & low vitamin B12 group, low folate & high vitamin B12 group, high folate & low vitamin B12 group, and high folate & high vitamin B12 group.
MASLD, Metabolic dysfunction associated steatotic liver disease; HR, hazard ratio; CIs, confidence intervals.

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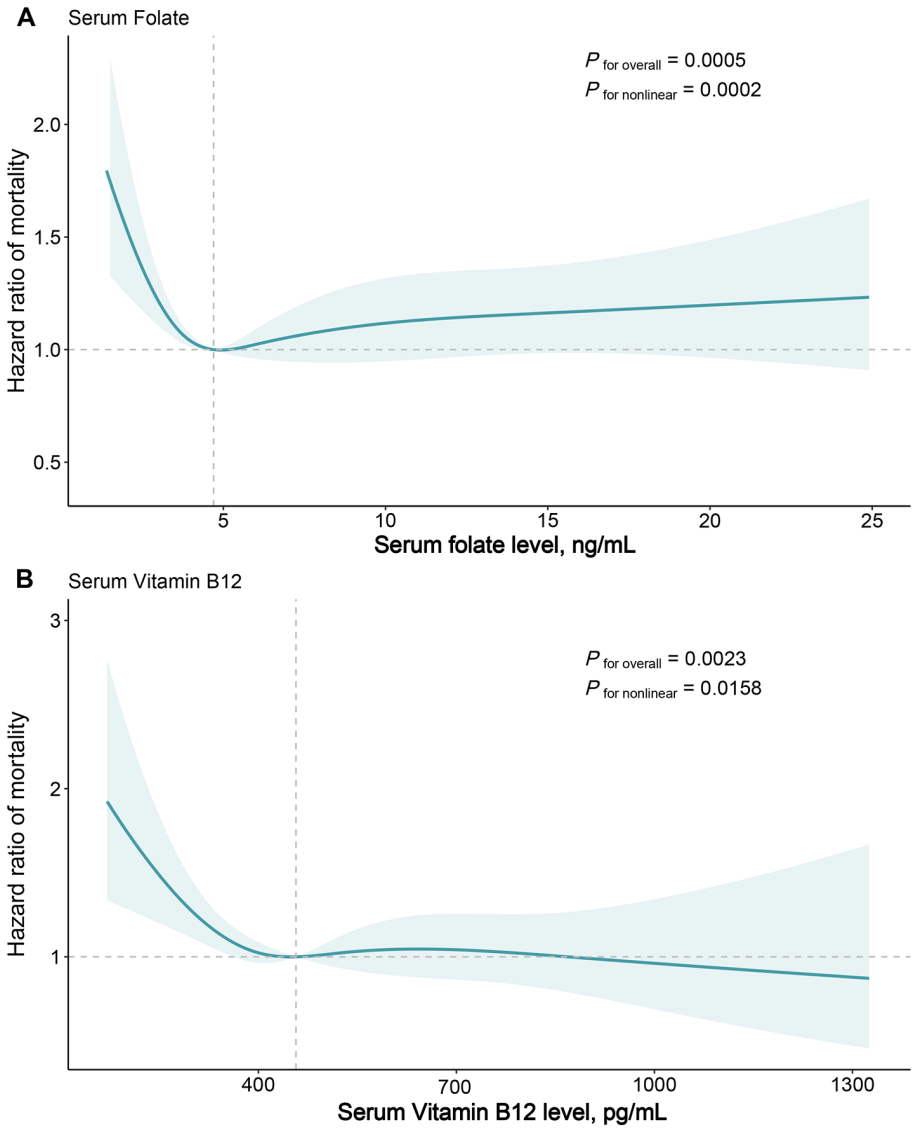

FIGURE 2
Dose-response Association of Serum Folate **(A)** and Vitamin B_{12} **(B)** Levels with All-cause Mortality in Patients with Metabolic Dysfunction-associated Fatty Liver Disease. Hazard ratios were estimated by multivariable restricted cubic spline models, with knots placed at 5th, 35th, 65th, and 95th percentiles. Solid line represents hazard ratios and shaded areas represents 95% CIs. The reference points are the median values for serum folate (4.7 ng/mL) and serum vitamin B12 (457.0 pg/mL) level. Risk estimates were adjusted for baseline age, sex, and race/ethnicity, educational level, marital status, family income level, smoking status, physical activity, Healthy Eating Index, FIB-4 index, triglycerides, C-reactive protein, body mass index, waist circumference, self-reported general health, diabetes mellitus, hypertension, and history of heart attack.

Discussion

To our knowledge, this is the first study to explore the effect of serum folate and vitamin B12 levels on all-cause mortality in patients with MASLD. In this large population-based prospective cohort study with a follow-up of more than 20 years, we found that both serum folate and vitamin B12 concentrations were significantly associated with all-cause mortality in individuals with MASLD. Low serum folate and vitamin B12 concentrations implied worse long-term outcomes for individuals with MASLD. Interestingly, we found that participants with both high folate and high vitamin B12 levels exhibited a more pronounced reduction in mortality compared to those with elevated levels of either folate or vitamin B12 alone. Sensitivity analyses confirmed the robustness of these findings from this study.

Of note, the association between serum folate and mortality shows inconsistent trends between men and women with MASLD. Some previous studies have suggested that estrogen influences folate metabolism and effects, but it is unclear whether this interaction contributes to the observed differences in MASLD patients (49, 50). Additionally, the association between serum vitamin B12 and mortality shows opposite trends in individuals under and over 40 years of age. This may be because MASLD patients over 40 tend to have poorer baseline health and more comorbidities, making the antioxidant, anti-inflammatory, and cardiovascular protective roles of vitamin B12 more critical (51, 52). In contrast, in patients under

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TABLE 3 Association of serum folate levels with all-cause mortality in different subgroups of patients with MASLD.

	HR (95% CIs) by quartile[a]				*P-value* for interaction
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Gender					0.098
Male	1 (reference)	1.16 (0.76-1.78)	0.88 (0.59-1.30)	1.19 (0.81-1.76)	
Female	1 (reference)	0.56 (0.42-0.74)	0.59 (0.46-0.77)	0.74 (0.56-0.98)	
Age					0.124
20-39 years	1 (reference)	0.90 (0.29-2.83)	0.57 (0.28-1.15)	0.17 (0.07-0.43)	
40-59 years	1 (reference)	0.77 (0.48-1.22)	0.60 (0.38-0.95)	1.01 (0.61-1.67)	
60-74 years	1 (reference)	0.97 (0.68-1.37)	0.96 (0.72-1.28)	1.10 (0.83-1.44)	
Race/ethnicity					0.415
Non-Hispanic white	1 (reference)	0.82 (0.57-1.18)	0.72 (0.55-0.94)	0.91 (0.68-1.21)	
Non-Hispanic black	1 (reference)	0.88 (0.56-1.40)	0.87 (0.57-1.32)	1.30 (0.85-1.99)	
Mexican-American	1 (reference)	0.73 (0.50-1.06)	0.90 (0.57-1.43)	0.93 (0.57- 1.58)	
Other	1 (reference)	1.17 (0.23-5.93)	0.14 (0.03-0.64)	0.07 (0.02-0.33)	

[a]Multivariable Cox proportional regression analysis adjusted for age, sex, and race/ethnicity, educational level, marital status, family income level, smoking status, physical activity, Healthy Eating Index, FIB-4 index, serum triglycerides, C-reactive protein, body mass index, waist circumference, self-reported general health, diabetes mellitus, hypertension, history of heart attack, vitamin C intake, and vitamin E intake.
MASLD, Metabolic dysfunction associated steatotic liver disease; HR, hazard ratio; CIs, confidence intervals.

40, high folate levels do not appear to offer similar protective effects. In summary, current evidence is insufficient to clarify the specific mechanisms of interaction between age/gender and the effects of these two nutrients in MASLD patients. However, it is important to note that the impact of folate and vitamin B12 on patient outcomes is not always consistent across different demographic groups.

Previous studies have shown that patients with NAFLD have lower serum folate and vitamin B12 concentrations compared with the general population (27–30). Furthermore, higher concentrations of folate/vitamin B12 and severity of liver fibrosis, steatosis, and nonalcoholic steatohepatitis were inversely correlated in patients with fatty liver (26, 53, 54). Thus, our findings may be

TABLE 4 Association of serum vitamin B_{12} levels with all-cause mortality in different subgroups of patients with MASLD.

	HR (95% CIs) by quartile[a]				*P-value* for interaction
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Gender					0.412
Male	1 (reference)	0.88 (0.61-1.29)	0.81 (0.54-1.21)	0.79 (0.52-1.20)	
Female	1 (reference)	0.55 (0.31-0.98)	0.47 (0.26-0.87)	0.61 (0.38-1.00)	
Age					0.071
20-39 years	1 (reference)	1.78 (0.51-6.15)	1.68 (0.62-4.59)	2.47 (0.53-11.5)	
40-59 years	1 (reference)	0.66 (0.34-1.30)	0.56 (0.28-1.12)	0.58 (0.31-1.06)	
60-74 years	1 (reference)	0.67 (0.47-0.96)	0.50 (0.33-0.75)	0.65 (0.47-0.90)	
Race/ethnicity					0.498
Non-Hispanic white	1 (reference)	0.74 (0.50- 1.09)	0.55 (0.36- 0.82)	0.70 (0.51- 0.97)	
Non-Hispanic black	1 (reference)	0.60 (0.33- 1.07)	1.17 (0.65- 2.10)	0.91 (0.53- 1.59)	
Mexican-American	1 (reference)	0.82 (0.59- 1.13)	0.69 (0.42- 1.11)	0.83 (0.50- 1.37)	
Other	1 (reference)	1.35 (0.21- 8.46)	0.25 (0.03- 2.43)	0.10 (0.02- 0.44)	

[a]Multivariable Cox proportional regression analysis adjusted for age, sex, and race/ethnicity, educational level, marital status, family income level, smoking status, physical activity, Healthy Eating Index, FIB-4 index, serum triglycerides, C-reactive protein, body mass index, waist circumference, self-reported general health, diabetes mellitus, hypertension, history of heart attack, vitamin C intake, and vitamin E intake.
MASLD, Metabolic dysfunction associated steatotic liver disease.

TABLE 5 Association of serum folate & vitamin B_{12} levels with all-cause mortality in different subgroups of patients with MASLD.

	HR (95% CIs) by quartile[a]				*P-value* for interaction
	Low folate & low vitamin B12	Low folate & high vitamin B12	High folate & low vitamin B12	High folate & high vitamin B12	
Gender					0.493
Male	1 (reference)	0.78 (0.44-1.37)	0.70 (0.39-1.23)	0.68 (0.42-1.09)	
Female	1 (reference)	0.85 (0.47-1.54)	1.05 (0.73-1.51)	0.64 (0.42-0.98)	
Age					0.679
20-39 years	1 (reference)	1.89 (0.88-4.07)	0.41 (0.14-1.22)	0.36 (0.06-2.00)	
40-59 years	1 (reference)	0.47 (0.22-0.99)	0.55 (0.27-1.12)	0.64 (0.38-1.09)	
60-74 years	1 (reference)	0.66 (0.45-0.98)	0.96 (0.70-1.30)	0.64 (0.47-0.87)	
Race/ethnicity					0.079
Non-Hispanic white	1 (reference)	0.64 (0.38- 1.07)	0.79 (0.53- 1.17)	0.61 (0.41- 0.89)	
Non-Hispanic black	1 (reference)	1.35 (0.89- 2.05)	0.95 (0.53- 1.73)	1.35 (0.79- 2.30)	
Mexican-American	1 (reference)	0.86 (0.51- 1.46)	1.08 (0.52- 2.23)	0.85 (0.43- 1.70)	
Other	1 (reference)	1.28 (0.06- 27.3)	0.31 (0.01- 9.29)	0.06 (0.01- 0.40)	

[a]Multivariable Cox proportional regression analysis adjusted for age, sex, and race/ethnicity, educational level, marital status, family income level, smoking status, physical activity, Healthy Eating Index, FIB-4 index, serum triglycerides, C-reactive protein, body mass index, waist circumference, self-reported general health, diabetes mellitus, hypertension, history of heart attack, vitamin C intake, and vitamin E intake.
MASLD, Metabolic dysfunction associated steatotic liver disease.

due to the fact that low folate and vitamin B12 concentrations in MASLD imply a more severe disease status. Folate and vitamin B12 may be useful as biomarkers to independently predict mortality in individuals with MASLD, but more evidence from other geographic areas or ethnicities is needed to support this.

Several published studies have investigated the correlation between folate/vitamin B12 concentrations and mortality in the general population, but the results have been inconsistent. For example, Wolffenbuttel et al. showed that low serum vitamin B12 concentrations were significantly associated with increased all-cause mortality (55), but Flores-Guerrero et al. found that high vitamin B12 concentrations represented increased all-cause mortality in the general population of the city of Groningen, the Netherlands (56). In an elderly population in China, the correlation between serum vitamin B12 and all-cause mortality showed a J-shaped pattern (57). Existing studies exploring the association between folate concentrations in the body and mortality in the general population tend to report beneficial health effects of folate (58). For example, Peng et al. and Song et al. found that higher folate levels were associated with lower all-cause and cause-specific mortality (59, 60). Interestingly, several studies have shown that folate intake is associated with a reduced risk of mortality, whereas vitamin B12 intake did not have this effect (55, 61–63).

Some cross-sectional investigations have found an inverse association between folate intake and the prevalence of NAFLD (28, 29). However, there is no evidence from longitudinal studies that increased folate intake reduces the risk of mortality in individuals with fatty liver disease. Although our study suggests that low folate levels *in vivo* may be associated with a higher risk of mortality and studies from the general population have shown the benefits of folate intake, more direct clinical evidence is still needed as to whether folate supplementation can be used as a dietary intervention strategy for patients with MASLD. Compared to vitamin B12 deficiency, folate deficiency is more common because the body stores only a small amount of folate, so when the ingested diet is deficient in folate, the body can exhibit folate deficiency within a few months (64). However, insufficient intake is only part of the reason for folate or vitamin B12 deficiency in the body; low serum concentrations of both nutrients may also represent absorption disorders such as celiac disease, pancreatic disease, small bowel resection, endogenous factor deficiencies, or the effects of certain medications such as metformin, methotrexate, and antibiotics (65). Therefore, further studies are still needed to determine whether folate or vitamin B12 supplementation is truly effective as an intervention.

The potential mechanisms by which folate/vitamin B12 affects mortality in MASLD remain to be elucidated. Both play a core role in 1C metabolism, affecting a wide range of physiological activities such as protein and nucleic acid synthesis, methylation of DNA, and post-translational modification of proteins (11, 12). A recent study found that folate/vitamin B12 reduced circulating concentrations of homocysteine and improved autophagy through transmethylation, while serum homocysteine levels were significantly associated with worse liver inflammation and degree of fibrosis (66). Thus, dietary folate/vitamin B12 supplementation in the mouse model slows the progression of non-alcoholic steatohepatitis and reverses inflammation and fibrosis (66). In addition, folate/vitamin B12 is strongly associated with lipid metabolism (17, 67); low folate/vitamin B12 levels are associated with a high prevalence of metabolic syndromes, and their deficiency

increases lipid accumulation in adipocytes, leptin production, and inflammatory factors, and thus may influence the progression of fatty liver (17, 67). Furthermore, deficiencies in vitamin b12 and folate increase oxidative stress by raising homocysteine levels (51, 68–70). Since oxidative stress plays an important role in the progression of fatty liver (71), ensuring adequate levels of vitamin b12 and folate may improve the prognosis of patients through this mechanism.

As the first large-scale investigation into the association of serum folate and vitamin B12 levels with all-cause mortality in patients with MASLD, the main strengths of the current study are that it has a follow-up time of more than 20 years, adjusts for a variety of potential confounders, and takes into account the complex sampling design to enable the included samples to be more representative and to facilitate the generalization of the current findings. However, some limitations need to be considered. First, data on serum folate and vitamin B12 concentrations were based on a single measurement, so we were unable to assess the impact of dynamic changes in both concentrations on mortality. Second, because of the lack of histologic examination and natural history information, we were unable to analyze whether the effect of folate/vitamin B12 on mortality was confounded by the severity and duration of MASLD. Third, we did not exclude drug-induced hepatic steatosis because we could not establish a causal association between the two in the current cohort. Fourth, some of the non-statistically significant findings, especially in stratified analyses, may be related to the limited sample size. More studies are needed in the future to minimize type 2 error. Fifth, due to the limited availability of medication information, we cannot rule out the potential impact of drugs on vitamin B12 and folate levels. Additionally, the vitamin B12 profile of vegetarians may differ from that of the general population. Since we were unable to identify vegetarians within our cohort, it is unclear whether the current findings can be generalized to this group. Finally, despite our attempts to eliminate reverse causality by excluding participants who died within two years of follow-up, the current results are still not representative of a causal association between serum folate/vitamin B12 and mortality due to the inherent limitations of observational studies.

Conclusions

Our results suggest a nonlinear association of serum folate and vitamin B12 levels with all-cause mortality in MASLD. Avoiding low serum folate and vitamin B12 concentrations may be potentially beneficial for reducing the risk of mortality in patients with MASLD.

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 Institutional Review Board (https://www.cdc.gov/nchs/nhanes/irba98.htm). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JZ: Writing – review & editing, Data curation, Methodology, Formal analysis, Validation, Investigation, Software. XL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. LD: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing, Software. PL: Writing – review & editing, Methodology, Supervision, Project administration, Validation, Investigation. JD: Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing, Investigation, Visualization.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors would like to thank the participants and staff of NHANES for their valuable contributions to the scientific study.

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1426103/full#supplementary-material

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 13 January 2025
DOI 10.3389/fendo.2024.1506953

OPEN ACCESS

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RECEIVED 06 October 2024
ACCEPTED 20 December 2024
PUBLISHED 13 January 2025

CITATION
Hong S, Liu Z, Li P, Zhang J and Wei H (2025) Golgi protein 73: charting new territories in diagnosing significant fibrosis in MASLD: a prospective cross-sectional study.
Front. Endocrinol. 15:1506953.
doi: 10.3389/fendo.2024.1506953

Golgi protein 73: charting new territories in diagnosing significant fibrosis in MASLD: a prospective cross-sectional study

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Objectives: To explore the correlation between serum Golgi protein 73 (GP73) levels and the degree of fibrosis in Metabolic dysfunction associated steatotic liver disease (MASLD); to establish a non-invasive diagnostic algorithm based on serum GP73 and liver elasticity.

Methods: This is a prospective cross-sectional study, including 228 patients diagnosed with MASLD from May 2018 to January 2024 at two tertiary hospitals. Clinical data and hepatic pathological features and the correlation between serum GP73 and liver fibrosis were assessed. A new algorithm was conducted after logistic regression. Receiver Operating Characteristic (ROC) curve was used to compare its diagnostic performance with traditional models.

Results: Significant fibrosis was diagnosed in 37.2% (85/228) patients. Serum GP73 levels were markedly higher in patients with significant fibrosis than in those without (128 ng/mL v.s 46 ng/mL, $p< 0.001$). Serum GP73 levels independently predicted significant liver fibrosis (adjusted odds ratio, aOR 1.028, $p< 0.001$). A new algorithm based on GP73 was developed with a higher area under ROC (AUC) of 0.840 than that of Fibrosis index-4 ($p< 0.001$).

Conclusions: Serum GP73 is an independent risk factor for significant liver fibrosis in MASLD, and the GFA (GP73-Fibroscan-Age) model has good diagnostic efficacy for significant liver fibrosis.

KEYWORDS

MASLD, metabolic diseases, liver biopsy, diagnosis, GP73

Highlights

- Serum GP73 levels correlate with the severity of hepatic fibrosis in patients diagnosed with MASLD.
- Measurement of serum GP73 enhances the diagnostic accuracy for detecting significant fibrosis in individuals with MASLD.

1 Introduction

Metabolism-Associated Steatotic Liver Disease (MASLD), previously recognized as non-alcoholic fatty liver disease (NAFLD), ranks as the foremost liver disease etiology globally (1). The escalating prevalence, notwithstanding the majority of patients not progressing to liver-related complications, is a concerning trend (2). In certain regions, MASLD emerges as the principal cause of cirrhosis and the secondary indication for liver transplantation (3). The prevalence of MASLD, in accordance with the most recent findings from a retrospective, cross-sectional study conducted in regional China using data from the health management database between 2017 and 2022, is 36.91% (4). Due to a lack of sufficient diagnostic tools and effective pharmacological treatments, many patients remain undiagnosed and untreated (5). As MASLD progresses, it can precipitate a cascade of hepatic complications, starting with inflammation (metabolic dysfunction associated steatohepatitis, MASH), advancing through stages of liver fibrosis, and potentially culminating in cirrhosis (6). Since 2023, studies have demonstrated a high degree of consistency between MASLD and NAFLD (7, 8). Given this overlap, pathological grading, and other relevant concepts from NAFLD can be applied to MASLD. This approach allows us to leverage the well-established frameworks and insights from NAFLD research to better understand and define the characteristics of MASLD.

Liver fibrosis serves as a pivotal prognostic indicator, offering predictions for the progression towards cirrhosis and hepatocellular carcinoma (HCC) (9–13). Although liver biopsy is not one of the diagnostic criteria for diagnosing MASLD, the elucidation of histopathological characteristics facilitates a precise evaluation of the condition. It remains the best standard for the accurate staging of liver fibrosis and inflammation in most chronic liver diseases (14). However, the routine use of liver biopsy is limited by the risk of complications, sampling error (15). Since a liver biopsy is still needed to establish the diagnosis, accurate assessment of fibrosis stage is labor-intensive and prone to error. Moreover, many patients are reluctant to get biopsies because of the hazards involved, which include excruciating pain and serious complications. Due to the rising incidence of MASLD and the previously mentioned limitations of liver biopsy, a number of non-invasive tests (NITs) for precise staging and risk assessment have been developed. NITs that were initially developed for staging fibrosis, are also increasingly used to determine liver-related prognosis (6). The use of NITs in risk stratification to identify patients who are more prone to experience severe liver events shows increasing promise. The constraints of a biopsy in terms of patient stratification would be overcome by using markers that are more trustworthy than a biopsy. Prognostic markers that function optimally have the potential to someday take the role of a biopsy, support clinical decision-making, and make it easier to enroll patients who will benefit from clinical trial participation. The European Association for the Study of the Liver (EASL), the European Association for the Study of Diabetes (EASD), and the European Association for the Study of Obesity (EASO) Clinical Practice Guidelines propose using basic non-invasive panels such the NAFLD Fibrosis Score (NFS) and Fibrosis Index-4 (FIB-4) as part of the diagnostic process to rule out advanced fibrosis (16). According to a systematic review (17), the performance of FIB-4, NFS in risk stratifying patients for liver-related morbidity and mortality is comparable to that of a liver biopsy. However, their usage is limited by a significant proportion of false positives and false negatives (18–21). It is acknowledged that in order to track the advancement or regression of MASLD, new NIT are required (22).

Golgi Protein 73 (GP73), identified on the Golgi apparatus and alternatively termed Golgi membrane protein 1 or Golgi phosphoprotein 2, owes its nomenclature to its molecular weight of approximately 73 kDa (23). Predominantly localized to biliary epithelial cells, the efficacy of GP73 as a serum biomarker for HCC diagnosis surpasses that of the conventional marker alpha-fetoprotein in both sensitivity and specificity (24–29). Further research has found that serum GP73 levels increase with the progression of chronic liver disease and decrease as liver pathology improves (30). Emerging evidence supports the utility of serum GP73 as a fibrosis biomarker in chronic liver diseases (31, 32). Previous studies by our team shed light on the potential role of serum GP73 in reflecting disease severity in chronic hepatitis B (CHB) (33, 34). Currently, there are some single-center studies exploring the diagnostic accuracy of serum GP73 in MASLD patients (35). The diagnostic value of GP73 for MASLD merits further investigation.

In this context, this prospective study aims to: 1) quantify serum GP73 levels in biopsy-proven MASLD and evaluate their correlation with significant fibrosis;2) establish and validate a novel algorithm combining serum GP73 and TE for diagnosis of significant fibrosis in MASLD.

Abbreviations: GP73, Golgi protein 73; MASLD, metabolic dysfunction associated steatotic liver disease; NAFLD, non-alcoholic fatty liver disease; ROC, Receiver Operating Characteristic; aOR, adjusted odds ratio; MASH, Metabolic dysfunction associated steatohepatitis; HCC, hepatocellular carcinoma; NITs, non-invasive tests; CHB, chronic hepatitis B; NFS, NAFLD Fibrosis Score; FIB-4, Fibrosis index-4; CMRF, cardiometabolic risk factors; WC, waist Circumference; BMI, body mass index; DM, Type 2 diabetes; GFA, GP73-Fibroscan-Age; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LSM, liver stiffness measurement; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAS, NAFLD activity score; PLT, platelet count; ELISA, enzyme-linked ; immunosorbent test; HRP, horseradish peroxidase; PPV, positive predictive value (PPV); NPV, negative predictive value; AUC, area under the curve; CI, Confidence intervals.

2 Subjects and methods

2.1 Study design

This dual-center, cross-sectional study prospectively recruited patients with diagnosis of steatotic liver disease in Beijing Ditan Hospital and Beijing Youan Hospital, Capital Medical University between May 2018 and Jan 2024 (Figure 1). Inclusion criteria were as follows: 1) the ability to give informed consent; 2) aged 18-75 years; 3) presence of hepatic steatosis detected by ultrasonography, computed tomography or other imaging techniques, and confirmed by liver biopsy. 4) patients with at least one of five cardiometabolic risk factors (CMRF). CMRFs were defined as follows: (1) Body mass index (BMI) ≥ 24kg/m^2 OR Waist Circumference (WC) > 90 cm (M)/80 cm (F) (36, 37); (2) A fasting serum glucose ≥ 5.6 mmol/L OR 2-hour post-load glucose levels ≥ 7.8mmol/L OR haemoglobin A1c (HbA1c) ≥ 5.7% OR diagnosis of Type 2 diabetes (DM) OR treatment for DM; (3) Blood pressure ≥130/85 mmHg OR acceptance of any antihypertension treatment; (4) Triglycerides ≥ 1.70mmol/L OR specific lipid-lowering treatment; (5) Plasma High-Density Lipoprotein Cholesterol (HDL-C) ≤1.0 mmol/L for men and ≤1.3 mmol/L for women OR use of lipid-lowering treatment.

Exclusion criteria were as follows: 1) history of excessive alcohol consumption >30 g/day for men and >20 g/day for women) or other steatogenic drugs; 2) individuals of known other liver diseases (e.g., viral hepatitis, drug-induced liver disease or autoimmune liver disease); 3) individuals of known or suspicious malignant disease or HIV infection.

2.2 Ethics

The study was approved by the Ethics Committee of Beijing Ditan Hospital and Beijing Youan Hospital, and each participant provided written informed consent for the use of their data. All methods were performed following the relevant Declaration of Helsinki. This study has been registered with the China Clinical Trials Registry, under the registration number ChiCTR1800015157, to ensure the transparency and traceability of the research.

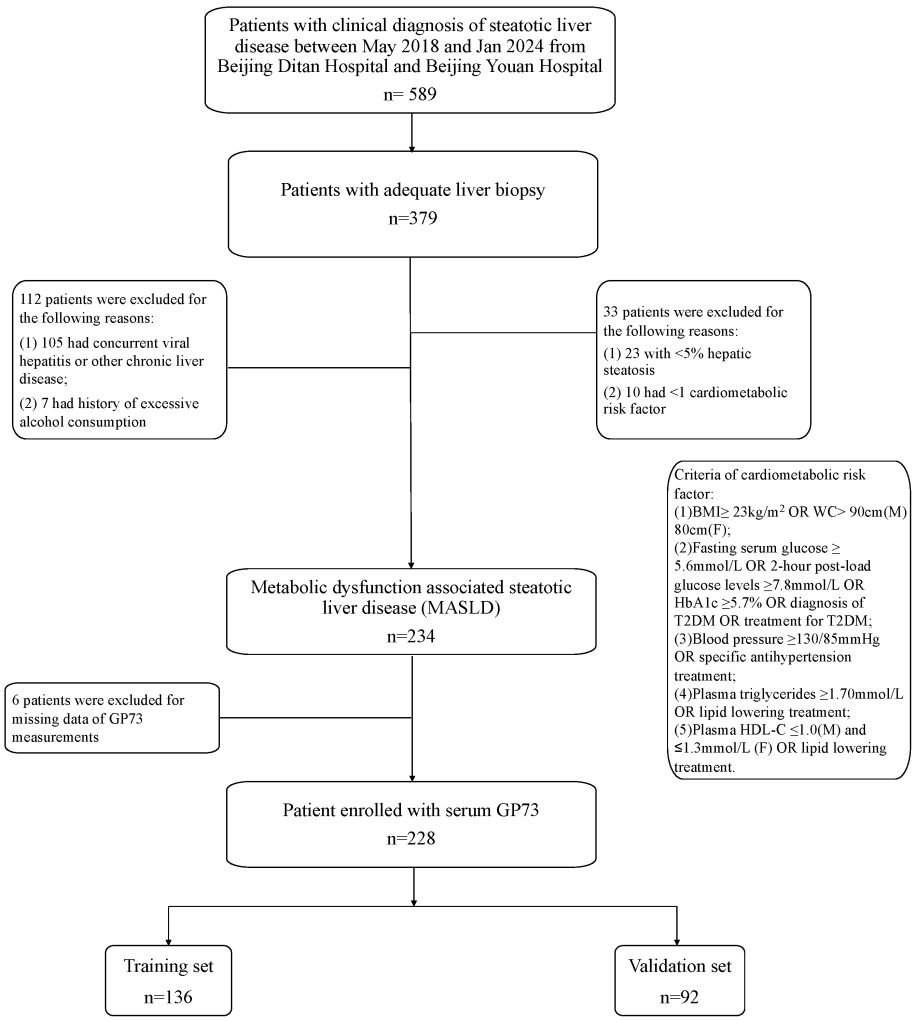

FIGURE 1
Study design. BMI, body mass index; T2DM, Type 2 diabetes; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; MASLD, metabolic dysfunction associated steatotic liver disease; GP73, Golgi protein 73.

2.3 Demographic variables and laboratory parameters

Data acquisition, encompassing a wide range of parameters such as height, weight, waist circumference, blood pressure, medication history, and past medical conditions, was carried out close to the time of liver biopsy procedures, either on the same day or within 48 hours prior. The calculation of BMI for each participant was based on their height and weight measurements. Additionally, participants' fresh samples underwent an extensive battery of standardized laboratory tests aimed at evaluating liver health and potential risk factors for associated diseases. This comprehensive panel included assessments for viral hepatitis, autoimmune antibodies, a full spectrum of liver function tests, complete blood count, coagulation function, lipid profiles, uric acid levels, blood glucose, and glycated hemoglobin. Liver stiffness evaluation was performed using Transient Elastography (TE, FibroScan) by an operator without knowledge of the participants' clinical backgrounds. To ensure precise measurements across diverse body types, the examination utilized an M probe as standard, with an XL probe for individuals with a BMI ≥30 kg/m^2. The procedure involved scanning the right liver lobe in a supine position with the right arm fully extended, aiming to achieve at least 10 valid readings. The median value of these readings was considered the final result, contingent upon the interquartile range to median ratio being ≤30%.

2.4 Quantitative detection of serum GP73

By applying a double-antibody sandwich enzyme-linked immunosorbent test (ELISA) kit (Hotgen Biotech Inc., Beijing, China), serum GP73 was quantitatively evaluated per the manufacturer's instructions. To sum up, wells precoated with monoclonal anti-GP73 were filled with sample dilution buffer (50 µL) and 20 µL serum, then incubated for one hour at 37°C. After washing, the wells were incubated for 30 minutes at 37°C with 100 µL of horseradish peroxidase (HRP) -conjugated anti-human antibody. Tetramethylbenzidine was used to develop the plate after five washings, and a microplate reader (Spectramax M2; Molecular Devices, Sunnyvale, CA, USA) was used to measure the OD450. As a calibration reference, parallel assays were performed on purified recombinant GP73. Every assay was conducted twice in triplicate. In this study, all samples were subjected to two rounds of testing, conducted in August 2022 and January 2024, respectively. Each sample was tested twice to ensure the reproducibility and reliability of the results. The coefficient of variation during the testing process was maintained at less than 15% to ensure the precision of the experiment. All testing procedures were carried out by the same experienced technician to minimize the impact of operational variability on the outcomes, while also ensuring that the clinical information of the participants was kept confidential from the technician. According to the data provided by the reagent kit manufacturer, the upper reference limit for serum GP73 in a healthy population is 53 ng/mL.

2.5 Liver biopsy and histological evaluation

Percutaneous liver biopsies were performed with real-time transabdominal ultrasonography guidance. Masson trichrome and hematoxylin-eosin stains were applied to each specimen. A biopsy specimen was deemed adequate if it measured at least 10 mm in length and had six or more portal tracts. Three seasoned histopathologists who were blind to the clinical data reevaluated the presence of ballooning and NAFLD activity score (NAS) (38). The NAS is based on a standard grading system: steatosis (on a scale of 0-3), lobular inflammation (on a scale of 0-3), and ballooning (on a scale of 0-2). Finally, an agreement had to be set in. Patients were regarded as "missing data" if the opinions of the three pathologists' evaluations were not in agreement. NAS ≥5 was used to define MASH. For analysis purposes, fibrosis stages 1a, 1b, and 1c were regarded as stage 1. Fibrosis stages 2-4 and 3–4 were used to define significant and advanced fibrosis, respectively.

2.6 Statistical analysis

Student's t-test was used to analyze normally distributed data expressed as mean ± SD. The Mann–Whitney U or Kruskal–Wallis H test was used to analyze non-normal data which were shown as median (interquartile range). The chi-square test was used to analyze counts of categorical data. Spearman's correlation was used to examine continuous variable relationships. A binary logistic regression analysis was conducted based on the presence of significant liver fibrosis as indicated by liver biopsy pathology. The sample was divided into a training group (60%, n=136) and a validation group (40%, n=92) using a random number generation method. Variables with a P-value of<0.10 in the univariate analysis were selected and incorporated into the multivariate model using a forward stepwise method. In the training group, we initially conducted univariate logistic regression analysis to select variables. Subsequently, we utilized multivariate logistic regression to further screen and refine the variables. Based on the results of the multivariate logistic regression, we formulated an equation, which constitutes our non-invasive diagnostic model. Based on the results of the multivariate logistic regression, which provided adjusted odds ratios and p-values for each variable, we formulated an equation. This equation constitutes our non-invasive diagnostic model, which was designed to predict the likelihood of significant liver fibrosis based on serum GP73 levels and other selected variables. This model was then evaluated for its diagnostic efficacy in the validation group. The diagnostic threshold (cut-off value) was determined by the maximum Youden index. The performance characteristics of the non-invasive diagnostic algorithm, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were assessed. The diagnostic performance of the non-invasive diagnostic algorithms was evaluated by analyzing the area under the receiver operating characteristic (ROC) curves. The area under the curve (AUC) provides a measure of the overall performance of a diagnostic test, with values closer to 1 indicating higher diagnostic accuracy.

Comparisons between the AUCs of different diagnostic algorithms were conducted to assess their relative performance in diagnosing the condition of interest. To compare the AUCs derived from the ROC curves, the DeLong et al. (39) method was employed, which provides a non-parametric approach to test for differences between two correlated ROC curves. This method is suitable for comparing the diagnostic accuracy of different tests applied to the same set of samples. A p-value of less than 0.05 was considered statistically significant for all analyses. Confidence intervals (95% CI) for the AUC were calculated to provide an estimate of the precision of the diagnostic accuracy measures. Additionally, sensitivity and specificity values were derived from the ROC curves to further describe the diagnostic performance of the algorithms at optimal cut-off points determined by the Youden index. A two-tailed P-value of<0.05 was considered statistically significant.

Sample Size Calculation: This is a single-sample diagnostic trial aimed at minimizing invasive procedures for patients. With an anticipated specificity of 80%, an allowable error of 0.1, a two-sided test, α=0.05, and a power of 1-β=0.9, the formula for calculating the sample size of a diagnostic study is as follows: Data were regarded as statistically significant when P< 0.05.

$$n = \left(\frac{Z_{1-\alpha/2} \times \sqrt{p_0 \times (1-p_0)} + Z_{1-\beta} \times \sqrt{p \times (1-p)}}{\delta} \right)^2$$

Z(1-α/2) and Z(1-β) are respectively represented by 1.96 and 1.28, as found in standard statistical tables. p_0 denotes the anticipated specificity, while pindicates the minimum acceptable specificity. With the expected specificity (p) set at 0.8 and the acceptable lowest specificity (p_0) at 0.7, and accounting for a 10% loss to follow up, the study required the inclusion of at least 207 participants.

Statistical analyses were conducted using SPSS 25.0 (IBM Corp., Armonk, NY, USA), MedCalc Statistical Software version 19.2 (MedCalc Software Ltd, Ostend, Belgium) and GraphPad Prism 9.3.1 (GraphPad Software, San Diego, CA, USA).

3 Results

3.1 Study population

Between May 2018 and January 2024, the investigation was carried out at Beijing Ditan Hospital, Capital Medical University and Beijing You'an Hospital, Capital Medical University. During this period, 379 participants provided informed consent and underwent liver biopsy. Initial assessments revealed that 91.3% (346 individuals) fulfilled the criteria for MASLD. The study then excluded individuals with other conditions: 101 were diagnosed with chronic hepatitis B, 3 presented with drug-induced liver injury, 1 was identified with autoimmune hepatitis, and 7 exceeded the recommended alcohol intake levels. A further 6 individuals were removed from the cohort due to inadequate serum samples for GP73 analysis. Consequently, the study focused on a cohort of 228 MASLD patients as shown in Figure 1.

3.2 Clinical and pathological characteristics

Among the patients meeting MASLD criteria, men represented 57.5% (131/228) as shown in Table 1. Significant fibrosis was diagnosed in 37.2% (85/228) patients. Higher BMI or waist circumference emerged as the predominant CMRF. Specifically, 54 participants (23.7%) exhibited at least one CMRF, whereas 103 participants (45.7%) had three or more CMRFs.

TABLE 1 Comparison of clinical characteristics of patients with metabolic associated fatty liver disease by fibrosis staging.

Characteristic	No Significant Fibrosis (n=143)	Significant Fibrosis (n=85)	p-value	Total (n=228)
Age (years)	38 (30-48)	45 (31-57)	0.025	40 (30-52)
Female, n (%)	55 (38.5%)	42 (49.4%)	0.017	97 (42.5%)
ALT (U/L)	72 (42-164)	92 (50-206)	0.216	83 (48-175)
AST (U/L)	54 (33-128)	81 (42-149)	0.044	63 (35-143)
PLT (10^9/L)	209 (164-259)	199 (154-251)	0.305	206 (165-256)
Total Cholesterol (mmol/L)	4.74 (4.09-5.41)	4.86 (4.34-5.60)	0.606	4.80 (4.22-5.55)
TG (mmol/L)	1.46 (0.98-2.17)	1.74 (1.20-2.73)	0.625	1.56 (1.05-2.45)
HDL-C (mmol/L)	1.06 (0.90-1.21)	1.02 (0.84-1.32)	0.768	1.04 (0.92-1.22)
LDL-C (mmol/L)	3.04 (2.66-3.92)	3.23 (2.53-3.90)	0.333	3.07 (2.61-3.90)
LSM (kPa)	6.1 (5.3-7.1)	7.6 (4.6-12.8)	<0.001	7.0 (5.5-9.2)
GP73 (ng/mL)	46.1 (32.8-77.1)	128.2 (80.9-183.6)	<0.001	66.1 (38.5-130.8)

ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; LSM, Liver Stiffness measurement; PLT, Platelet Count; GP73, Golgi Protein 73; TG, Triglycerides
Values are presented as median (interquartile range) or number (percentage) as appropriate.

3.3 Association between significant fibrosis and serum GP73 levels

The study participants were categorized based on the degree of liver fibrosis into two groups: those with significant fibrosis (F≥2) and those without significant fibrosis (F0-1) for comparative analysis. The findings indicated that patients with significant fibrosis were older on average (45 years v.s 38 years, p= 0.025) and had a higher proportion of females (49.4% v.s 38.5%, p= 0.017). With the progression of liver fibrosis, GP73 levels gradually increase. Additionally, serum GP73 levels were markedly higher in patients with significant fibrosis than in those without (128 ng/mL v.s 46 ng/mL, p< 0.001) as shown in Figure 2. However, no statistically significant differences were observed between the two groups in terms of ALT or HDL-C. A correlation coefficient of 0.269 (p< 0.001) was identified between GP73 levels and NAS.

3.4 Pathological characteristics

Liver fibrosis was graded as F0-1 in 143 cases, accounting for 62.7%; F2 was observed in 49 cases, representing 21.5%; and patients reaching advanced liver fibrosis (F3-4) totaled 36, comprising 15.8% of the cohort. Consequently, the proportion of patients diagnosed with significant liver fibrosis (F2-4) was 37.3%. A diagnosis of MASH was made in 80 cases (35.1%). A statistically significant correlation was identified between the diagnosis of MASH and the presence of significant liver fibrosis, with a Pearson correlation coefficient of 0.402 (p< 0.001). The AUC for MASH in predicting significant liver fibrosis was 0.699 (95% CI: 0.626-0.772).

Furthermore, patients were stratified into three groups based on the number of CMRFs they possessed (1, 2, ≥3) to investigate the relationship between the quantity of risk factors and the incidence of significant liver fibrosis. The incidence of MASH significantly escalated with an increasing number of risk factors (Supplementary Figure S1), recorded at 20.4%, 23.9%, and 50.5%, respectively, with this difference being statistically significant (p< 0.001).

3.5 GP73 as an independent predictor of significant liver fibrosis

The degree of liver fibrosis (stages F2-4) was served as the positive outcome of interest in training set (n=136). Univariate and multivariate logistic regression analyses were conducted, and factors associated with significant liver fibrosis were identified. The analysis indicated that patient age, liver stiffness measurement (LSM) values, and serum GP73 levels each independently predicted significant liver fibrosis as shown in Table 2. Based on the results of the multivariate analysis, we developed a novel non-invasive diagnostic algorithm, named "GFA" (GP73-Fibroscan-Age), which integrates information on GP73, LSM, and age. The formula for the GFA algorithm is as follows:

$$-8.757 + 1.048 \times Agey + 1.028 \times GP73ng/mL + 1.588 \times LSM(kpa)$$

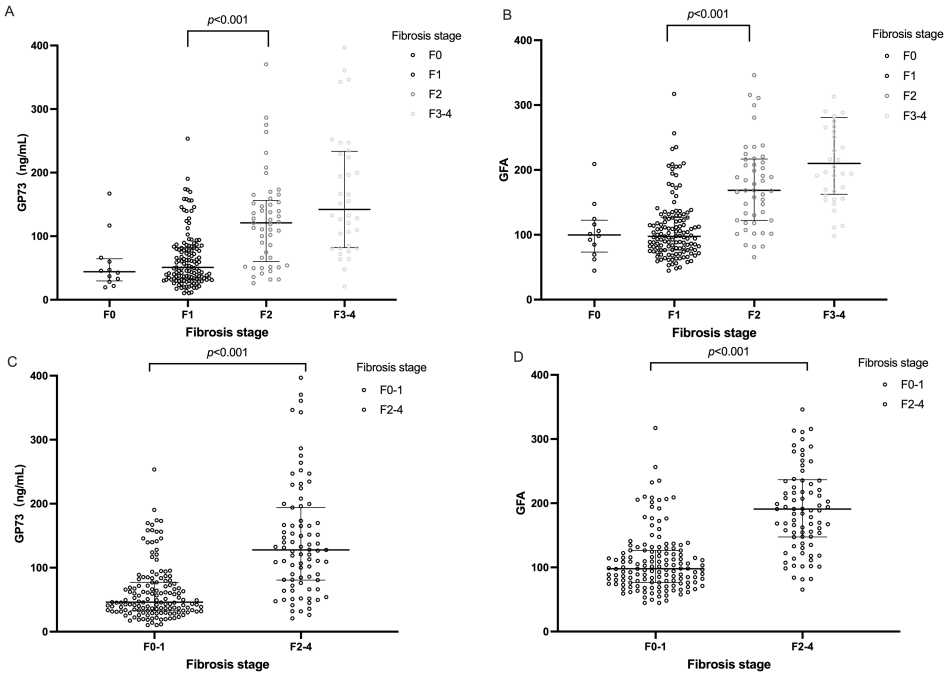

FIGURE 2
Distributions of serum GP73 value and GFA score according to fibrosis stage. The horizontal bar inside the scatterplots represents the median value. Comparisons between each two groups were performed using Mann–Whitney U test. **(A)** Distributions of serum GP73 levels according to fibrosis stage. **(B)** GFA score values according to fibrosis stage. **(C)** Distribution of serum GP73 levels in patients with and without significant hepatic fibrosis. **(D)** Distribution of GFA score values in patients with and without significant hepatic fibrosis. Due to the small sample size of the F4 fibrosis stage, we combined and presented the samples from stages F3-F4 for analysis. GFA= -8.757+1.048\times Agey+1.028×GP73ng/mL+1.588×LSM(kpa).

TABLE 2 Univariate and multivariate logistic regression analysis results for significant liver fibrosis.

Variable	Significant fibrosis			
	Univariate analysis		Multivariate analysis	
	OR (95%CI)	*p*-value	aOR (95%CI)	*p*-value
Male gender		0.096		
Age		0.072	1.048 (1.005-1.092)	0.029
BMI		0.053		
ALT		0.390		
AST		0.380		
ALB	0.874 (0.811-0.943)	<0.001		
TG		0.988		
HDL-C		0.059		
GP73	1.020 (1.012-1.028)	<0.001	1.028 (1.017-1.039)	<0.001
PLT		0.374		
LSM	1.496 (1.254-1.784)	<0.001	1.588 (1.306-1.931)	<0.001

BMI, Body Mass Index; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; HDL-C, High-Density Lipoprotein Cholesterol; LSM, Liver Stiffness measurement; PLT, Platelet Count; GP73, Golgi Protein 73; TG, Triglycerides; ALB, albumin

Consistent with GP73 levels, the GFA algorithm values show a gradual increasing trend with the progression of liver fibrosis stages as shown in Figure 2.

3.6 Comparison of diagnostic accuracy between the GFA model and traditional NITs

To further compare the diagnostic performance of the new diagnostic algorithm with traditional algorithms, we compared the diagnostic performance of GFA, which was based on LSM and GP73, and serological-based models such as FIB-4 and NFS levels across the entire sample by constructing ROC curves. The AUC for the GFA model was 0.860 (95% Confidence Interval: 0.811-0.909). The difference in AUC between the GFA model and liver stiffness measurements (an incremental increase of 0.070) did not reach statistical significance (p=0.301). However, the diagnostic performance of the GFA model was significantly superior to that of the FIB-4 and NFS (p< 0.001). This conclusion was also reflected in the comparison within the validation group (92/228), where the GFA model's AUC for significant liver fibrosis was superior to LSM, FIB-4, and NFS as shown in Table 3 and Figure 3.

4 Discussion

This study prospectively established a non-invasive diagnostic algorithm based on GP73, and for the first time compared the diagnostic efficacy of the GP73-based diagnostic algorithm and conversional NITs for significant liver fibrosis in MASLD patients confirmed by liver biopsy. A key finding of the study is that GP73 is an independent risk factor for significant liver fibrosis in MASLD.

The first hallmark of our study is that serum GP73 was independently associated with higher risk of fibrosis. Zheng et al., 2020 (40) conducted serum GP73 and cytokeratin-18 M30 fragment testing on 105 patients with biopsy-confirmed MASLD despite persistently normal alanine aminotransferase levels, establishing a non-invasive diagnostic algorithm for nonalcoholic steatohepatitis (NASH) using both markers sequentially, achieving an accuracy of 82.9%. Current research on the correlation between GP73 levels and liver fibrosis in the MASLD population is scarce. A study with a

TABLE 3 Comparison of performance of different non-invasive diagnostic models in diagnosing significant liver fibrosis in validation set.

Model	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P-value (compared to GFA)
LSM	0.807 (0.711-0.881)	82.86	73.68	65.90	87.50	0.286
GFA	0.840 (0.748-0.908)	82.86	82.46	74.36	88.68	–
NFS	0.527 (0.419-0.632)	52.94	64.91	48.08	69.20	<0.001
FIB-4	0.575 (0.466-0.679)	51.43	70.91	52.94	69.64	<0.001

AUC, Area Under the Receiver Operating Characteristic Curve; CI, Confidence Interval; LSM, liver stiffness measurement; NFS, NAFLD Fibrosis Score; FIB-4, Fibrosis-4; GFA, GP73-Fibroscan-Age; PPV, Positive Predictive Value; NPV, Negative Predictive Value. Values are presented with sensitivity, specificity, positive predictive value, and negative predictive value percentages. P-values are calculated in comparison to the GFA model.

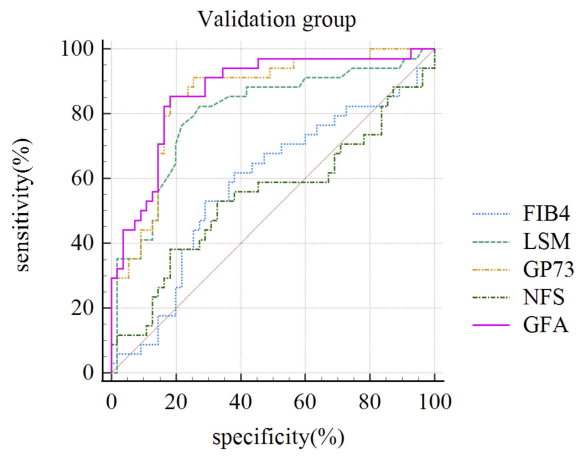

FIGURE 3
ROC Curves of Different Non-Invasive Diagnostic Models for Diagnosing Significant Liver Fibrosis in the Validation Group (n=92). FIB-4, Fibrosis index-4; LSM, liver stiffness measurement; GP73, Golgi protein 73; NFS, NAFLD Fibrosis Score; GFA, GP73-Fibroscan-Age.

sample size of 91 (35) found a positive correlation between serum GP73 concentration and significant liver fibrosis in MASLD, consistent with our findings. A systematic review analyzed the sensitivity and specificity of GP73 for liver fibrosis in chronic liver disease as 0.63 (95% CI = 0.60–0.65) and 0.79 (95% CI = 0.76–0.81), respectively, with an AUC of 0.818, although the study did not perform stratified analysis by different liver disease etiologies (41). In a retrospective analysis of 497 chronic hepatitis B virus-infected individuals (42), found a significant correlation between serum GP73 levels and liver fibrosis staging (r=0.539). A retrospective analysis of untreated chronic hepatitis B found a positive correlation between serum GP73 levels and liver fibrosis grading, unaffected by e antigen, HBV DNA viral load, or ALT levels (43). This study using 267 cases for model development and validated in a group of 133 confirmed that serum GP73's diagnostic performance was superior to FIB-4 (AUC 0.76 vs. 0.66) and combining G73 with liver elasticity achieved an AUC of 0.85. These results above all suggest the significant diagnostic value of GP73 in liver fibrosis.

Elevated serum GP73 levels were found to have a good correlation with MASH in our study, and MASH has good consistency with the diagnosis of significant liver fibrosis (69.9%), with patients with severe liver inflammation showing elevated serum GP73. A point of debate centers on the source of elevated GP73 levels in MASLD patients: is it hepatocyte damage or liver fibrosis that primarily contributes to this increase? Iftikhar et al. (44) revealed that the main trigger for GP73 expression is progressive tissue remodeling and fibrogenesis in chronic liver disease. Many studies including our previous research have explored that serum GP73 concentration is correlated with liver fibrosis. While attempting to clarify the mechanisms underlying, efforts are also being made to disseminate and implement the findings in clinical settings (38, 45–48). Intracellularly, GP73 expression is influenced by transcriptional and post-translational modifications, including Furin-mediated cleavage. *In vitro*, serum GP73 levels possibly upregulated under inflammatory conditions (49) and *in vivo*, the elevation of serum GP73 level was triggered by liver inflammatory injury (50). Extracellularly, GP73 levels are influenced by factors such as inflammation, liver injury, and changes in liver function that affect protein shedding and clearance. Within the spectrum of chronic liver diseases, the expression levels of GP73 protein and mRNA rise progressively, manifesting in both hepatocytes and in stellate cells that have been activated, with the latter being particularly implicated in the development of liver fibrosis (51, 52). Whether its influence interferes with the diagnosis of liver fibrosis still requires further stratified analysis of inflammation levels, which affect the accuracy of this serum marker as a single biomarker for significant liver fibrosis.

The second hallmark our study is that a new algorithm was conducted with a good diagnostic accuracy. Currently, serological markers for liver fibrosis are divided into two types: indirect and direct. Indirect serum markers include combinations of routine laboratory parameters and demographic data such as age (53). Recently, Agile 3+ and 4 scores were established, combining LSM with inherent demographic data (age, sex, and the presence of type 2 diabetes) and serum biomarkers (ALT, AST, and platelets) to better diagnose advanced fibrosis and cirrhosis in MASLD, with diagnostic efficacy AUC of 0.85, similar to that of LSM (AUC 0.83, p= 0.142) (54–57). This algorithm is similar to the GFA explored in this study, and both are significantly superior to FIB-4, but its calculation method is more complex, and the inclusion of more reference indicators limits its application. GFA can be simply applied using a single serological and liver elasticity measurement, which is easy to operate. Serum GP73 can be measured using commercial ELISA kits, which can be established in hospitals or clinics with biochemical laboratories, with the cost of a single test only about 100 RMB and can be conducted simultaneously with other liver function tests. The GFA model only requires knowledge of the patient's age, LSM, and GP73 level, low cost, and high accuracy in predicting significant liver fibrosis, and dynamic monitoring may also predict the occurrence of liver cancer, with further cost-effectiveness ratio and predictive effect on prognosis that can be quantified by subsequent research.

There is a growing recognition of the intricate interplay between metabolic risk factors such as obesity, diabetes, chronic kidney disease, and cardiovascular disease, highlighting their shared pathophysiological underpinnings (58). Though MASLD is prevalent as a chronic liver condition often progressing to cirrhosis, empirical evidence suggests that cardiovascular issues are the leading contributors to mortality in patients with this disease (59). Previous study provides significant evidence that GP73 is causally associated with incident coronary artery disease and other atherosclerotic events sharing similar etiology based on proteomics and 2-sample Mendelian randomization design, suggesting its role in the pathogenesis of coronary artery disease (60). Elevated vascular GP73 expression has been validated through the examination of both murine models and human tissue samples. In the realm of translational medicine, it is anticipated that forthcoming foundational studies that substantiate and elucidate the fundamental pathological mechanisms of GP73 or the GFA algorithm proposed in this study based on GP73 could contribute to the development of efficacious strategies for the management of MASLD.

The incorporation of these non-invasive techniques holds great potential to transform the diagnostic environment as the field develops, enabling prompt interventions and enhancing patient

outcomes for MASLD patients. Patients with MASLD can now benefit from precise diagnosis and risk evaluations without invasive liver biopsies due to the development of reliable NIT based on GP73. Future studies will further explore whether GP73 could serve as a valuable prognostic tool for assessing the risk of liver-related events and overall mortality.

The limitations of the study include the following: This is a dual-center study, with research samples from two tertiary hospitals focusing on liver disease, and all patients underwent liver biopsy, as patients undergoing liver biopsy in large hospitals are usually considered to have a higher risk of adverse outcomes, which may have caused selection bias. To minimize potential variability related to differences in sample storage duration, an interim analysis was performed in August 2022, followed by a final assessment in January 2024, after the completion of sample collection. These measures were taken to mitigate any effects associated with prolonged serum storage, ensuring consistency in the data. Due to the current lack of widespread testing for GP73, the clinical sample size is limited, and our new algorithm has not been externally validated. However, despite the small sample size, the percentage of significant liver fibrosis and demographic characteristics are very similar to those of previous larger sample MASLD studies conducted by our team (7), suggesting that the results of this study can be extrapolated and generalized. Furthermore, due to the small number of cases of significant liver fibrosis (n=85), further stratified analysis of the population for the proportion of positive outcomes could not be conducted. Therefore, further large-scale, multi-center prospective validation of this diagnostic algorithm is needed before it can be widely applied.

5 Conclusion

In summary, serum GP73 is an independent risk factor for significant liver fibrosis in MASLD, and the GFA model based on GP73 combined with LSM has good diagnostic efficacy for significant liver fibrosis, effectively reducing the need for liver biopsy pathology in MASLD patients.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethics Committee of Beijing Ditan Hospital, Capital Medical University [No. 2018(024)] and Beijing Youan Hospital, Capital Medical University [No.2019(025)]. This study has been registered with the China Clinical Trials Registry, under the registration number ChiCTR1800015157, to ensure the transparency and traceability of the research. 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

SH: Data curation, Formal analysis, Methodology, Validation, Writing – original draft. ZL: Conceptualization, Data curation, Formal analysis, Writing – original draft, Visualization. PL: Formal analysis, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing. JZ: Funding acquisition, Investigation, Supervision, Validation, Writing – review & editing. HW: Supervision, Writing – review & editing, Funding acquisition.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. In the submitted manuscript, we declare that the funding for the research, specifically for the experiments involving the detection of Golgi protein 73 (GP73), was provided by the Capital Foundation for Clinical Characteristic Applied Research Projects. The grant number associated with this funding is Z181100001718084. This funding source played a pivotal role in enabling the completion of the GP73 detection experiments, which are a central component of our study.

Conflict of interest

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

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1506953/full#supplementary-material

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 14 January 2025
DOI 10.3389/fendo.2024.1499735

OPEN ACCESS

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RECEIVED 21 September 2024
ACCEPTED 20 December 2024
PUBLISHED 14 January 2025

CITATION
Wang R, Wu N, Qu H, Zheng X, Zhang H, Zhu L, Wang X, Yao X and Zhang L (2025) The association between working hours and working type with non-alcoholic fatty liver disease: results from the NHANES 1999-2014. *Front. Endocrinol.* 15:1499735. doi: 10.3389/fendo.2024.1499735

The association between working hours and working type with non-alcoholic fatty liver disease: results from the NHANES 1999-2014

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Background: Previous research has indicated that long working hours are connected to a variety of health conditions, including nonalcoholic fatty liver disease (NAFLD). However, this association which has been observed in more population is limited. Our research is designed to evaluate the association between working hours, working type, and NAFLD.

Methods: The study comprised adults with complete details on working hours, working type, and NAFLD from the NHANES 1999-2014. We employed the hepatic steatosis index (HSI) to evaluate NAFLD and examined the relationship between working hours or working type and hepatic steatosis using weighted multiple-variable regression models and restricted cubic spline (RCS) analysis. In addition, further subgroup analysis was performed based on sex, age, ratio of family income to poverty (PIR), education, and diabetes.

Results: Long working hours were significantly linked to an elevated risk of NAFLD (OR: 1.57, 95%CI: 1.21-2.05), even after controlling for confounding factors. RCS analysis suggested that there was no nonlinear relationship between them. When weekly working hours > 50, the likelihood of NAFLD among the population heightened to 57% and this risk increased to 99% in the female population. As for working type, increasing physical intensity of work was associated with higher NAFLD risk, but only heavy manual labor continued to show significance after adjustment (OR:1.39, 95%CI: 1.06-1.81). We observed that the relationship between heavy manual labor and NAFLD was more significant in the older and male populations.

Conclusion: Our results indicate that long working hours and engaging in heavy physical labor are independent risk factors for NAFLD. As working hours increase and individuals engage in heavy physical labor for extended periods, the risk of developing NAFLD significantly rises.

KEYWORDS

working hours, working type, hepatic steatosis index, NAFLD, NHANES

Introduction

Long working hours can have negative health consequences (1), and research has indicated that long working hours may lead to an heightened risk of hypertension (2), diabetes (3), cardiovascular disease (4, 5), obesity (6), even depression and suicidal tendencies (7). Therefore, it is crucial to plan work hours reasonably. The statutory limit on weekly work hours is less than 48 hours in most European nations (8), and about half of these nations have set a 40-hour workweek cap. Nevertheless, approximately one-third of the global labor force still works more than 48 hours per week.

It is beneficial for health to engage in exercise during free time, and it is also essential for sustaining and enhancing physical strength and work performance (9, 10). One might think that physically demanding work has an advantageous impact on health (11). However, the physical demands of work may actually be detrimental. Reports suggest that jobs with high physical demands are linked to greater levels of disability, reduced body function, and decreased in muscle power (12–14).The differences in physical activity patterns between work and leisure time might be a crucial factor in clarifying this occurrence (15). Furthermore, intense manual labor could contribute to a lack of exercise during leisure time (16).

Non-alcoholic fatty liver disease (NAFLD) is a prevalent metabolic condition with an increasing global incidence and has become a significant factor in chronic liver disease in many parts of the world (17, 18). As people's understanding of the disease deepens, it has been discovered that NAFLD is actually a disease related to metabolic dysfunction. It was renamed metabolic dysfunction-associated fatty liver disease (MAFLD) in 2020 and subsequently renamed metabolic dysfunction-associated steatotic liver disease (MASLD) in 2023, and a new category, metabolic dysfunction and alcohol-associated liver disease (MetALD), was proposed (19–21). The natural course of NAFLD includes a wide range of pathological conditions, from simple steatosis to steatohepatitis (NASH), as well as with varying levels of fibrosis and cirrhosis (22). Many factors are associated with NAFLD, including obesity, diabetes, hypertension, and dyslipidemia (23, 24). The main cause of death in patients with NAFLD is cardiovascular disease (25). NAFLD is becoming a more significant public health challenge (17), making prevention crucially important.

Both long working hours and heavy physical labor may impact people's health, therefore we aim to analyze the connection between working hours, working type, and NAFLD. Previous studies have shown that working long hours is strongly linked to NAFLD (26), but they did not indicate whether the association is linear or a dose-response. Additionally, the Korean data may not be representative of the situation in other regions, as it only represents a subset of the Asian population. This study aims to expand the population to include the United States to understand whether the link between long working hours and NAFLD exists in this context. Additionally, no one has explicitly studied the relationship between working type and NAFLD. We will classify occupations based on work intensity to investigate whether different types of occupations are associated with NAFLD.

Methods

Research design and subjects

Data were obtained from the National Health and Nutrition Examination Survey (NHANES), which aims to evaluate the health and nutritional status of both children and adults in the United States (27, 28). We screened 43793 participants aged 20 years and older, which was collected from the NHANES 1999-2014. Exclusion criteria included: (1) other current hepatic disorders or factors leading to chronic liver disease, including hepatitis resulting from hepatitis B virus (HBV), hepatitis c virus (HCV), and liver damage caused by iron overload and liver tumors (n = 5976); (2) individuals who consume more than 1 alcoholic drink per day for women or 2 for men (n = 3711); (3) missing data on the hepatic steatosis index (HSI) and alcohol (n = 459), (4) pregnant participants (n = 222). (5) other missing values (n = 1067). Finally, we filtered out 5210 participants with working hours data and 5116 participants with working type. Figure 1 shows the flow of participants. The protocol was approved by National Center for Health Statistics (NCHS) Research Ethics Review Board, and all participants provided informed consent. Detailed information can be found at https://www.cdc.gov/nchs/nhanes/.

Survey and laboratory analyses

Basic demographic features included age, sex, race (non-Hispanic White, non-Hispanic Black, Mexican American, or other), ratio of

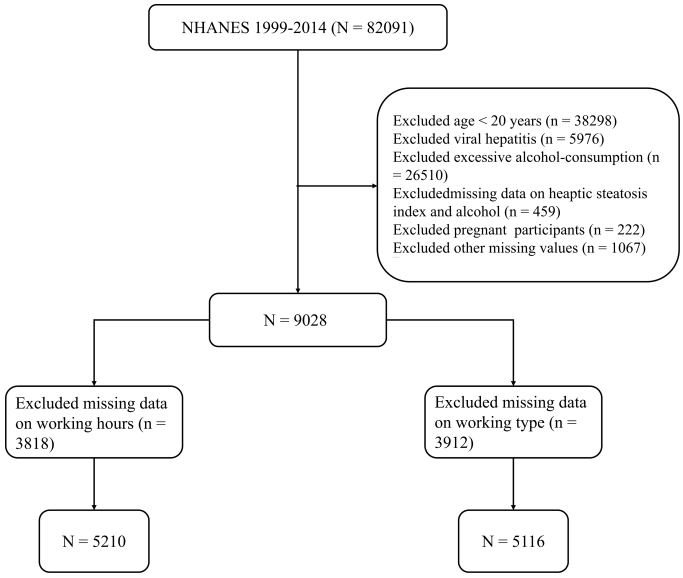

FIGURE 1
Flowchart of participants selection.

family income to poverty (PIR), education level (less than high school, high school and some college or above), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), smoking status, and diabetes. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), and triglycerides (TG) were extracted. The SBP and DBP data were obtained as the average of three measurements, while data on smoking and diabetes were collected from questionnaires. Detailed laboratory testing methods such as TC, TG, ALT, AST can be found at: https://www.cdc.gov/nchs/data/series/sr_01/sr01_056.pdf. The PIR was used to categorize family income levels into low (PIR< 1.3), middle (PIR: 1.3–3.5), and high (PIR > 3.5) groups. The definition of smoking was established as having smoked 100 or more cigarettes in one's lifetime or being a current smoker. Diabetes was defined as being informed by a doctor of having diabetes or having a fasting blood glucose concentration of 126 mg/dL or more. We classified working hours into the following categories: less than 30 hours per week, 30-40 hours, 40-50 hours, and more than 50 hours. Working type was classified into four categories: mental labor, light physical labor, medium physical labor, and heavy physical labor (Supplementary Table S1). We used the HSI (29) to diagnose NAFLD in this study due to the absence of abdominal ultrasound data:

$$HSI = \frac{\text{alanine aminotransferase (ALT)}}{\text{aspartate aminotransferase (AST)}} + \text{body mass index (BMI)} + 2 \text{ (if diabetic)} + 2 \text{ (if female)}.$$

Prior research has shown a strong correlation between NAFLD and the extent of hepatic steatosis; therefore, we define NAFLD as an HSI greater than 36 (30).

Statistical analysis

In descriptive analysis, data for categorical variables were reported as frequency (percentages), while continuous variables were reported as medians (IQR) due to their skewed distribution. Kruskal-Wallis tests were conducted to compare differences in continuous variables among groups, while categorical variable differences were examined using chi-square tests. Multivariate logistic regression incorporating weights was used to evaluate the association between working hours or working type and NAFLD across different models. Model 1: No adjustment was made for confounding variables. Model 2: Consideration was given to age, sex, race, PIR, and education in the adjustments. Model 3: Based on model 2, additional factors were considered, including ALT, TC, TG, DBP, SBP, smoking status, and diabetes. We employed restricted cubic spline (RCS) analysis to examine the potential non-linear relationship between working hours and NAFLD. Subgroup analysis was conducted to explore the relationship between working hours or type and NAFLD. Stratification factors included gender (male/female), age (≤ 40/40-60/> 60years), PIR (≤ 1.3/1.3-3.5/> 3.5), education level (less than high school, high school and some college or above), and diabetes (yes/no). We conducted interaction analysis to examine the heterogeneity of the relationship among various subgroups. All of our data analyses were conducted using R version 4.3.2. A two-sided *P* value less than 0.05 was considered statistically significant.

Results

Baseline characteristics of participants

After excluding participants with significant alcohol intake, viral hepatitis, other liver conditions, and those who missed key parameters, we identified 5210 participants for the working hours analysis and 5116 participants for the working type analysis. Our study found that for working hours, the average age of the total population was 45.4 years, with males accounting for 62.7%. In

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terms of working type, the average age of participants was 57.0 years, with males accounting for 57.3%.

The working hours showed significant differences among participants based on sex, age, race, education level, PIR, and ALT. As working hours increased, the proportion of males gradually increased. The median age was 50.0 years in the ≤ 30 hours group, 44.0 years in the 30-40 hours group, 45.0 years in the 40-50 hours group, and 44.0 years in the > 50 hours group. This indicates that younger participants may work longer hours than middle-aged and older participants. In the ≤ 30 hours group, 24.6% of participants were from low-income households, 32.2% from middle-income households, and 43.2% from high-income households. In the > 50 hours group, only 13.6% of participants were from low-income households, 27.9% from middle-income households, and 58.4% from high-income households. The proportion of smokers and individuals with diabetes was higher in the< 30 hours work duration group (Table 1).

TABLE 1 Baseline characteristics of participants in the NHANES 1999-2014 cycles.

Characteristics	Working hours [a]				*P*
	≤ 30 (n = 965)	30-40 (n = 2094)	40-50 (n = 1227)	> 50 (n = 924)	
Sex, n (%)					**< 0.001**
Male	485 (50.3)	1221 (58.3)	848 (69.1)	712 (77.1)	
Female	480 (49.7)	873 (41.7)	379 (30.9)	212 (22.9)	
Age, median (IQR), years	50.0 (33.0, 64.0)	44.0 (34.0, 54.0)	45.0 (36.0, 54.0)	44.0 (34.0, 54.0)	**< 0.001**
Race, n (%)					**< 0.001**
Mexican American	107 (11.1)	315 (15.0)	165 (13.4)	117 (12.7)	
Non-Hispanic White	573 (59.4)	972 (46.4)	721 (58.8)	495 (53.6)	
Non-Hispanic Black	161 (16.7)	472 (22.5)	171 (13.9)	187 (20.2)	
Other	124 (12.8)	335 (16.0)	170 (13.9)	125 (13.5)	
Education, n (%)					**< 0.001**
Less than high school	308 (31.9)	733 (35.0)	341 (27.8)	275 (29.8)	
High school	310 (32.1)	657 (31.4)	345 (28.1)	282 (30.5)	
Some college or above	347 (36.0)	704 (33.6)	541 (44.1)	367 (39.7)	
Family income [b], n (%)					**< 0.001**
Low	237 (24.6)	327 (15.6)	135 (11.0)	126 (13.6)	
Medium	311 (32.2)	725 (34.6)	313 (25.5)	258 (27.9)	
High	417 (43.2)	1042 (49.8)	779 (63.5)	540 (58.4)	
BMI (kg/m^2), median (IQR)	26.9 (23.5, 30.9)	27.5 (24.3, 31.6)	27.4 (24.6, 30.8)	27.6 (24.7, 32.1)	0.466
TC (mmol/L), median (IQR)	5.0 (4.4, 5.7)	5.0 (4.4, 5.7)	5.1 (4.5, 5.7)	5.0 (4.4, 5.7)	0.532
TG (mmol/L), median (IQR)	1.3 (0.8, 2.0)	1.3 (0.8, 1.9)	1.3 (0.9, 1.9)	1.3 (0.8, 2.1)	0.666
ALT (U/L), median (IQR)	20.0 (16.0, 27.0)	22.0 (17.0, 30.0)	23.0 (18.0, 31.0)	23.0 (18.0, 32.0)	**< 0.001**
AST (U/L), median (IQR)	23.0 (20.0, 27.0)	23.0 (20.0, 27.0)	23.0 (20.0, 28.0)	23.0 (20.0, 28.0)	0.506
SBP (mmHg), median (IQR)	120.0 (110.0, 134.0)	118.0 (110.0, 130.0)	118.0 (110.0, 128.0)	118.0 (110.0, 128.0)	0.066
DBP (mmHg), median (IQR)	70.0 (64.0, 78.0)	72.0 (66.0, 78.0)	72.0 (66.0, 80.0)	72.0 (66.0, 80.0)	0.255
Smoking, n (%)					0.061
No	579 (60.0)	1333 (63.7)	769 (62.7)	547 (59.2)	
Yes	386 (40.0)	761 (36.3)	458 (37.3)	377 (40.8)	
Diabetes, n (%)					**0.007**
No	863 (89.4)	1934 (92.4)	1145 (93.3)	854 (92.4)	
Yes	102 (10.6)	160 (7.6)	82 (6.7)	70 (7.6)	

[a] working hours/week; [b] Categorized into the following 3 levels based on the family poverty income ratio: low income (≤ 1.3), medium income (1.3 to 3.5), and high income (> 3.5). BMI, body mass index; TC, total cholesterol; TG, triglyceride; ALT, alanine aminotransferase; AST, aspartate transaminase; SBP, systolic blood pressure; DBP, diastolic blood pressure; IQR, interquartile range.
Bold indicates significant results.

Association between different working hours and NAFLD

Compared to the reference group (≤ 30 hours), the risk of NAFLD increased with longer working hours after adjusting for confounding factors, with the significant increase in the > 50 hours group (OR: 1.57, 95%CI: 1.21-2.05, $P = 0.006$) (Table 2). We found that even after adjusting for age, gender, race, education level, household income, TC, TG, SBP, DBP, ALT, smoking, and diabetes, the relationship between working hours and NAFLD still persisted. In the RCS analysis, no significant nonlinear association of working hours with NAFLD was found ($P_{nonlinear} = 0.162$), but a linear correlation may exist (Figure 2).

Relationship between working type and NAFLD

The type of work was significantly associated with NAFLD before adjusting for confounding factors, and the risk of developing NAFLD increased with higher work intensity. In light physical labor, the probability of NAFLD heightened by 33% (OR: 1.33, 95%CI: 1.08-1.65, $P = 0.008$). In medium physical labor, the risk heightened by 62% (OR: 1.62, 95%CI: 1.26-2.08, $P< 0.001$). For heavy physical labor, the risk heightened by 76% (OR: 1.76, 95%CI: 1.44-2.51, $P< 0.001$). After correcting for age, sex, race, education level, and PIR, no significant connection existed between light physical labor and NAFLD ($P = 0.299$). Medium physical labor and heavy physical labor were significantly associated with NAFLD. Heavy physical labor showed a 37% increased risk (OR: 1.37, 95%CI: 1.07-1.76, $P = 0.014$). Further adjustment for TC, TG, SBP, DBP, ALT, smoking status, and diabetes showed that this significant association was only observed in heavy physical labor (OR: 1.39, 95%CI: 1.06-1.81, $P = 0.018$). Additionally, there was a tendency toward an association between medium physical labor and NAFLD ($P = 0.087$) (Table 3).

Relationship between working hours and NAFLD in various subgroups

In subgroup analyses stratified by sex, our findings indicated a notable positive correlation between working hours and NAFLD in

TABLE 2 The association between working hours and NAFLD in participants from NHANES 1999-2014.

Characteristic	Working hours						
	≤ 30	30-40		40-50		> 50	
		OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Model 1[a]	Ref.	**1.37 (1.14, 1.65)**	**0.001**	**1.27 (1.01, 1.60)**	**0.039**	**1.44 (1.11, 1.87)**	**0.006**
Model 2[b]	Ref.	**1.45 (1.20, 1.77)**	**< 0.001**	**1.42 (1.12, 1.81)**	**0.004**	**1.61 (1.21, 2.14)**	**0.001**
Model 3[c]	Ref.	**1.38 (1.12, 1.69)**	**0.003**	**1.36 (1.06, 1.75)**	**0.017**	**1.57 (1.21, 2.05)**	**0.001**

[a] unadjusted; [b] adjusted for age, sex, race, educational level, family income; [c] Adjusted for Model 2, smoking, diabetes, total cholesterol, triglyceride, systolic blood pressure, diastolic blood pressure, alanine aminotransferase. NAFLD, nonalcoholic fatty liver disease.
Bold indicates significant results.

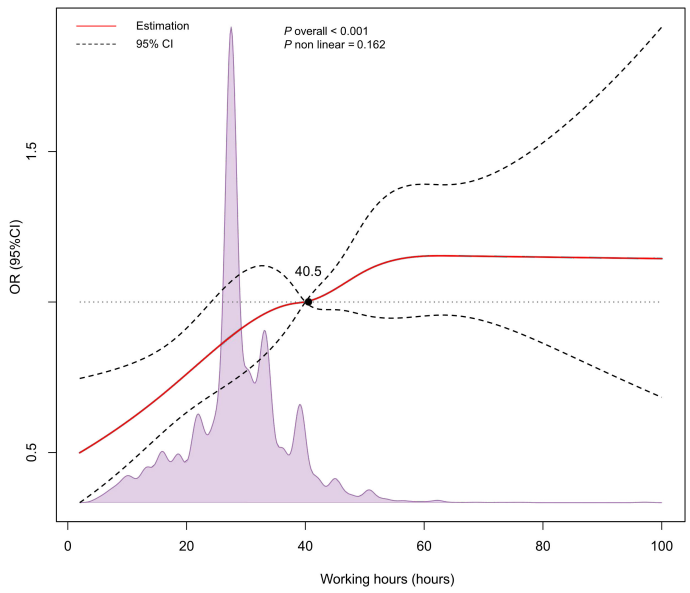

FIGURE 2
Restricted cubic spline (RCS) analysis for the relationship between working hours and NAFLD.

10.3389/fendo.2024.1499735

TABLE 3 The association between working type and NAFLD in participants from NHANES 1999-2014.

Characteristic	Working type					
	Mental labor	Light physical labor		Medium physical labor		Heavy physical labor	
		OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Model 1[a]	Ref.	**1.33 (1.08, 1.65)**	**0.008**	**1.62 (1.26, 2.08)**	**< 0.001**	**1.76 (1.44, 2.15)**	**< 0.001**
Model 2[b]	Ref.	1.13 (0.90, 1.41)	0.299	**1.33 (1.02, 1.74)**	**0.037**	**1.37 (1.07, 1.76)**	**0.014**
Model 3[c]	Ref.	1.18 (0.92, 1.51)	0.194	1.30 (0.96, 1.75)	0.087	**1.39 (1.06, 1.81)**	**0.018**

[a] unadjusted; [b] adjusted for age, sex, race, educational level, family income; [c] adjusted for Model 2, smoking, diabetes, total cholesterol, triglyceride, systolic blood pressure, diastolic blood pressure, alanine aminotransferase.
Bold indicates significant results.

females ($P< 0.050$), but no statistically association was detected in male models. There was no statistically association between working hours and NAFLD in the age-stratified analysis. In subgroup analyses stratified by family income, the risk of NAFLD increased in medium-income and high-income populations. In analysis stratified by education level, it is apparent that longer working hours are independently correlated with NAFLD in individuals with a high school education or above ($P< 0.050$), but not in those with lower educational levels. Among people without diabetes, there is a significant association between working hours and NAFLD (Table 4).

Association between working type and NAFLD in various subgroups

In subgroup analyses stratified by sex, men were at greater risk for NAFLD with medium physical labor and heavy physical labor, while no significant difference was observed in females. In subgroup analyses stratified by age, NAFLD risk increased among older individuals with higher work intensity (OR: 1.49, 95%CI: 1.11-2.00). In the family income layered analysis, we did not detect a correlation between the type of work and NAFLD. There was no significant difference in the risk of NAFLD among individuals with

TABLE 4 Associations of working hours in various subgroups among participants with NAFLD in NHANES 1999-2014.

Characteristic	Working hours				*P* for interaction
	≤ 30	30-40	40-50	> 50	
Sex					0.181
Male	Ref.	1.15 (0.89, 1.47)	1.21 (0.93, 1.58)	1.26 (0.96, 1.66)	
Female		**1.61 (1.25, 2.10)**	**1.69 (1.24, 2.30)**	**1.99 (1.38, 2.89)**	
Age [a]					0.338
Young	Ref.	1.27 (0.95, 1.72)	1.30 (0.93, 1.84)	1.27 (0.89, 1.82)	
Middle		1.21 (0.91, 1.62)	1.30 (0.95, 1.77)	**1.60 (1.15, 2.23)**	
Old		1.44 (0.99, 2.09)	1.54 (0.97, 2.46)	1.46 (0.85, 2.54)	
PIR [b]					0.556
Low	Ref.	1.28 (0.86, 1.91)	1.16 (0.69, 1.93)	1.16 (0.69, 1.96)	
Medium		1.32 (0.97, 1.81)	**1.45 (1.00, 2.09)**	**1.85 (1.25, 2.75)**	
High		**1.45 (1.11, 1.88)**	**1.51 (1.15, 1.99)**	**1.50 (1.11, 2.04)**	
Education					0.823
Less than high school	Ref.	1.13 (0.83, 1.54)	1.10 (0.77, 1.57)	1.11 (0.75, 1.63)	
High school		**1.56 (1.14, 2.14)**	**1.77 (1.23, 2.55)**	**1.87 (1.28, 2.76)**	
Some College or above		**1.42 (1.05, 1.93)**	**1.49 (1.08, 2.05)**	**1.70 (1.20, 2.42)**	
Diabetes					0.696
No	Ref.	**1.37 (1.14, 1.65)**	**1.44 (1.17, 1.77)**	**1.50 (1.21, 1.88)**	
Yes		0.82 (0.33, 1.97)	0.73 (0.27, 1.95)	1.64 (0.54, 5.41)	

[a] Age was classified into 3 groups: 40 years or younger, 41to 60 years and 61 years or older. [b] Categorized into the following 3 levels based on the family poverty income ratio: low income (≤ 1.3), medium income (1.3 to 3.5), and high income (> 3.5).
Bold indicates significant results.

a high school education or below in light and moderate physical labor. Finally, in the diabetes stratified analysis, there was no meaningful connection between the type of work and NAFLD. Furthermore, the results of the interaction analysis indicated no notable interaction between working hours and the various subgroups (Table 5).

Discussion

In this study, we found that long working hours significantly increased the risk of NAFLD even after adjusting for confounding factors. Previous research indicated that long working hours raise the likelihood of NAFLD among Koreans (26). In our study, we expanded the population to the United States and found that working hours continued to show a significant relationship with NAFLD even after adjusting for confounding variables, which is consistent with the findings in Koreans. The risk in the US population (before correcting for confounding variables) was relatively higher (OR: 1.57, 95%CI: 1.21-2.05) compared to Korea. This may be attributed to the higher prevalence of NAFLD in North America (31.2%) compared to East Asia (29.7%) (31). Using unconstrained cubic splines based on the Korean study, we demonstrated a linear relationship between working hours and NAFLD ($P_{nonlinear} = 0.162$) (26). A Chinese study showed that prolonged and high-frequency night shift work increases the risk of NAFLD in male steel workers by 27% (32). Furthermore, the 2024 study by Robert Maidstone et al. on biobanks in the UK demonstrates that long-term night shift work elevates the risk of fatty degeneration by 8% (33). These findings are consistent with our research. Night shift work has been shown to be associated with various liver diseases. The study by Wang Feng et al. indicates that night shift work among Chinese workers is positively correlated with liver function abnormalities (34). Similarly, a study involving the Korean population found that night shift work is positively correlated with NAFLD among young female workers with poor sleep quality (35). Moreover, prolonged night shift work increases the risk of dyslipidemia and liver and kidney function abnormalities among nurses (36). Since there is no data on night shifts in the NHANES database, we used weekly working hours to investigate its association with NAFLD. These studies consistently indicate that poor work patterns can lead to the occurrence of various liver diseases.

TABLE 5 Associations of working type in various subgroups among participants with NAFLD in NHANES 1999-2014.

Characteristic	Working type				*P* for interaction
	Mental labor	Light physical labor	Medium physical labor	Heavy physical labor	
Sex					0.593
Male	Ref.	1.27 (0.95, 1.69)	**1.73 (1.26, 2.38)**	**1.55 (1.17, 2.07)**	
Female		1.01 (0.78, 1.30)	1.07 (0.79, 1.45)	1.24 (0.87, 1.76)	
Age [a]					0.840
Young	Ref.	0.86 (0.55, 1.34)	1.14 (0.73, 1.78)	0.97 (0.59, 1.58)	
Middle		1.14 (0.78, 1.66)	1.37 (0.90, 2.09)	1.36 (0.90, 2.05)	
Old		1.26 (0.97, 1.63)	**1.69 (1.23, 2.33)**	**1.49 (1.11, 2.00)**	
PIR [b]					0.617
Low	Ref.	0.53 (0.31, 0.88)	0.96 (0.58, 1.60)	0.88 (0.53, 1.45)	
Medium		1.14 (0.83, 1.57)	1.42 (0.99, 2.02)	**1.52 (1.08, 2.15)**	
High		**1.33 (1.01, 1.75)**	1.39 (0.98, 1.97)	1.37 (0.98, 1.93)	
Education					0.211
Less than high school	Ref.	0.60 (0.38, 0.95)	1.01 (0.63, 1.63)	0.87 (0.55, 1.37)	
High school		1.16 (0.84, 1.62)	1.33 (0.91,1.95)	**1.56 (1.07, 2.28)**	
Some College or above		**1.33 (1.00, 1.77)**	1.34 (0.93, 1.92)	1.42 (0.95, 2.10)	
Diabetes					0.085
No	Ref.	1.12 (0.78, 1.61)	1.43 (0.96, 2.13)	1.45 (0.98, 2.15)	
Yes		1.22 (0.16, 8.05)	1.50 (0.15, 13.27)	0.26 (0.03, 1.72)	

[a] Age was classified into 3 groups: 40 years or younger, 41 to 60 years and 61 years or older. [b] Categorized into the following 3 levels based on the family poverty income ratio: low income (≤ 1.3), medium income (1.3 to 3.5), and high income (> 3.5).
Bold indicates significant results.

Currently, there is no research indicating that heavy physical labor increases the risk of NAFLD. Existing studies have shown that heavy physical labor raises the likelihood of work absence (37, 38), musculoskeletal diseases (39), hypertension (40), cardiovascular disorders (41, 42), and even the risk of mortality from all causes in men (43). In addition, moderate-intensity aerobic exercise can lead to a 2-4% absolute reduction in liver fat degeneration in adults with MAFLD (44). We considered that heavy physical labor may affect the risk of NAFLD through some underlying mechanisms. To verify this hypothesis, we categorized participants into groups based on work intensity: mental labor, light manual work, moderate manual work, and heavy manual work. We observed that the risk of NAFLD increased with greater work intensity. This indirectly suggests that work-related exercise does not provide equivalent health benefits compared to free-time physical activity (45). When adjusting for age, sex, race, education level, PIR, TC, TG, SBP, DBP, ALT, smoking, and diabetes, only heavy physical labor was notably linked to NAFLD (OR: 1.39, 95%CI: 1.06-1.81). One possible explanation for this phenomenon is that while appropriate physical activity can improve mood, excessive physical activity may not only fail to enhance mood, but can also lead to mood deterioration (46, 47). This deterioration can manifest as sleep disturbances, weight and appetite loss, fatigue, irritability, emotional instability, and even depression. Sleep disturbances (48), emotional instability, and depression (49, 50) have been established as risk factors for NAFLD. Additionally, heavy manual work may contribute to a lack of leisure exercise, which is vital for promoting health and enhancing physical fitness and work capacity (9, 51). Furthermore, heavy physical labor can increase the cardiovascular burden on male construction workers, significantly raising the incidence of NAFLD (52). The specific mechanisms still need to be confirmed through extensive research.

Our subgroup analysis showed that the relationship between working hours and NAFLD was more significant in women and high-income individuals. The distribution of body fat in women changes with hormonal cycles compared to men (53), which results in a relatively higher probability of obesity in women (54). This physiological difference may contribute to an increased risk of NAFLD in women. The connection between working type and NAFLD was more pronounced in men and the elderly. This may be due to the higher proportion of male workers in heavy physical labor, as age increases, the consequences of prolonged heavy physical work become more apparent (55, 56).

Long working hours and heavy physical labor are associated with the development of various diseases. Research indicates that for every 10-hour increase in weekly working hours, the likelihood of sleep deprivation increases by approximately 50%, and the risk of difficulty falling asleep also rises significantly (57). Long working hours can lead to an increased probability of obesity (6), which may be caused by the impact of prolonged work on metabolic response mechanisms (58). Existing evidence indicates that long working hours contribute to coronary heart disease, stroke, hypertension, depression, and other chronic diseases (59). The cumulative effect of these factors greatly increases the risk of developing NAFLD. Therefore, it is crucial to properly arrange working hours and avoid intense physical labor.

However, certain limitations exist. Firstly, because this study employed a cross-sectional design, the observed connection does not inherently indicate a cause-and-effect connection. Secondly, we employed a non-direct method (the HSI assessment tool) instead of imaging studies or pathological assessments to determine NAFLD. However, the HSI has been thoroughly confirmed and can be used to predict the existence and severity of NAFLD in numerous extensive studies (29). Thirdly, since our research only consisted of the demographic in the United States, our findings ought to be corroborated in various racial groups. Ultimately, even though we tried to adjust for several potential risk variables, there could still be unaccounted confounding factors or biases beyond our control, such as participants' specific sleep habits, daily exercise time, and dietary habits. Finally, we investigated the relationship between working hours and NAFLD; however, the associations of other steatotic liver disease categories, such as MASLD and MetALD, remain unexplored. We hope to further explore the connections between working hours, working type and other liver diseases in future research. Regardless of these limitations, this study offers important strengths, such as a large representative sample from across the nation, standardized exceptional clinical and laboratory data gathering, and thorough details on different confounding influences.

Conclusion

The findings of this cross-sectional study suggest significant connection between long working hours and heavy physical labor with NAFLD. Furthermore, our research indicates that these factors heighten the likelihood of developing NAFLD, which may offer insights into innovative interventions and approaches for reducing the risk of NAFLD.

Data availability statement

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

Ethics statement

The studies involving humans were approved by The NCHS Research Ethics Review Board approved the questionnaire, ensuring that all participants consented with full awareness. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/ next of kin in accordance with the national legislation and institutional requirements.

Author contributions

RW: Formal analysis, Methodology, Writing – original draft. NW: Data curation, Methodology, Writing – original draft. QH: Data curation, Methodology, Project administration, Writing – original draft. XZ: Methodology, Writing – review & editing. HZ: Project administration, Supervision, Writing – original draft. LiZ: Resources, Writing – review & editing. XW: Project administration, Resources, Writing – review & editing. XY: Conceptualization, Methodology, Project administration, Writing – review & editing. LeZ: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by Top medical expert team of Wuxi Taihu Talent Plan (Grant No. DJTD202106, GDTD202105, YXTD202101), Medical Key Discipline Program of Wuxi Health Commission (Grant No. ZDXK2021007, CXTD2021005), Top Talent Support Program for young and middle-aged people of Wuxi Health Committee (Grant No.BJ2023090), Scientific Research Program of Wuxi health Commission (Grant No. Z202109, M202208), and Wuxi Science and Technology Development Fund (Grant No. N20202003, Y20222001, K20241001).

Acknowledgments

All authors thank NHANES for providing the publicly available data.

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1499735/full#supplementary-material

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 17 February 2025
DOI 10.3389/fendo.2025.1505050

OPEN ACCESS

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RECEIVED 02 October 2024
ACCEPTED 29 January 2025
PUBLISHED 17 February 2025

CITATION
Hu M-Y, Sun D-Q, Yang F, Zheng X-W, Wu N-X, Zhang H-Y, Yao X-D, Zhang J-H and Zhang L (2025) Impact of segmental body composition on metabolic *dysfunction-associated* fatty liver disease in Chinese children.
Front. Endocrinol. 16:1505050.
doi: 10.3389/fendo.2025.1505050

Impact of segmental body composition on metabolic *dysfunction-associated* fatty liver disease in Chinese children

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Purpose: This study aimed to assess the relationship between regional body composition and metabolic dysfunction-associated fatty liver disease (MAFLD) in Chinese children.

Methods: In this study, 1399 children aged 7–14 years were included. Liver steatosis was assessed using the controlled attenuation parameter (CAP) measured through Fibroscan. MAFLD is defined as the presence of liver steatosis along with either overweight/obesity, prediabetes/diabetes, or at least two metabolic index abnormalities. Regression analyses were applied to assess the relationship between regional body composition and MAFLD in children. Subgroup analyses were performed based on sex and weight.

Results: The participants had a mean age of 9 years, with 52.11% being boys. Among them, 134 (9.57%) were diagnosed with MAFLD, and 17 (1.22%) had severe fatty liver disease. We found an inverse correlation between the muscle percentage in each region and MAFLD, with the extremities demonstrating the most significant negative correlation (OR: 0.732; 95% CI: 0.634–0.844). Conversely, regional fat was positively associated with MAFLD, with the strongest correlation found in the upper limbs (OR: 3.104; 95% CI: 2.023–4.764). Subgroup analyses showed similar results.

Conclusion: The decrease in regional muscle percentage, particularly in the limbs, along with the increase in regional fat percentage, especially in the upper limbs, is associated with a higher probability of developing MAFLD in prepubertal children. Additional prospective studies are needed to strengthen and validate these findings.

KEYWORDS

BIA, MAFLD, children, regional body composition, Fibroscan

1 Introduction

In recent years, the global prevalence of childhood obesity has surged, emerging as a significant public health challenge. It not only contributes to short-term health issues, such as metabolic disorders and psychological problems, but also markedly increases the risk of chronic diseases in adulthood, including metabolic syndrome, diabetes, and cardiovascular diseases. Childhood obesity is closely associated with metabolic-associated fatty liver disease (MAFLD), which is the leading cause of chronic liver disease in children (1). The estimated prevalence of MAFLD in children is approximately 10% in the general population and up to 34% in children who are obese (2). Moreover, the prevalence of MAFLD varies across different regions. In obese children, MAFLD prevalence is higher in Asia (52.1%) than in Europe (39.7%) and North America (23.0%) (3). Pediatric MAFLD is associated with hepatic and extrahepatic comorbidities, including hypertension, dyslipidemia, gallstones, diabetes, chronic kidney disease, and depression (4–6). Research has shown that pediatric fatty liver disease may persist into adulthood and significantly reduce life expectancy (7).

Recently, there has been growing interest in assessing body composition in MAFLD patients. Epidemiological studies indicate that low skeletal muscle mass is linked to a higher likelihood of developing MAFLD and its severity in adults (8–11). Guo et al. reported that participants in the lowest tertiles of skeletal muscle mass had an approximately fourfold higher risk of developing MAFLD and an approximately fourfold higher risk of advanced fibrosis compared to those in the highest tertile (9). A possible explanation is that skeletal muscle mass affects liver metabolic health by modulating insulin-mediated glucose disposal, which plays a critical role in the pathogenesis of MAFLD (12). In addition, increased body fat mass, especially abdominal fat deposition, is linked to a higher incidence of MAFLD (13–15). A large observational study predicted that for every 1 kg increase in fat mass, participants had a 27%–40% increased risk of MAFLD (13). The researchers also confirmed a correlation between muscle and fat mass in adults and MAFLD risk.

Despite the abundant data linking body composition to MAFLD in adults, research in pediatric populations remains limited. Therefore, the study was designed to assess muscle and fat content in various body regions and explore their relationship with MAFLD in children using bioelectrical impedance analysis (BIA).

2 Methods

2.1 Study design and participants

This cohort study was conducted in Wuxi City, Jiangsu Province, China, and involved approximately 2086 students aged 7 to 14 years, from March to April 2023. Initially, these students were required to undergo the routine elementary school physical examination, and participants were recruited from this group. Subsequently, 687 children were excluded for the following reasons: (1) 618 children had parents who did not sign the informed consent form; (2) 52 children dropped out during the Fibroscan and/or BIA examination; and (3) 17 children were excluded because of incomplete core data. Ultimately, a total of 1399 children were enrolled in this study (Figure 1). Personal information was omitted, and personal identifiers were replaced with health examination numbers. This study was approved by the Wuxi Children's Hospital, Affiliated Hospital of Jiangnan University. Written informed consent was obtained from the guardians of the participants before their participation in the study.

2.2 Anthropometric measurements and body composition evaluation

Standard procedures were followed by trained technicians to obtain anthropometric data. After an overnight fast, all participants were asked to wear light clothing for BIA measurements. BIA was conducted using a fixed multifrequency, eight-point device (InBody 370, InBody, Seoul, Korea). Participants stood on the scale's footpads (with two electrodes per foot) and held a handle in each hand (with two electrodes per hand) for approximately 1 minute. The device, which measured fat and muscle mass for each region (bilateral upper and lower limbs and trunk), recorded height, sex and age. Muscle mass percentage was defined as the ratio of muscle mass to body weight, calculated as [muscle mass (kg)/weight (kg)] × 100 (16). Percentage of regional muscle mass, e.g. upper extremity, was calculated by dividing sum of left and right upper extremity muscle mass by body weight. The following formula was used to calculate body fat percentage: [fat mass (kg)/weight (kg)] × 100. For assessing regional body fat percentage, we used the following formula: [regional body fat mass (kg)/weight (kg)] × 100. The lower limb fat mass was calculated by adding the fat mass on both sides of the lower limbs, and the same method was applied for other regions.

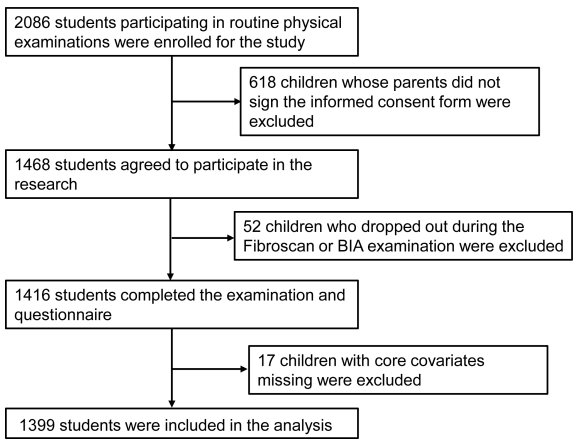

FIGURE 1
Flowchart of participants selection.

2.3 Clinical and biochemical measurements

The data were collected by trained medical staff at the school. General examinations included measurements of height, weight, and blood pressure (BP). Weight was measured in kilograms, height in centimeters, and BP in millimeters of mercury (mmHg). Venous samples were collected after an 8-hour overnight fast. Serum levels of alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG), fasting glucose, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were measured. The homeostasis model assessment of insulin resistance (HOMA-IR) score (17) was calculated as follows: HOMA-IR = fasting insulin [μIU/mL] × fasting plasma glucose [mmol/L])/22.5.

2.4 Liver steatosis and MAFLD definition

Transient elastography using the Fibroscan device (Echosens, Paris, France) was conducted by operators using a 3.5 MHz M probe. All operators were trained according to a standardized procedure and had each conducted more than 50 examinations prior to the study. Ten successful measurements were performed to obtain the average controlled attenuation parameter (CAP) scores. According to the user manual, participants were classified based on the CAP value into the following groups: non-liver steatosis (CAP < 238 dB/m), mild liver steatosis (238 dB/m ≤ CAP value < 259 dB/m), moderate liver steatosis (259 dB/m ≤ CAP value < 292 dB/m), or severe liver steatosis (CAP value ≥ 292 dB/m) groups (18). In line with the most recent consensus, the diagnosis of MAFLD in children includes the presence of steatosis, assessed using the CAP value, along with at least one of the following criteria: overweight/obesity, prediabetes, or diabetes, and at least two metabolic abnormalities (19). Overweight and obesity were defined based on the BMI z-score, according to the age and sex-specific criteria proposed by the World Health Organization (20). Abdominal obesity was diagnosed when waist circumference exceeded the 90th percentile for age and gender (21). Prediabetes and diabetes were defined according to international guidelines (22). Diagnostic criteria included: (1) a previous diagnosis of diabetes; (2) a hemoglobin A1c level of 5.7% (48 mmol/mol) or higher; and (3) a fasting plasma glucose level of 100 mg/dL or higher. These metabolic abnormalities were accompanied by elevated BP, elevated TG levels, low high-density lipoprotein levels, and high TG/HDL ratios (23). Elevated BP was defined as systolic or diastolic BP exceeding the 90th percentile, while elevated TG levels were defined as TG levels above the 90th percentile. A low HDL level was defined as an HDL level ≤ 10th percentile. The diagnostic criteria for children aged 10–15 years were as follows: elevated BP (defined as a systolic reading > 130 mmHg or diastolic reading > 85 mmHg); TG levels ≥ 150 mg/dL; HDL cholesterol levels < 40 mg/dL; and a TG-to-HDL cholesterol ratio > 2.25 (19).

2.5 Questionnaire

Parents completed a questionnaire on their children's lifestyles. The questionnaire gathered basic information regarding the children's screen time and physical activity status. Screen time was categorized as < 30 min/day, 1–2 hour/day, or > 2 hours/day. For physical activity, exercise habits were classified as yes or no; exercise frequency was classified as seldom, 1–3 times/week, 4–6 times/week or every day; exercise duration was classified as < 30 min, 30–90 min or 90–120 min. Based on the examination results, valid questionnaires were grouped into MAFLD and non-MAFLD categories. The relationship between pediatric MAFLD and lifestyle habits was investigated.

2.6 Statistical analysis

Continuous variables are presented as mean ± standard deviation. Statistical comparisons across groups were conducted using either Student's t-test or one-way analysis of variance. Categorical variables expressed as percentages, were compared using the chi-squared test. The logistic regression model was used to evaluate the associations between regional muscle mass percentage, regional fat mass percentage, and the risk of MAFLD. Three models were established, and adjustments were made for various factors. These factors included age, sex, prediabetes or diabetes status, overweight status, central obesity status, elevated BP, elevated TG, low HDL, and a high TG/HDL ratio. Stratified analyses were conducted based on sex (male and female) and weight (normal weight or overweight/obesity). All statistical analyses were performed using SPSS (version 26.0). The significance level was set at two-side $P < 0.05$.

3 Results

3.1 Participant characteristics

As shown in Table 1, the study included 1,399 children with a mean age of 9 ± 2 years. Of these, 52.11% were boys and the mean BMI z score was 0.58 ± 1.19. Fatty liver disease was observed in 178 participants (12.71%), with 17 (1.22%) having severe fatty liver disease. Among these patients, 134 (9.57%) were diagnosed with MAFLD. Compared to the non-MAFLD group, they were more likely to have significantly greater systolic and diastolic BP, decreased HDL cholesterol levels, increased TG levels, and a greater prevalence of prediabetes or diabetes. They also had significantly greater BMI, waist circumferences, TG levels, fasting plasma glucose levels, ALT levels, GGT levels, HOMA-IR scores, and lower HDL cholesterol levels than those in the non-MAFLD group ($P < 0.05$). In terms of body composition parameters, patients with MAFLD had significantly lower total muscle mass percentage (34.34 ± 2.91% *vs.* 39.34 ± 3.75%, $P < 0.05$) and greater total body fat

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percentage (34.53 ± 6.92% *vs.* 22.57 ± 7.06%, $P < 0.05$) than patients in the non-MAFLD group. As shown in Figure 2, compared to the non-MAFLD group, the MAFLD group had significantly lower muscle mass percentages in the lower limbs (18.67 ± 1.80% *vs.* 20.40 ± 2.64%, $P < 0.05$), extremities (24.23 ± 2.20% *vs.* 25.9 ± 3.18%, $P < 0.05$), and trunk (28.22 ± 1.96% *vs.* 31.74 ± 2.55%, $P < 0.05$). Furthermore, the fat mass percentages in the upper limbs (5.13 ± 1.08% *vs.* 3.53 ± 0.90%, $P < 0.05$), lower limbs (11.65 ± 1.66% *vs.*

TABLE 1 Baseline characteristics of participants.

Variables	All (N = 1399)	Non-MAFLD (N = 1265)	MAFLD (N = 134)	*P*
Age (years)	9 ± 2	9 ± 2	10 ± 2	< **0.001**
Sex, male, n (%)	729 (52.11)	627 (49.57)	102 (76.12)	< **0.001**
BMI z-score	0.58 ± 1.19	0.41 ± 1.10	2.15 ± 0.78	< **0.001**
SBP (mm Hg)	107.79 ± 12.3	106.54 ± 11.7	116.9 ± 12.85	< **0.001**
DBP (mm Hg)	64.12 ± 8.49	63.7 ± 8.43	67.21 ± 8.36	< **0.001**
HOMA-IR score	1.45 ± 1.05	1.31 ± 0.81	2.62 ± 1.87	< **0.001**
Waist circumference (cm)	62.41 ± 8.02	61.18 ± 6.67	74.59 ± 9.93	< **0.001**
Prediabetes or diabetes (%)	19 (1.65)	11 (1.06)	8 (6.90)	< **0.001**
Overweight or Obesity (%)	498 (35.90)	371 (29.59)	127 (95.49)	< **0.001**
Central obesity (%)	108 (7.76)	47 (3.74)	61 (45.86)	< **0.001**
Elevated BP (%)	51 (5.48)	43 (5.26)	8 (7.14)	0.411
Elevated triglycerides (%)	122 (10.36)	100 (9.43)	22 (18.64)	**0.002**
Low HDL (%)	17 (1.48)	11 (1.07)	6 (5.17)	< **0.001**
High triglyceride/HDL ratio (%)	109 (9.62)	81 (7.96)	28 (24.14)	< **0.001**
CAP value (dB/m)	193.92 ± 39.11	186.63 ± 32.91	262.71 ± 22.71	< **0.001**
LSM value (kPa)	4.46 ± 1.06	4.44 ± 1.05	4.7 ± 1.13	**0.006**
ALT (U/L)	14.71 ± 8.69	14.17 ± 7.6	19.76 ± 14.63	< **0.001**
GGT (U/L)	13.77 ± 3.80	13.40 ± 2.95	17.25 ± 7.48	< **0.001**
AST (U/L)	25.82 ± 5.89	25.96 ± 5.81	24.43 ± 6.50	**0.013**
TG (mmol/L)	0.75 ± 0.32	0.73 ± 0.30	0.95 ± 0.44	< **0.001**
TC (mmol/L)	4.72 ± 0.84	4.74 ± 0.84	4.62 ± 0.82	0.110
LDL (mmol/L)	2.28 ± 0.61	2.28 ± 0.60	2.26 ± 0.62	0.735
HDL (mmol/L)	1.67 ± 0.34	1.69 ± 0.34	1.42 ± 0.27	< **0.001**
FPG (mmol/L)	4.88 ± 0.32	4.86 ± 0.32	5.04 ± 0.33	< **0.001**
Total muscle mass percent (%)	38.86 ± 3.96	39.34 ± 3.75	34.34 ± 2.91	< **0.001**
Total body fat percent (%)	23.72 ± 7.82	22.57 ± 7.06	34.53 ± 6.92	< **0.001**
Daily screen viewing time, n (%)				0.991
< 30 min	1182 (86.72)	1069 (86.80)	113 (85.61)	
1-2 hour	156 (11.40)	138 (11.21)	18 (13.64)	
>2 hours	25 (1.83)	24 (1.95)	1 (0.76)	
Exercise habits, n (%)				0.089
No	148 (10.62)	128 (10.17)	20 (14.90)	
Yes	1245 (89.38)	1131 (89.83)	114 (85.07)	

(Continued)

TABLE 1 Continued

Variables	All (N = 1399)	Non-MAFLD (N = 1265)	MAFLD (N = 134)	*P*
Exercise frequency, n (%)				0.970
Seldom	153 (11.47)	134 (11.07)	19 (15.32)	
1-3 per week	787 (59.00)	717 (59.26)	70 (56.45)	
4-6 per week	255 (19.12)	239 (19.75)	16 (12.90)	
Everyday	139 (10.42)	120 (9.92)	19 (15.32)	
Exercise duration (min), n (%)				0.071
< 30	474 (35.56)	436 (36.06)	38 (30.65)	
30-90	750 (56.26)	678 (56.08)	72 (58.06)	
90-120	91 (6.83)	81 (6.70)	10 (8.06)	

Continuous variables are shown as mean ± SD. Categorical values are shown as n (%). Bold values indicate if $P < 0.05$. BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; MAFLD, metabolic dysfunction–associated fatty liver disease; SBP, systolic blood pressure; PBF (%), percent body fat; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, g-glutamyl transferase; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose.

8.90 ± 1.99%, $P < 0.05$), extremities (16.78 ± 2.63% *vs.* 12.44 ± 2.83%, $P < 0.05$), and trunk (16.32 ± 4.05% *vs.* 8.21 ± 4.77%, $P < 0.05$) were significantly greater in the MAFLD group than in the non-MAFLD group.

3.2 Relationship between segmental body composition and MAFLD

Figure 3 shows the total muscle and fat mass percentages divided into four quartiles. The prevalence of MAFLD gradually decreased as the muscle mass percentage quartiles increased (28.03% *vs.* 8.05% *vs.* 2.30% *vs.* 0.00%), whereas it increased progressively with higher fat mass percentage quartiles (0.57% *vs.* 1.15% *vs.* 5.22% *vs.* 31.14%).

Logistic regression analysis was conducted to further understand the relationship between MAFLD and segmental body composition (Table 2). In the unadjusted model, the muscle mass percentage of each body segment was negatively correlated with MAFLD. In contrast, the risk of MAFLD was significantly associated with the fat mass percentage in each body segment. Importantly, even after adjusting for age, sex, prediabetes or diabetes status, overweight status, central obesity status, elevated BP, elevated TG, low HDL, and a high TG/HDL ratio (Model 3), this relationship remained robust. Furthermore, in Model 3, the percentage of muscle mass in the extremities (OR: 0.732; 95% CI: 0.634–0.844, $P < 0.001$) and lower limbs (OR: 0.682; 95% CI: 0.571–0.813, $P < 0.001$) had a more significant impact on MAFLD than in other body regions. The increased risk of MAFLD was associated with an increase in the percentage of fat mass in the upper limbs (OR: 3.104; 95% CI: 2.023–4.764, $P < 0.001$), lower limbs (OR: 1.794; 95% CI: 1.425–2.257, $P < 0.001$), extremities (OR: 1.499; 95% CI: 1.285–1.749, $P < 0.001$) and trunk (OR: 1.506; 95% CI: 1.299–1.744, $P < 0.001$).

Sensitivity and subgroup analyses were conducted for sex and weight. When stratified by sex (Table 3), similar results and trends

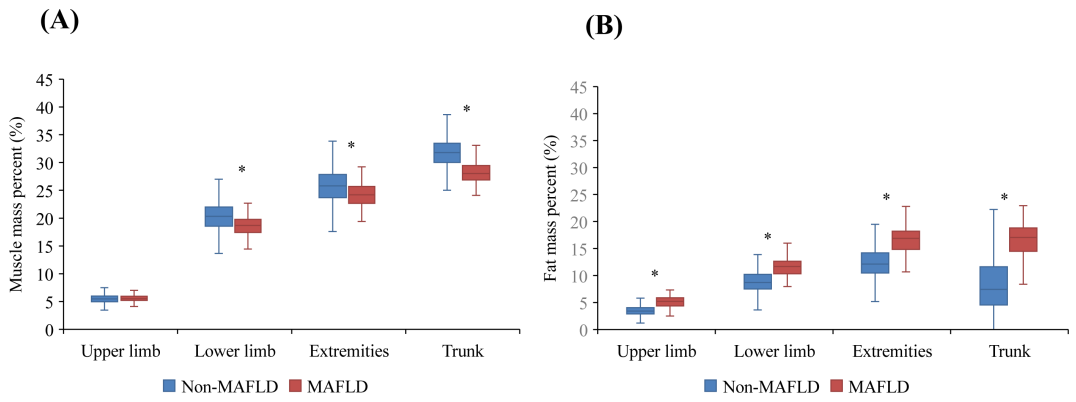

FIGURE 2
Regional muscle mass percentage (%)/fat mass percentage (%) between the MAFLD and non-MAFLD groups. **(A)** Regional muscle mass percentage (%) between the MAFLD and non-MAFLD groups. **(B)** Regional fat mass percentage (%) between the MAFLD and non-MAFLD groups. * indicate $P < 0.05$.

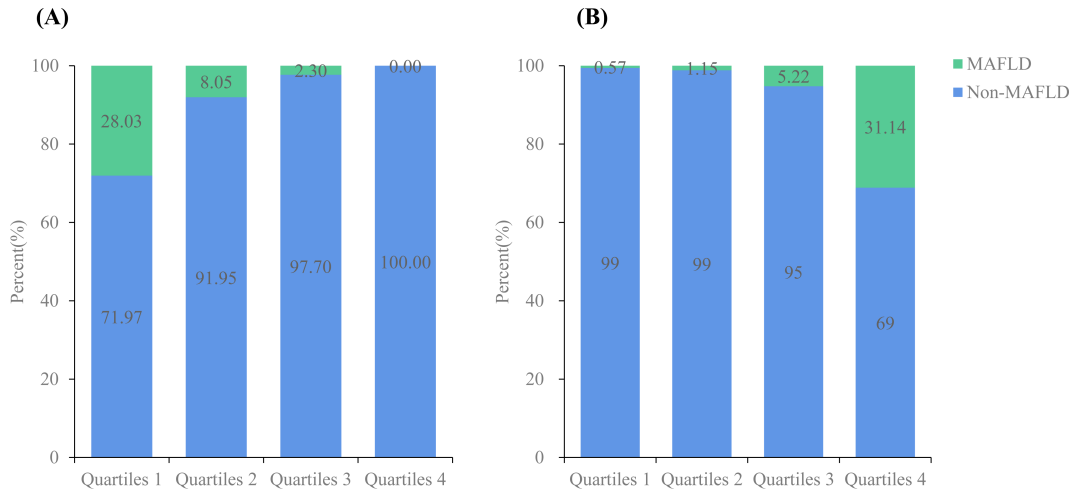

FIGURE 3
Prevalence of MAFLD according to total muscle mass percentage (%)/fat mass percentage (%). **(A)** The prevalence of non-MAFLD and MAFLD according to total muscle mass percentage (%); **(B)** The prevalence of non-MAFLD and MAFLD according to fat muscle mass percentage (%).

were observed for both men and women. Conversely, no significant association was found in the normal-weight subgroup (Table 4).

3.3 Relationship between physical activity habits and MAFLD

Regarding the relationship between physical activity habits and MAFLD, although the difference was not statistically significant, a greater proportion of individuals in the MAFLD group reported no exercise habits (14.93% *vs.* 10.17%). Similarly, the MAFLD group had a higher proportion of individuals with a lower frequency of exercise (15.32% *vs.* 11.07%) (Table 1). The associations between physical activity habits and body composition in children with MAFLD are presented in Figure 4. Compared to individuals who rarely exercise, those who engage in daily exercise had a greater overall muscle percentage (34.05 ± 3.11% *vs.* 34.26 ± 3.43%, P = 0.029), extremity muscle percentage (23.98 ± 2.58% *vs.* 25.1 ± 1.79%, P = 0.049), trunk muscle percentage (28.21 ± 2.03% *vs.* 29.62 ± 1.86%, P = 0.022), overall fat percentage (35.72 ± 6.06% *vs.* 35.53 ± 6.66%, P = 0.027), and lower limb fat percentage (11.71 ± 1.45% *vs.* 10.77 ± 1.33%, P = 0.040).

TABLE 2 Multivariate analysis for the relationship between regional muscle mass (%)/fat mass (%) and MAFLD.

Variables	Model 1		Model 2		Model 3	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Muscle mass percent (%)						
Total	0.695 (0.655-0.738)	< **0.001**	0.685 (0.642-0.729)	< **0.001**	0.719 (0.634-0.815)	< **0.001**
Upper limb	1.125 (0.893-1.418)	0.317	0. 789 (0.611-1.020)	0.070	0.407 (0.241-0.685)	< **0.001**
Lower limb	0.751 (0.695-0.813)	< **0.001**	0.592 (0.535-0.654)	< **0.001**	0.682 (0.571-0.813)	< **0.001**
Extremities	0.833 (0.783-0.886)	< **0.001**	0.687 (0.635-0.744)	< **0.001**	0.732 (0.634-0.844)	< **0.001**
Trunk	0.553 (0.503-0.608)	< **0.001**	0.569 (0.516-0.627)	< **0.001**	0.626 (0.516-0.758)	< **0.001**
Fat mass percent (%)						
Total	1.257 (1.214-1.300)	< **0.001**	1.240 (1.198-1.283)	< **0.001**	1.235 (1.144-1.333)	< **0.001**
Upper limb	4.561 (3.646-5.704)	< **0.001**	4.101 (3.275-5.134)	< **0.001**	3.104 (2.023-4.764)	< **0.001**
Lower limb	1.964 (1.762-2.188)	< **0.001**	1.990 (1.773-2.232)	< **0.001**	1.794 (1.425-2.257)	< **0.001**
Extremities	1.647 (1.526-1.778)	< **0.001**	1.615 (1.494-1.746)	< **0.001**	1.499 (1.285-1.749)	< **0.001**
Trunk	1.452 (1.372-1.537)	< **0.001**	1.424 (1.345-1.508)	< **0.001**	1.506 (1.299-1.744)	< **0.001**

Model 1: unadjusted; Model 2: Model 1 additionally adjusted for age and sex; Model 3: Model 2 additionally adjusted for prediabetes or diabetes, overweight, central obesity, elevated BP, elevated triglycerides, low HDL, high triglyceride/HDL ratio. Bold values indicate if $P < 0.05$.
MAFLD, metabolic dysfunction-associated fatty liver disease; OR, odds ratio; CI, confidence interval.

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TABLE 3 Associations between regional muscle mass (%)/fat mass (%) and MAFLD stratified by sex.

Variables	Male		Female	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Muscle mass percent (%)				
Total	1.227 (1.179-1.277)	< **0.001**	1.277 (1.193-1.367)	< **0.001**
Upper limb	0.739 (0.549-0.994)	**0.046**	0.974 (0.581-1.632)	0.920
Lower limb	0.588 (0.521-0.663)	< **0.001**	0.593 (0.488-0.719)	< **0.001**
Extremities	0.681 (0.619-0.748)	< **0.001**	0.698 (0.598-0.815)	< **0.001**
Trunk	0.577 (0.514-0.647)	< **0.001**	0.534 (0.441-0.646)	< **0.001**
Fat mass percent (%)				
Total	0.692 (0.642-0.745)	< **0.001**	0.662 (0.585-0.748)	< **0.001**
Upper limb	3.844 (2.964-4.987)	< **0.001**	4.993 (3.190-7.816)	< **0.001**
Lower limb	1.939 (1.695-2.218)	< **0.001**	2.145 (1.717-2.679)	< **0.001**
Extremities	1.584 (1.446-1.735)	< **0.001**	1.709 (1.468-1.990)	< **0.001**
Trunk	1.392 (1.305-1.486)	< **0.001**	1.518 (1.349-1.708)	< **0.001**

The covariate was age; Bold values indicate if P <0.05.
MAFLD, metabolic dysfunction-associated fatty liver disease; OR, odds ratio; CI, confidence interval.

TABLE 4 Associations between regional muscle mass (%)/fat mass (%) and MAFLD stratified by weight.

Variables	Normal		Overweight/obesity	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Muscle mass percent (%)				
Total	0.826 (0.620-1.100)	0.191	0.768 (0.710-0.830)	< **0.001**
Upper limb	0.471 (0.159-1.398)	0.175	0.582 (0.411-0.824)	**0.002**
Lower limb	0.755 (0.510-1.118)	0.161	0.704 (0.623-0.797)	< **0.001**
Extremities	0.791 (0.582-1.076)	0.136	0.766 (0.695-0.846)	< **0.001**
Trunk	0.710 (0.497-1.015)	0.061	0.687 (0.612-0.772)	< **0.001**
Fat mass percent (%)				
Total	1.129 (0.939-1.357)	0.198	1.183 (1.131-1.236)	< **0.001**
Upper limb	2.706 (0.713-1.273)	0.143	2.654 (2.051-3.433)	< **0.001**
Lower limb	1.431 (0.775-2.643)	0.252	1.583 (1.375-1.823)	< **0.001**
Extremities	1.336 (0.867-2.059)	0.189	1.392 (1.267-1.529)	< **0.001**
Trunk	1.171 (0.871-1.575)	0.296	1.346 (1.246-1.455)	< **0.001**

The covariate was age, sex; Bold values indicate if $P < 0.05$.
MAFLD, metabolic dysfunction-associated fatty liver disease; OR, odds ratio; CI, confidence interval.

4 Discussion

In this study, we evaluated the relationship between segmental body composition and MAFLD in children. Our analysis revealed an inverse association between muscle mass and MAFLD, as well as an independent positive association with fat mass, especially with the muscle mass in the extremities and the fat mass in the upper limbs. Previous studies have shown that puberty has a significant impact on the prevalence of MAFLD across genders, with the prevalence in boys increasing as puberty progresses, while in girls, it peaks during the early stages of puberty (24). However, this study did not observe any gender differences, likely because the participants had not yet entered puberty. These findings underscore the significance of incorporating segmental body composition in assessing MAFLD risk.

MAFLD, formerly known as non-alcoholic fatty liver disease (NAFLD), underwent a nomenclature change in 2020 due to several limitations of the term "NAFLD" (25). These include potential confusion caused by the term "non-alcoholic", its inability to account for adolescent alcohol misuse, and its failure to accurately reflect the underlying pathophysiology of metabolic dysfunction driven by insulin resistance. In 2023, the term was further updated to metabolic dysfunction-associated steatotic liver disease (MASLD) due to the potentially stigmatizing nature of the word "fatty" (26). Over the past few years, research have confirmed the link between low skeletal muscle mass and hepatic steatosis in both adults and children (27–34). For instance, Yodoshi et al. (35) showed that lower muscle mass was associated with a greater steatosis score in children (OR: 0.73, 95% CI: 0.56–0.96). A cross-sectional study of biopsy-confirmed NAFLD patients revealed that the lowest tertiles of relative muscle mass at baseline were correlated with an increased risk of developing NAFLD in children (OR: 2.80; 95% CI: 1.57–5.02) (36). In adults, Du et al. (37) found that greater lower limb, extremity and trunk muscle mass reduced the risk of NAFLD. However, research on the relationship between regional skeletal muscle mass and MAFLD in children is limited. Our findings demonstrated an inverse association between muscle mass across various regions and the occurrence of MAFLD, with the most significant effect observed in the muscle mass percentage in the extremities and lower limbs.

The pathogenic mechanisms underlying the negative correlation between muscle mass and hepatic steatosis involve multiple aspects. First, insulin resistance is strongly linked to the accumulation of ectopic fat in the liver. Skeletal muscle, the most efficient target organ for insulin-mediated glucose disposal in the body, is closely associated with insulin resistance (38, 39). Reduced muscle mass is also associated with a lower basal metabolic rate, which further exacerbates insulin resistance (8). In addition, chronic inflammation and enhanced oxidative stress are closely associated with muscle mass and the occurrence and progression of MAFLD (40). Lastly, skeletal muscle, which acts as an endocrine organ, plays a crucial role by secreting myokines, such as interleukin-6 and irisin, to regulate metabolic processes. These myokines are involved in the development of MAFLD (41). However, the relationship between the regional distribution of

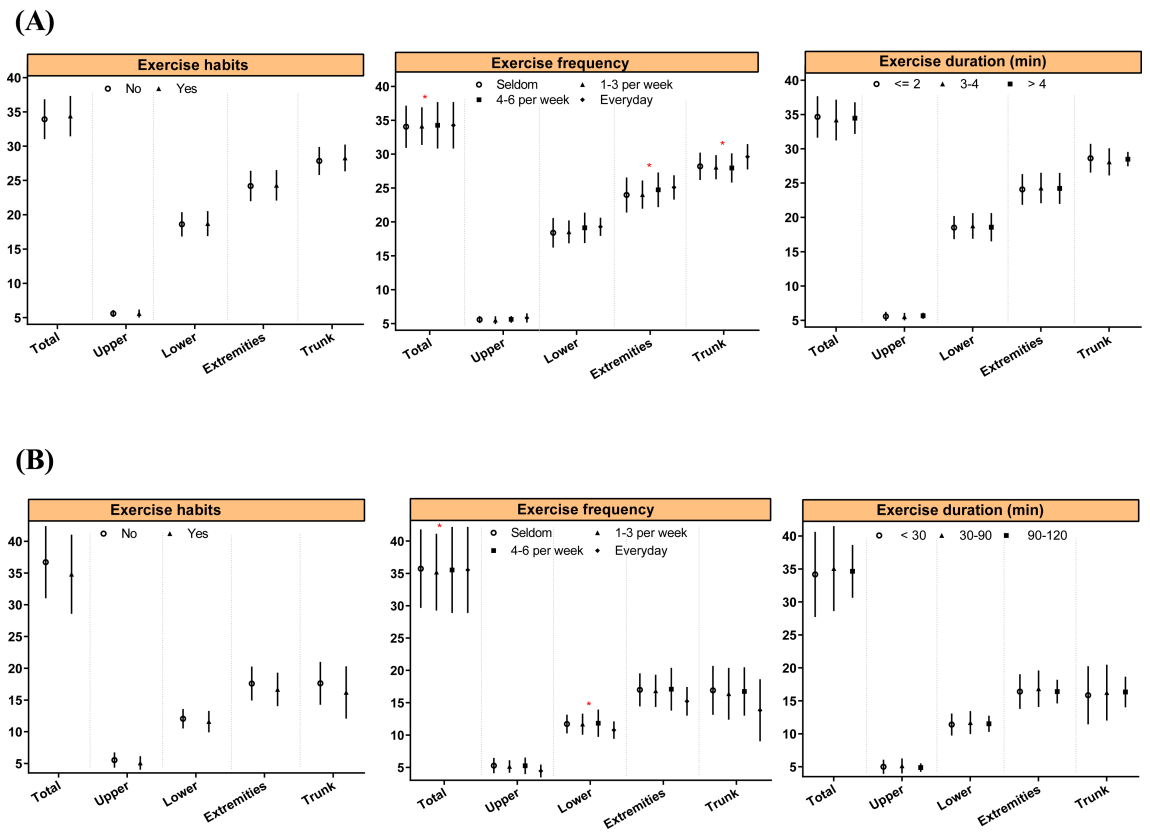

FIGURE 4
Associations between physical activity habits and body composition in MAFLD children. * indicate $P < 0.05$.

muscle mass and MAFLD remains unclear. One possible explanation is that the association between lower limb muscle mass and insulin resistance may be stronger than the association between upper limb muscle mass and insulin resistance.

Similarly, the role of body fat distribution in the development of MAFLD has garnered significant attention in the field of hepatology. Numerous studies have consistently highlighted the crucial role of abdominal fat deposition in the development of MAFLD (42, 43). Ciardullo et al. revealed a remarkable correlation between the android/gynoid ratio and hepatic steatosis (14). A significant correlation has also been found between a higher android-to-gynoid fat ratio and an increased risk of MAFLD in the Danish pediatric population (44). Our study confirmed the association between abdominal fat percentage and pediatric MAFLD and revealed for the first time that the correlation between upper limb fat percentage and pediatric MAFLD is even more significant. Recent studies exploring the relationship between upper limb fat and fatty liver have primarily been conducted in adults. Zhang et al. reported a positive correlation between adiposity levels in the upper limbs (measured using triceps and biceps skinfold thicknesses) and histological liver damage in patients with MAFLD (45). Studies have shown that upper limb obesity is a significant risk factor for various metabolic disorders (46). In metabolically unhealthy individuals with normal weight, fat tends to accumulate preferentially in the upper body (subcutaneous and visceral fat in the abdomen) and liver (47). As the liver plays an important role in the metabolism of carbohydrates and lipids (48), we hypothesize that the concentration of free fatty acids (FFA) in the portal vein after lipolysis may be significantly higher than in the arterial circulation. Thus, the liver may be exposed to higher concentrations of FFAs, which could increase the risk of developing MAFLD.

In patients with chronic liver disease, abnormal autophagy can impair muscle synthesis and increase protein degradation (49). Physical exercise can rescue impaired mTORC1 signaling by stimulating phosphatidic acid, thereby maintaining muscle mass through protein synthesis activation and autophagy inhibition (50). In particular, resistance exercise has been shown to effectively stimulate skeletal muscle protein synthesis, contributing to the treatment of chronic liver disease (51). Compared to diets high in unsaturated fats, those rich in saturated fats resulted in elevated intrahepatic triglyceride levels, which exacerbated hepatic lipid accumulation and impaired metabolic function (52). Therefore, for patients with MAFLD, high-quality diets, such as the Mediterranean diet, have become a crucial therapeutic approach. Our study aims to investigate regional body composition in relation to CAP-confirmed MAFLD in children (53). This approach provides a more accurate and comprehensive analysis of the association between these two factors, overcoming the limitations of previous studies that primarily focused on abdominal and overall body composition. This study holds clinical significance because it supports the use of body composition measurements obtained via BIA to assess the risk of

MAFLD in adolescents. Moreover, our study provides a theoretical basis for developing treatment strategies to reduce the risk of MAFLD based on the association between regional body composition and MAFLD. For instance, protein supplementation (54) and resistance exercise (55) can increase muscle mass, particularly in the limbs, and reduce body fat, especially in the upper limbs and abdominal region, thus lowering the risk of MAFLD.

However, this study has several limitations. First, the study utilizes a cross-sectional design, meaning that data were collected at a single point in time. This design restricts our ability to make causal inferences, as we cannot determine the directionality or temporal sequence of the relationships between variables. To address this limitation, future research should consider longitudinal designs to better explore causal relationships. Additionally, incorporating experimental or randomized controlled trial methodologies could further validate our findings. Second, the potential selection bias arising from the specific recruitment process, including the inclusion of participants with chronic diseases and those using certain medications, should be acknowledged as a limitation of this study. Furthermore, we used BIA, a noninvasive and straightforward method, to assess body composition. The BIA method is widely used due to its rapid, simple, cost-effective, non-invasive, and safe characteristics. However, the accuracy of measurements obtained through BIA can be influenced by factors such as the BIA device, the subject's body composition, hydration status, and health conditions, leading to potential measurement error. Therefore, further research is needed to explore the relationship between body composition and MAFLD using more accurate measurements, such as densitometry. Moreover, when selecting variables for inclusion, some may have been omitted or missing, which could introduce potential confounders that weren't accounted for in the models. Finally, while CAP has been proven to be an effective diagnostic tool for detecting liver fat, it might be affected by factors, such as skin-to-capsule distance, age, or intercostal space width.

5 Conclusion

This study demonstrates that lower regional muscle percentages, particularly in the extremities, and higher regional fat percentages, especially in the upper limbs, are significantly associated with an increased risk of MAFLD in prepubertal children. Therefore, clinicians should incorporate segmental body composition analysis into routine risk assessments for pediatric MAFLD, with a particular focus on limb muscle mass and upper limb fat distribution. Moreover, personalized exercise interventions aimed at reducing upper limb fat while preserving muscle mass may represent an effective approach for managing MAFLD in children. Further studies are needed to validate these findings and explore stratified intervention strategies based on specific body composition characteristics, thereby providing more precise guidance for clinical practice.

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 ethical approval for this study was provided by the Affiliated Children's Hospital of Jiangnan University (WXCH2022-09-044). 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

MH: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. DS: Conceptualization, Formal analysis, Visualization, Writing – original draft. FY: Data curation, Methodology, Writing – original draft. XZ: Supervision, Writing – review & editing. NW: Data curation, Methodology, Writing – review & editing. HZ: Supervision, Writing – review & editing. XY: Formal analysis, Visualization, Writing – review & editing. JZ: Conceptualization, Supervision, Writing – review & editing. LZ: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Top medical expert team of the Wuxi Taihu Talent Program (Grant No. DJTD202106, GDTD202105, YXTD202101); Medical Key Discipline Program of Wuxi Health Commission (Grant No. ZDXK2021007, CXTD2021005); Top Talent Support Program for young and middle-aged people of Wuxi Health Committee (Grant No. BJ2023090, BJ2023023); Scientific Research Program of Wuxi health Commission (Grant No. Z202109, M202208); Wuxi Science and Technology Development Fund (Grant No. N20202003, Y20222001, K20241001); scientific technological innovation and venture capital fund in Wuxi (Y20232011); Jiangsu Medical Association Pediatric Medicine Phase II Scientific Research Special Fund Project (Grant No. SYH-32034-0106 (2024010)); and China Postdoctoral Science Foundation (2023M732681).

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.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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frontiers | Frontiers in Endocrinology

TYPE Case Report
PUBLISHED 26 May 2025
DOI 10.3389/fendo.2025.1501984

OPEN ACCESS

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RECEIVED 26 September 2024
ACCEPTED 25 April 2025
PUBLISHED 26 May 2025

CITATION
Oe Y, Omori T, Aimono E, Furukawa S, Kitakawa H, Tateno M, Sakai K and Cho KY (2025) Case Report: Amelioration of severe metabolic dysfunction-associated steatohepatitis after switching from conventional GLP-1RAs to tirzepatide. *Front. Endocrinol.* 16:1501984. doi: 10.3389/fendo.2025.1501984

Case Report: Amelioration of severe metabolic dysfunction-associated steatohepatitis after switching from conventional GLP-1RAs to tirzepatide

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Metabolic dysfunction-associated steatohepatitis (MASH) has cardiometabolic risk factors, such as obesity and type 2 diabetes, and has been reported to have a potentially higher risk of mortality than conventional steatotic liver diseases. Liver fibrosis develops and can progress to cirrhosis and hepatocellular carcinoma. Although some antidiabetic agents have been reported to ameliorate the condition, no specific medical treatment has been developed to date. Tirzepatide is a dual glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 (GLP-1) receptor agonist (GLP-1RA) that has shown efficacy against MASH in some clinical trials. However, these trials were limited to those with mild-to-moderate fibrosis and their history of treatment was often unclear. Here, we report the case of a 50-year-old man with a 16-year history of diabetes. He demonstrated poor control of his diabetes with elevated liver enzymes. A liver biopsy was performed and he was diagnosed with steatohepatitis. Liraglutide was administered for 3 years but his liver function and glycemic control deteriorated gradually and a second liver biopsy was performed in 2023. The histological examination found cirrhosis and liraglutide was switched to tirzepatide. Over 6 months of administration of tirzepatide, the patient's glycated hemoglobin and elevated liver enzyme levels improved. A third biopsy was performed, which showed a marked improvement in histology, with the amelioration of liver fibrosis. A diagnosis of steatotic liver disease was made. Although some previous studies had demonstrated an amelioration of liver fibrosis and an improvement in the prognosis of patients following GLP-1RA treatment, effective medications for patients with severe fibrosis or who are refractory to treatment with GLP-1RAs has not been identified to date. We reported a case with severe MASH whose condition had ameliorated by switching from conventional GLP-1RAs to tirzepatide.

KEYWORDS

glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1 receptor, type 2 diabetes, metabolic dysfunction-associated steatohepatitis, obesity

1 Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a new concept for steatotic liver disease (SLD), taking cardiometabolic risk factors into account (1). Recent studies have shown that MASLD is associated with higher risks of mortality and metabolic comorbidities than non-alcoholic fatty liver disease (NAFLD) (2). In patients with SLD, the progression of fibrosis can develop severe complications, including hepatocarcinoma (3). Several drugs for the treatment of this challenging disease are in development, but no breakthrough has been made to date (4). Tirzepatide was launched as the world's first dual glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor agonist (GLP-1RA) in April 2023 in Japan. The GIP receptor (GIPR) is expressed in various organs, including the brain and adipose tissue, and the infusion of GIPs induces glucose uptake and free fatty acid (FFA) re-esterification secondary to an increase in adipose tissue blood flow ***in vivo***. However, the reported effects of fat mass reduction have been inconsistent and the mechanisms appear to vary over time and according to the nature of any concurrent therapy, especially in combination with GLP-1 RAs (5). In clinical trials, treatment with tirzepatide has been reported to result in an overwhelming reduction in body weight and glycated hemoglobin (HbA1c), implying that it may be beneficial for many obesity-related diseases (6), including MASLD. However, its therapeutic effects for MASLD have not been fully investigated in a clinical setting. Here, we report the case with severe MASLD who had ameliorated by switching from conventional GLP-1RAs to tirzepatide.

2 Case description

A 50-year-old man was diagnosed with type 2 diabetes by his primary physician in 2008 and administered medication. Because of a failure of compliance with the medication, diet, and exercise therapy, his HbA1c remained in the 53.0–64.0 mmol/mol range, and he was referred to our department in 2014. At his initial visit, his body weight was 98.9 kg (body mass index: 33.0 kg/m^2) with an HbA1c of 90.2 mmol/mol with elevated liver enzymes [aspartate aminotransferase (AST), 97 IU/L; alanine aminotransferase (ALT), 109 IU/L]. After referral to our hospital, sodium-glucose cotransporter-2 inhibitor (SGLT2i) was started in 2016 and his HbA1c improved to below 53.0 mmol/mol for some months. However, his glycemic control gradually deteriorated again and the administration of liraglutide was commenced in 2017. Though he had continued liraglutide of 0.9 mg/day for 1 year, it was switched to a dipeptidyl peptidase-4 inhibitor (DPP-4i) for financial reasons. This caused his glycemic control to deteriorate to an HbA1c of 80.3 mmol/mol; therefore, dulaglutide was administered from 2018 as an alternative. During this period of time, elevated liver enzymes persisted. There was no history of alcohol consumption and the results of various antibody tests were negative; therefore, a liver biopsy was performed for further examination in 2020.

The histological diagnosis made was steatohepatitis on the basis of the following evaluations: NAFLD activity score (NAS) of 3 (steatosis, 1; lobular inflammation, 2; ballooning, 0); Matteoni classification, type 2; and Brunt classification, stage 3. In response to this result, the GLP-1RA being administered was switched from dulaglutide to liraglutide 1.8 mg/day, but his HbA1c remained at approximately 70.0 mmol/mol and, finally, insulin therapy was started. Nevertheless, his HbA1c remained over 75.0 mmol/mol and liver enzymes remained high; therefore, a liver biopsy was performed again in 2023. The histological finding had deteriorated, and a diagnosis of cirrhosis was made on the basis of the following evaluations: NAS of 6 (steatosis, 2; lobular inflammation, 2; ballooning, 2), Matteoni classification, type 4; and Brunt classification, stage 4 (Figures 1A, B). Following the result, we decided to switch the patient from liraglutide to tirzepatide at 2.5mg weekly. After the initiation of tirzepatide, the patient's HbA1c improved steadily from 77.1 mmol/mol to 57.4 mmol/mol and he was able to reduce the total daily dose of insulin administered from 66 units to 40 units (Figure 2). Furthermore, his elevated liver enzyme levels also decreased (AST from 74 IU/L to 35 IU/L, and ALT from 84 IU/L to 61 IU/L) (Figure 2). The dose of tirzepatide being administered was increased to 5.0 mg after 3 months from its initiation (Figure 2). Tolerable gastroenterological side-effects were reported during monthly consultations. Although the patient's body weight temporarily decreased from 92.4 kg to 90.8 kg during the first 3 months of treatment, it returned to 92.0 kg over the following 6 months (Supplementary Figure 1). Nevertheless, his visceral fat area decreased from 421.8 cm^2 to 167.3 cm^2 over 6 months and his lean mass increased from 59.1 kg to 60.7 kg (Supplementary Figures 1, 2).

After 6 months of treatment, the elevated liver enzyme levels had improved and a third liver biopsy was performed. The histological finding had ameliorated remarkably and a diagnosis of steatotic liver disease was made on the basis of the following evaluations: NAS of 3 (steatosis, 1; lobular inflammation, 1; ballooning, 1), Matteoni classification, type 3; and Brunt classification, stage 2 (Figures 1C, D). Some non-invasive tests for liver fibrosis, including fibrosis 4 index (FIB-4 index), NAFLD fibrosis score (NFS), and aspartate aminotransferase-to-platelet ratio index (APRI), also improved (FIB-4 index from 2.99 to 1.66, NFS from 0.093 to -0.07, and APRI from 1.83 to 0.86). As concomitant medications, he had been prescribed antihypertensive drugs, i.e., an angiotensin II receptor blocker and a calcium channel blocker; a proton pump inhibitor; a xanthine oxidase inhibitor; a beta blocker; a histamine-1 receptor antagonist; and ursodeoxycholic acid at constant doses during this period. Diet and exercise therapy had also been continued, with the patient's energy intake being limited to 1,600 kcal/day (6,694,400 joules per day). The histological finding was evaluated by two professional pathologists in different institutions who were blinded to the laboratory data and there were no discrepancies in their evaluations. The histology was evaluated by representative methods, namely, NAS for steatosis, and Brunt and Matteoni classifications for fibrosis (7–9). Non-invasive tests for liver fibrosis were calculated using the formula presented in the previous report (10).

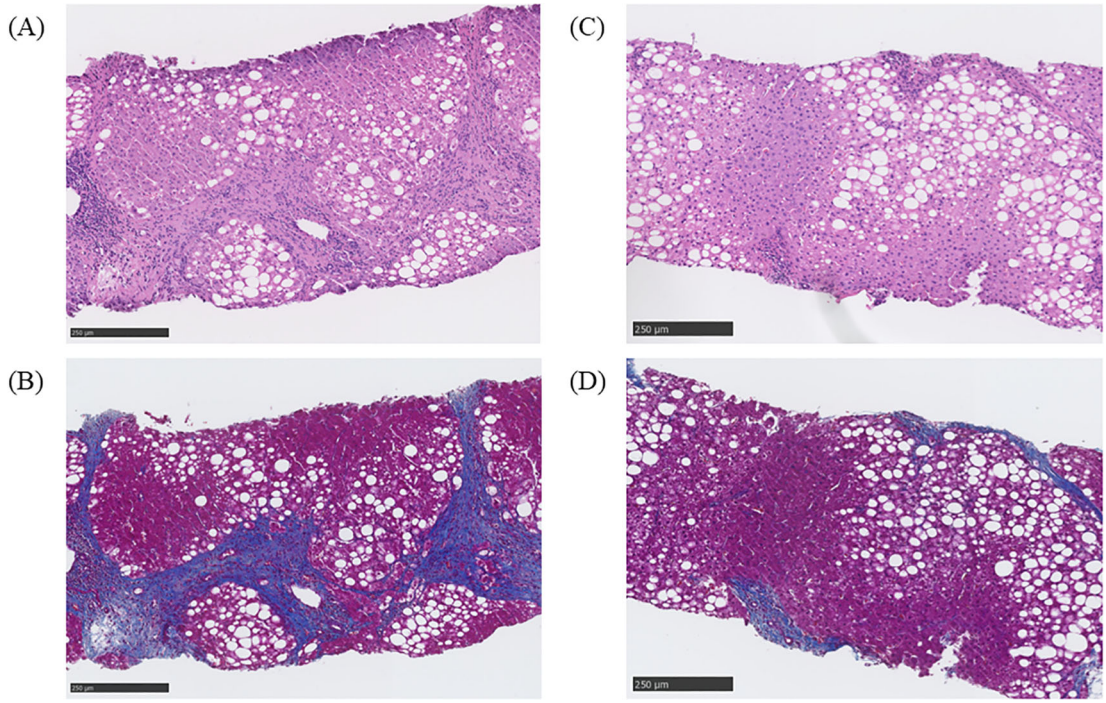

FIGURE 1
Histological findings before and after treatment with tirzepatide. **(A)** Evaluation of liver steatosis by hematoxylin and eosin staining before administration of tirzepatide. The steatosis was evaluated as follows: non-alcoholic fatty liver disease (NAFLD) activity score (NAS) of 6 (steatosis, 2; lobular inflammation, 2; ballooning, 2). **(B)** Evaluation of liver fibrosis using Masson's trichrome staining before the administration of tirzepatide.The fibrosis was evaluated as follows: Brunt classification, grade 3, stage 4. **(C)** Evaluation of liver steatosis by hematoxylin and eosin staining after the administration of tirzepatide. The steatosis was evaluated as follows: NAS of 3 (steatosis, 1; lobular inflammation, 1; ballooning, 1). **(D)** Evaluation of liver fibrosis by Masson's trichrome staining after administration of tirzepatide. The fibrosis was evaluated as follows: Brunt classification, grade 1, stage 2.

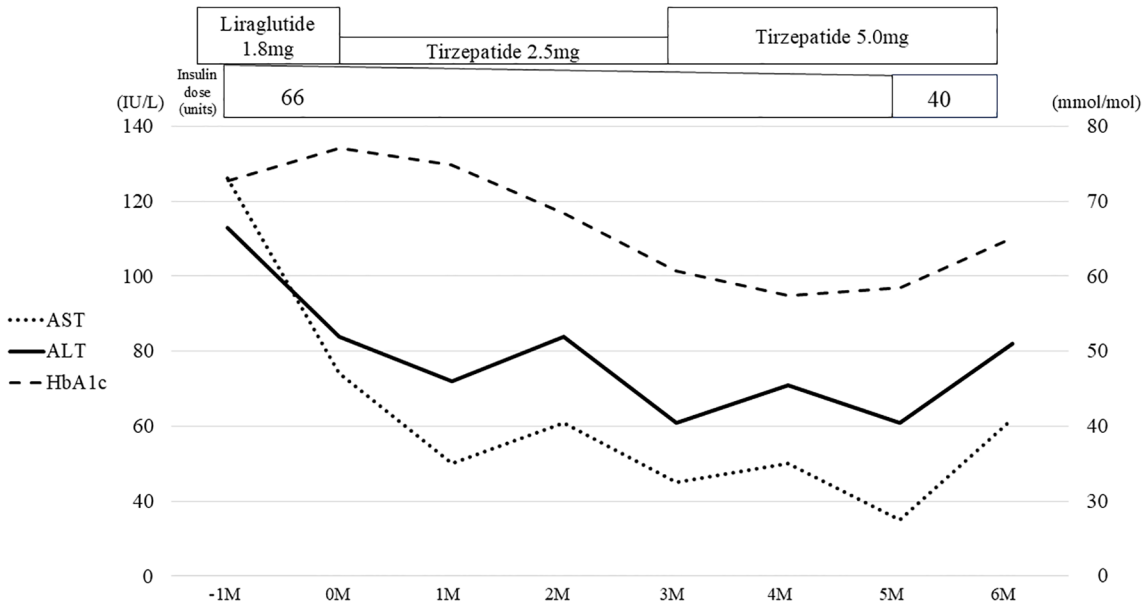

FIGURE 2
Clinical course associated with tirzepatide for 6 months. The gray bars represent the values of HbA1c, the solid line shows the changes in ALT, and the dotted line shows the changes in AST. Liraglutide was switched to tirzepatide and the concomitant insulin dose was reduced over time. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

3 Systematic review of the literature

To assess the uniqueness of this case, we conducted a systematic review of the literature in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. A systematic review was conducted using the PubMed, Web of Science, and Cochrane databases, searching using the terms "Tirzepatide" and "Liver" on 20 January 2025. We included studies that met the following inclusion criteria, using the Patient, Intervention, Comparison, and Outcome (PICO_ methodology: the targeted patients were all treated with tirzepatide and the intervention was the administration of tirzepatide. We recorded each comparator, but did not establish strict criteria and included case reports. The outcomes were changes in liver function and imaging findings, and adverse events involving the liver were also recorded. We excluded review articles, protocols, practical guidelines, animal studies, expert opinions, abstracts, and commentaries. A PRISMA flow diagram of the selected studies is presented in Supplementary Figure 3. A total of 217 publications were identified, from which 77 duplicates were removed. From the remaining 139 articles, 125 were excluded on the basis of a review of their titles and abstracts. From among these articles, studies of hepatic pharmacokinetics and those that did not include the measurement of specific indices of hepatic injury were excluded, even if they were clinical studies.

Following this screening, three case reports, four randomized controlled trials including *post-hoc* analysis, one retrospective observational analysis, and two database analyses remained for inclusion in the systematic review (Supplementary Table 1) (11–20). All of the case reports concerned adverse events involving the liver (11–13). The substudy of the SURPASS-3 trial demonstrated an approximate 5.0% reduction in liver fat content on magnetic resonance imaging following the administration of tirzepatide 5.0 mg (15). Hartman et al. reported that biomarkers of liver fibrosis were reduced by tirzepatide (16). Only Loomba et al. reported the resolution of metabolic dysfunction-associated steatohepatitis (MASH) in the SYNERGY-NASH trial, which involved histological investigations (14). However, these subjects were limited to patients with mild-to-moderate fibrosis (Brunt stage 2–3), and patients with cirrhosis were excluded. The only two studies that were not derived from clinical trials were those conducted by Sawamura et al. and Buckley et al. (19, 20). Both showed improvement in liver enzymes, including AST and ALT, but the criteria for determining whether the background status of the liver qualified as MASLD were not clearly defined.

4 Discussion

In this case, the switch from liraglutide to tirzepatide demonstrated an improvement in HbA1c with a reduction in the daily insulin dose and showed amelioration of the hepatic steatosis and fibrosis in the histological evaluations. Only a few clinical trials have shown the effect of tirzepatide on SLD to date (15, 16). Significant factors contributing to this change were the reductions in body weight and visceral fat volume (15, 16). However, none of the studies involved histological imaging. Recently, the SYNERGY-NASH trial showed the resolution of MASH by tirzepatide with histological findings (14). However, only a limited number of reports explicitly identified the participants as having MASLD, and very few included biopsy-derived data (14, 16). Thus, we are the first to report the potential utility of tirzepatide for severe MASH with histological evidence and discuss the mechanism involved.

A previous report showed that a body weight reduction of 7%–10% can ameliorate SLD (21). Although the existence of a dose-response relationship between improvements in hepatic steatosis and weight loss has been suggested, there is insufficient evidence that fibrosis is ameliorated by weight loss alone. In other words, the pathogenesis of liver fibrosis comprises various and complex factors from lipid metabolism to chronic inflammation (22, 23). How do the GIP or a combination of GLP-1 and GIP work against these? To date, the expression of GIPR has not been identified in the liver, suggesting that these actions on hepatic metabolism may be indirect (24). Of course, a reduction in energy intake could be expected due to appetite suppression (24, 25). GIPR is widely expressed in the central nervous system, including in the hypothalamus, and one previous study indicated that GIP may act directly by crossing the blood-brain barrier *in vivo* (25). There is a report of the administration of tirzepatide affecting lipid preference, albeit in rodents, which may cause changes not only in the quantity but also in the content of meals (26). The effect of GIP in adipose tissue may be important, but it is controversial. The function of white adipose tissue (WAT) is to regulate circulating lipids through the release or storage of FFAs; however, it can be impaired by various pathways, including decreased adipose tissue blood flow in type 2 diabetes (25, 27). Though an infusion of GIP can cause hypertrophy of adipocytes to occur instead of reducing spillover of FFAs, this may be able to restore the original lipid-buffering capacity by correcting WAT blood flow (28). This optimization of lipid metabolism may have led to a decrease in ectopic fat and improved MASLD. It is also possible that an amelioration of fibrosis was induced *via* the anti-inflammatory action of GIP (24). The administration of GIP reduces the expression of pro-inflammatory cytokines and chemokines, and consequently the inflammation in adipose tissue (29). Inflammatory signals stimulate apoptosis and contribute to the deterioration of liver fibrosis (30). Particularly, GIP may suppress macrophage-driven inflammation (24), and a previous report also showed that tirzepatide inhibits the apoptosis of hepatocytes (16). Even though the present case had severe fibrosis, this was ameliorated by tirzepatide, despite only a 2% weight loss being achieved (Supplementary Figure 1). Focusing on the change in fat mass, however, the patient had actually achieved a reduction of 7.0% (Supplementary Figure 1). This was consistent with the desired body weight loss, and visceral fat accumulation had also reduced by 15% (Supplementary Figure 2). Instead, lean mass had increased by 2.7%, which could explain there being no change in apparent body weight (Supplementary Figure 1). Although measurements of inflammatory markers were not made in the present study, it is possible that fat mass loss relieved the inflammation induced by adipose tissue. In addition to the reduction in insulin dosage with favorable glycemic control, the effect on and change in adipose tissue may have

contributed to the amelioration of fibrosis. Xiang et al. reported that GLP-1RAs ameliorate muscle atrophy (31), and GIP may help with this. GIPR and GLP-1 receptors are not expressed in skeletal myocytes, but these hormones may have had favorable effects on muscle indirectly (32). Nevertheless, the mechanisms are still uncertain, and therefore, additional research regarding these mechanisms and the durability of the effects of agonism at each receptor, separately and in combination, is warranted.

Of the established antidiabetic agents, pioglitazone, SGLT2is, and GLP-1RAs have been reported to be effective for liver fibrosis (33). The SYNERGY-NASH trial demonstrated the effects of tirzepatide on liver fibrosis, but its effects had not been adequately investigated to date with reference to the backgrounds of the patients. Nearly half of the subjects in this study had type 2 diabetes, but their previous treatments were not presented (14). The combination of type 2 diabetes and NAFLD aggravated each pathogenesis and information regarding the previous treatment is important to assess their potential refractoriness to specific treatments (34). In our case, empagliflozin and liraglutide had previously been administered, but his fibrosis was only ameliorated after the switch from liraglutide to tirzepatide. While the effect on SLD have been reported in SGLT2is and GLP-1RAs, a few studies have involved investigations of their effects on fibrosis using histological assessments (35, 36). Our case also had taken an SGLT2i for a long period of time, and there was no change before and after the administration of tirzepatide. With respect to GLP-1RAs, only liraglutide has been shown to ameliorate liver fibrosis at any stage; semaglutide showed no significant effectiveness in a placebo-controlled trial (36). In compensated liver cirrhosis, the administration of GLP-1RAs has been shown to improve the prognosis of patients, and tirzepatide may have benefits as well (37). In the scope of our systematic review, several studies showed that tirzepatide reduces the level of liver enzymes, even when GLP-1RAs have been used as a pre-treatment. However, the background status of the liver was not described in these reports (Supplementary Table 1) (19, 20). A clinical trial (NCT05751720) for severe fibrosis is ongoing, and we hope that this case will be replicated in this trial.

As for limitations, this was a case report and additional clinical trials are desirable in a clinical setting. Liver biopsies were performed using only one puncture at each time point, and there was a risk of sampling errors. We did not examine several biomarkers for the assessment of fibrosis, such as type IV collagen 7S or mac-2-binding protein glycosylation isomer. Quantitative image findings, including elastography or Fibroscan, were not performed either, as the facility did not have the equipment. However, histopathological findings are the most robust means of assessing the status of the liver. Some non-invasive tests, while considered supplementary, were included in the evaluation. Additionally, it should also be noted that there are no current guidelines recommending the use of tirzepatide for the treatment of cirrhosis. We also conducted a systematic review following the PRISMA guidelines, but we did not register it in the database. Though information was obtained from multiple databases, the number of reports extracted was small. These also included case reports, making it unsuitable for standardizing the quality of the studies. Furthermore, the screening was performed by several independent authors without the use of automated software, which may introduce a potential risk of bias. As a potential confounding factor, we could not entirely exclude the possibility of lifestyle changes accompanying medication adjustments, and the retrospective nature of the study made it challenging to minimize sources of bias. Although it is inexplicable that there was no history of pioglitazone administration in the present case, we have shown the efficacy of tirzepatide following treatment with SGLT2is and GLP-1RAs. From the report of Rosenstock et al., patients who exhibit greater improvements in HbA1c may also have more significant improvements in liver impairment (18). Tirzepatide may ameliorate MASH through its marked effects on glycemic control and weight loss, which occur regardless of the previous treatments.

In conclusion, the present case report shows that tirzepatide may be an effective treatment for cases with severe hepatic fibrosis that are refractory to conventional treatment with GLP-1RAs. The combination of type 2 diabetes and liver cirrhosis has a poor prognosis; thus, medications are desired to be effective for both conditions simultaneously. We hope that new solutions will be discovered for this challenging 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.

Ethics statement

The requirement of ethical approval was waived by Ethics Committee of Kushiro Red Cross Hospital for the studies on humans because it was not necessary for the reported investigations, as they were performed in routine clinical setting and therapeutic intention. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. Patient provided written consent in reporting his case in an international published medical journal. 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

YO: Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. TO: Investigation, Writing – review & editing. EA: Data curation, Investigation, Resources, Writing – review & editing. SF: Supervision, Writing – review & editing. HK: Funding acquisition, Writing – review & editing. MT: Data curation, Investigation,

Resources, Writing – review & editing. KS: Writing – review & editing. KC: Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Acknowledgments

The authors would like to express their gratitude to all the patients and staff who participated in this study. We thank Mark Cleasby, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Conflict of interest

YO has received honoraria for lectures from Novo Nordisk Pharma Ltd.

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

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2025.1501984/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Changes in body composition over 6 months. These data were obtained using a body composition analyzer (InBody 770; InBody USA, Cerritos, CA, USA). The bars show the mass of each tissue compartment; the dotted area, fat mass; the striped area, the lean mass; and the filled black area, other compartments. The mass of each compartment and the total mass are shown for each time point.

SUPPLEMENTARY FIGURE 2
Changes in each fat mass over 6 months. These data were measured by computed tomography. Within the pie chart, the dotted slice represents the subcutaneous fat mass and the striped area represents the visceral fat mass. The total mass (presented above) reduced over 6 months and, particularly, visceral fat mass predominantly decreased.

SUPPLEMENTARY FIGURE 3
Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. YO and KYC contributed to the screening and selection process of articles manually. Case reports were included.

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 03 June 2025
DOI 10.3389/fendo.2025.1500242

OPEN ACCESS

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RECEIVED 23 September 2024
ACCEPTED 06 May 2025
PUBLISHED 03 June 2025

CITATION
Taiwo A, Merrill RA, Wendt L, Pape D, Thakkar H, Maschek JA, Cox J, Summers SA, Chaurasia B, Pothireddy N, Carlson BB, Sanchez A, Ten Eyck P, Jalal D, Dokun A, Taylor EB and Sivitz WI (2025) Metabolite perturbations in type 1 diabetes associated with metabolic dysfunction-associated steatotic liver disease.
Front. Endocrinol. 16:1500242.
doi: 10.3389/fendo.2025.1500242

Metabolite perturbations in type 1 diabetes associated with metabolic dysfunction-associated steatotic liver disease

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly called non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome. Although MASLD has been widely studied in persons with Type 2 diabetes (T2D), far less in known about the pathogenesis and severity of MASLD in Type 1 diabetes (T1D).

Objectives: Determine metabolic perturbations associated with MASLD in persons with T1D.

Study Design: We conducted a cross-sectional study of 30 participants with T1D. Based on the results of a FibroScan, participants were stratified as cases (MASLD) or controls. Metabolomic analyses were performed on plasma obtained from all participants after an overnight (after midnight) fast.

Results: 17 of 30 participants were classified as cases (MASLD) and 13 as controls. Cases had higher BMI (p=<0.001) and were taking higher daily insulin doses than controls (p=0.003). Metabolomic analyses revealed that those with MASLD had elevated levels of gluconeogenic substrates pyruvate (p=0.001) and lactate (p=0.043), gluconeogenic amino acids alanine (p<0.001) and glutamate (p=0.004), phenylalanine (p=0.003), and anthranilic acid (p=0.015). Lipidomics revealed, elevated ceramides (P=0.02), diacylglycerols (p=0.0009) and triacylglycerols (P=0.0004) in MASLD group. In those with MASLD, the acylcarnitines, isovalerylcarnitine (CAR.5.0) (P=0.002) and L-Palmitoylcarnitine (CAR.16.0) (P=0.048), were elevated. Pathway analyses using MetaboAnalyst 5.0

Software revealed that, pathways including phenylalanine and tyrosine metabolism, tryptophan metabolism, glucose-alanine cycle, glutamate metabolism, and glutathione metabolism were significantly enriched in those with MASLD.

Conclusion: Participants with T1D and MASLD manifest features of insulin resistance and metabolite perturbations suggesting enhanced gluconeogenesis, dysfunctional fat synthesis, and perturbed TCA cycle activity.

KEYWORDS

NAFLD, MASLD, NASH, MASH, Type 1 diabetes, Type 2 diabetes, FibroScan

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly called non-alcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in the USA and developed countries with a rate in the adult population between 10-30% (1). MASLD consists of a spectrum of abnormalities with the earliest stage being simple steatosis, which progresses to metabolic dysfunction-associated steatohepatitis (MASH), formerly called non-alcoholic steatohepatitis (NASH). MASH consists of steatosis with hepatocyte ballooning, and inflammation with or without fibrosis and can further progress to cirrhosis, which is a risk factor for hepatocellular carcinoma (2). Cardiometabolic risk factors associated with the development of MASLD include obesity, insulin resistance and type 2 diabetes (T2D) (3). The global prevalence of MASLD among patients with T2D is estimated at 51-70% (3) and 22% in patients with Type 1 diabetes (T1D) (4). Younossi et al. estimated in 2017 that, the lifetime direct costs due to NASH in the United States would be $222.6 billion (5). The incidence of hepatic decompression, hepatocellular carcinoma, and death due to NASH cirrhosis are expected to increase 2 to 3-fold by 2030 (6).

The pathophysiology underlying MASLD is complex and incompletely understood. Hepatocyte fat accumulation is commonly associated with hyperinsulinemia and insulin resistance (7). The resultant lipotoxicity is believed to contribute to the severity of MASLD by inducing endoplasmic reticulum and oxidative stress, autophagy, apoptosis, and inflammation (7). Recent evidence indicates that obesity and insulin resistance in T1D are increasing over time (8), suggesting greater risk for MASLD. Although several studies have examined the pathogenesis of MASLD in T2D, studies of MASLD in T1D are limited and no study to date has evaluated the metabolomic profile associated with MASLD in TID. Studies of persons with T2D and obesity with MASLD showed that, increased levels of branched chain amino acids, aromatic amino acids, glutamate, alanine and ceramides, contribute to insulin resistance and hepatic dysfunction (9, 10).

To evaluate MASLD in T1D, we conducted a cross-sectional study. Participants with T1D were stratified as MASLD (cases) or controls, dependent on the results determined by FibroScan imaging. Key fasting intermediary metabolites, lipid species, and acylcarnitines were measured.

Given the lack of information regarding MASLD in type 1 diabetes and knowing that a substantial subset of individuals with type 1 diabetes are overweight or obese, we hypothesized that a random sample of participants with T1D would have a high prevalence of MASLD and those with MASLD would exhibit metabolites associated with insulin resistance including ceramides, glutamate, branched chain amino acids, and aromatic amino acids. We further hypothesize that these metabolites would correlate with the severity of MASLD as determined by FibroScan imaging.

Research design and methods

Study design

We conducted a cross-sectional study of 30 participants with T1D recruited from our university diabetes outpatient clinics. Inclusion criteria included: (i) age 18 and older and (ii) history of T1D with duration greater than 5 years. T1D was confirmed by evidence of either undetectable C-peptide or positive anti-glutamic acid decarboxylase antibody or typical history of T1D determined by an experienced diabetologist/endocrinologist in the setting of our diabetes specialty clinic. Exclusion criteria included: (i) alcohol consumption greater than 20 g/day in women or 30 g/day in men, (ii) hepatitis B or C infection, (iii) autoimmune liver disorders or other known metabolic causes of chronic liver disease such as hemochromatosis and Wilson's disease, (iv) current pregnancy or lactation, (v) medications known to cause steatosis or (vi) illicit drug use. The study protocol was approved by our Institutional Review Board (approval #: 202405130). 250

Abbreviations: MASLD, Metabolic dysfunction-associated steatotic liver disease; MASH, Metabolic dysfunction-associated steatohepatitis; NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; T1D, Type 1 diabetes; T2D, Type 2 diabetes; GC-MS, gas chromatography mass spectroscopy; LC-MS, liquid chromatography mass spectroscopy; CAP, controlled attenuation parameter; dB/m, decibels per meter; AIC, Akaike Information Criterion; TCA, tricarboxylic acid.

individuals were invited to participate by letter or by face-to-face contact in our Endocrinology and Metabolism clinics. 35 responded positively. Of those 35, 30 met inclusion and exclusion criteria and were enrolled. Participants were recruited over a 7-month period from September 2021 to March 2022.

Participants were classified as cases (MASLD) if they had a controlled attenuation parameter (CAP) score (see below) of >=248 (mild or greater steatosis) or as controls if the CAP score was < 248 (11). Since there is variability in recommended CAP score cutoff points (12–14), we performed a sensitivity analysis using a CAP score of > 267 (moderate or greater steatosis) rather than >=248 for classification of cases versus controls. The specific data adjusted in this way appear in supplemental information.

Participants attended a single study visit in our Institutional Clinical Research Unit (CRU). Informed consent was obtained, blood was drawn for plasma metabolites and a liver FibroScan was performed. Demographic data including age, race, gender, and BMI was extracted from the history or electronic medical record. The total daily insulin dose was obtained from chart review and confirmed by the participant.

Participants were asked to avoid food intake from midnight until completion of their study visit. They were to take their scheduled long-acting insulin on the morning of their visit but omit their short acting pre-breakfast insulin dose. Participants were taking insulin either through a subcutaneous pump or receiving multiple doses including evening and/or bedtime insulin. As clinical goals include achieving fasting AM glucose control, participants entering our study had been treated to provide adequate basal insulin for this purpose. Therefore, we made no adjustments in insulin therapy on the night or day prior to study. If the pre-visit AM glucose was less than 70 mg/dl or greater than 200 mg/dl, participants were to reschedule their visit. At their study visit, 3 ml of blood was drawn into an EDTA containing tube for separation into plasma for subsequent metabolomic, lipidomic, and acylcarnitine analyses. Participants then had a FibroScan (Transient Elastography) done at our Gastroenterology Procedure Unit.

Metabolite determinations

Fasting blood samples were placed on ice for 10 minutes, centrifuged, plasma extracted, and stored at -80C. All 30 plasma samples were processed and run together to prevent batch effects. Broad metabolite profiling was conducted using Gas Chromatography Mass Spectrometry (GC-MS). Lipidomics and acylcarnitines were measured using liquid chromatography tandem mass spectrometry (LC-MS). Comprehensive GC-MS and LC-MS methods are detailed in supplemental information. A total of 117 metabolites were measured by GC-MS and 14 acylcarnitines and 5 lipid species measured by LC-MS.

FibroScan (liver elastography)

FibroScan was performed using an Echosense instrument (France) with either M or XL probes. The standard M probe was used when the skin-liver capsule distance (SCD) was less than 25 mm. The XL probe was used for patients with a larger SCD, including obese patients (BMI of 30 or above) and patients with a SCD of > 25 mm and a thoracic circumference of 75 cm or more, where the M probe can be unreliable due to interference from fat tissue. The FibroScan software automatically recommends the right probe size.

A liver stiffness score in kPa (kilopascal) was determined from measurement of shear wave propagation and used as a quantitative index of liver fibrosis stage, ranging from absent: Stage F0-1 (score < 8.2 kPa) through severe fibrosis Stage F4 (13.6 kPa) (11). A controlled attenuation parameter (CAP) score in dB/m (decibels per meter) was determined and used as a quantitative index of hepatic steatosis ranging from absent (S0: < 248), mild steatosis (S1: 248-267), moderate steatosis (S2: 268-279) to severe steatosis (S3: > 280) (11). We used the FibroScan to diagnose MASLD as opposed to the gold standard liver biopsy, due to the invasive nature and possible morbidity associated with biopsies.

Statistical analyses

After metabolite peak areas and the NOREVA correction were performed as described in the supplementary methods, values for individual metabolites were normalized by per sample total metabolite signal. Per sample-normalized individual metabolite values were then divided by the mean of the control group for that metabolite, leading to individual metabolites being reported on a fold-of-control basis. Categorical variables were summarized using counts and percentages, while continuous demographic variables were summarized using either means and standard deviations or medians and inter-quartile ranges. The choice between the two methods depended on the normality of the distribution as determined by a Shapiro-Wilk test. Continuous variables with a Shapiro-Wilk p-value less than 0.05 were considered non-normally distributed, while the remaining continuous variables were considered to be approximately normal.

To assess differences in demographic variables between cases and controls, Fisher's Exact Test was utilized for categorical variables. For the non-normal and approximately normal continuous variables, the Wilcoxon rank sum test and Welch two sample t-test were employed respectively. In addition, correlations between continuous measures of interest were also evaluated using Spearman's correlation.

To construct regression models to predict fibrosis and steatosis scores, a stepwise selection algorithm was used to find the 3-predictor model for each outcome variable that has the lowest Akaike Information Criterion (AIC). All metabolites and acylcarnitines were considered as candidates for inclusion into the model, as well as BMI and 24-hour insulin dose. This algorithm began by fitting an intercept-only model and then examining whether the addition of independent variables would improve the AIC of the model. This process was continued until the model had 3 independent variables, and then the algorithm was terminated. A linear regression model was used to predict steatosis scores due to

their approximately normal distribution, while a log-transformed Gamma model was used to predict fibrosis scores due to the right-skewed nature of fibrosis scores in our study. Point and interval estimates were calculated for predictors in each optimal model, along with p-values.

P-values less than 0.05 were considered statistically significant. False Discovery Rate-adjusted p-values were calculated using the Benjamini & Hochberg correction and are presented to provide context for interpretation of the data given the many comparisons that were performed. Data analyses were carried out using R version 4.2.3, GraphPad Prism version 9, and by the MetaboAnalyst 5.0 software package.

Results

Demographics

Participants characteristics are listed in Table 1. Of 30 participants, 13 were controls and 17 were cases. Those with MASLD versus the controls were of comparable age and gender. The group with MASLD had a significantly higher BMI (p <0.001) and were taking higher daily insulin doses (p=0.003). There were no statistically significant differences in age, race, gender or hemoglobin A1C. The mean duration of diabetes was significantly longer in the controls than those with MASLD. Sensitivity analyses using a CAP score of ≤ 267, which encompassed 16 controls and 14 cases, showed that the duration of diabetes lost significance but was still longer in the case cohort (p =0.08). Moreover, not surprisingly the difference in fibrosis staging became significant (more controls in mild stage) when classified by CAP score ≤ 267.

TABLE 1 Participant demographics.

Characteristic	CONTROL, N = 13[1]	CASE, N = 17[1]	p-value[2]
AGE (yrs)	38 (34, 42) [Min: 25, Max: 67]	41 (29, 65) [Min: 23, Max: 72]	0.66
SEX			
Female	7 (54%)	8 (47%)	
Male	6 (46%)	9 (53%)	
Duration of DM (yrs)	25 (17, 39) [Min: 10, Max: 61]	13 (13, 17) [Min: 3, Max: 61]	**0.032**
Mean HbA1C (%)	7.10 (1.31)	7.58 (1.12)	0.299
BMI (kg/m^2)	28 (5)	37 (7)	**< 0.001**
24hrs Insulin dose (units)	44 (35, 54)	67 (57, 115)	**0.003**
Insulin unit per Kg	0.51 (0.47, 0.70) [Min: 0.34, Max: 1.35]	0.69 (0.59, 0.91) [Min: 0.38, Max: 2.61]	0.062
RACE			0.433
Black	1 (7.7%)	0 (0%)	
Caucasian	12 (92%)	17 (100%)	

[1]Median (IQR); n (%); Mean (SD).
[2]Wilcoxon rank sum test; Fisher's exact test; Welch Two Sample t-test.
The bold values are statistically significant with p <0.05.

Steatosis and fibrosis

FibroScan results (Table 2) showed that cases had a higher mean steatosis score than controls. All cases had steatosis, with 13 out of 17 (76%) having moderate to severe steatosis (Stage S2-S3). Four out of 17 (24%) in the case group had evidence of fibrosis of stage F2 or greater. A comprehensive list of our FibroScan results is shown in Supplementary Table 1.

Association between 24h insulin dosage and severity of steatosis or fibrosis: Among all participants, we observed a positive correlation between daily insulin dosing with both steatosis and fibrosis scores (Figures 1A, B). Upon stratification, the correlation among the case group for fibrosis score with daily insulin dosing remained significant while steatosis correlated at a p value of 0.052 (Figures 1C, D). Sensitivity analysis revealed that the correlation of steatosis with insulin dosing among the case group became significant at a p value of 0.025.

Metabolite determinations

117 metabolites were assessed by GC-MS. Figure 2; Supplementary Table 2 show that the aromatic amino acid, phenylalanine, was higher in the group with MASLD versus controls (p=0.003), but the other aromatic amino acids,

TABLE 2 FibroScan results.

Characteristic	Controls, N = 13[1]	Cases, N = 17[1]	p-value[2]
Steatosis Score	202 (20)	312 (44)	**< 0.001**
Steatosis Stage			**< 0.001**
S0	13 (100%)	0 (0%)	
S1	0 (0%)	4 (24%)	
S2	0 (0%)	3 (18%)	
S3	0 (0%)	10 (59%)	
Fibrosis score	4.5 (1.2)	7.4 (3.6)	**0.005**
Fibrosis Stage			0.492
F0-F1	13 (100%)	13 (76%)	
F2	0 (0%)	1 (5.9%)	
F3	0 (0%)	2 (12%)	
F4	0 (0%)	1 (5.9%)	

[1]Mean (SD); n (%).
[2]Welch Two Sample t-test; Fisher's exact test.
The bold values are statistically significant with p <0.05.

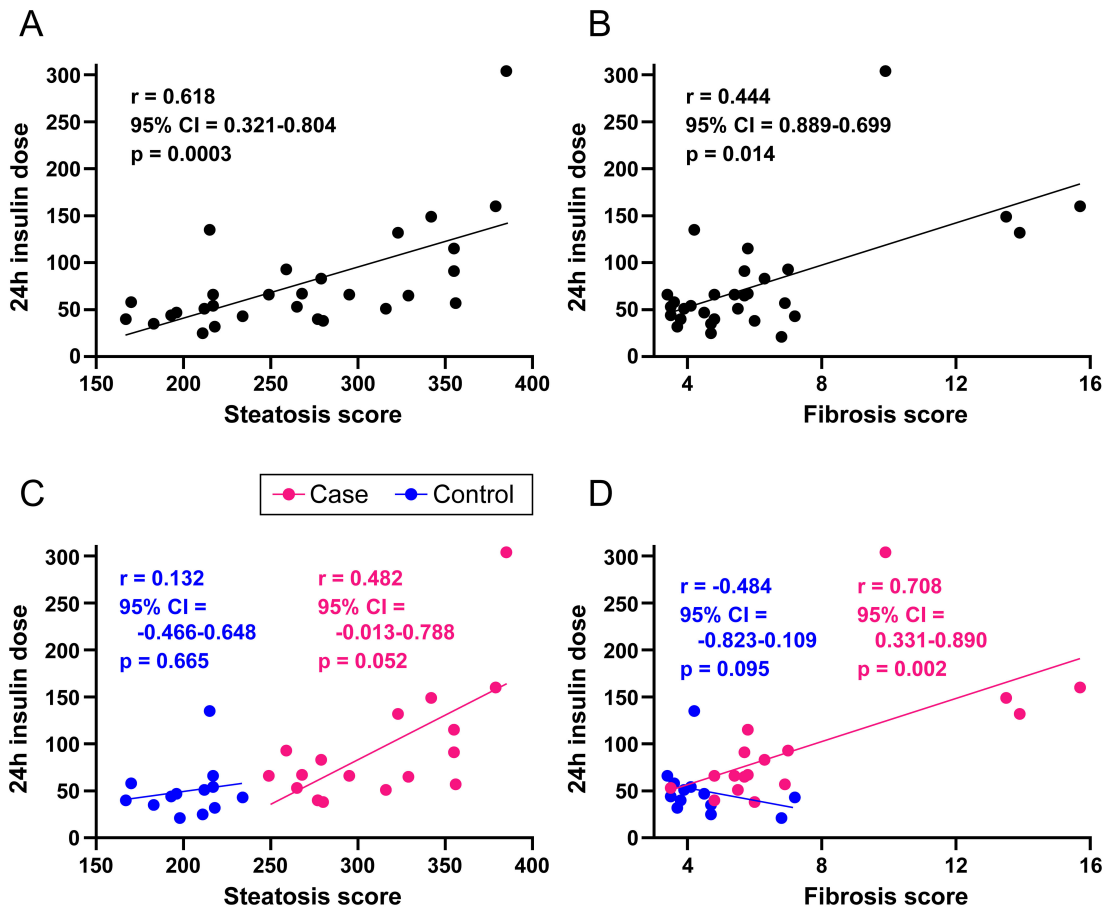

FIGURE 1
Correlations between 24h insulin dosage with steatosis and fibrosis scores. Data are shown separately for combined cases and controls **(A-B)** and individually for cases and controls **(C-D)**. r values by Spearman correlation. Lines by linear regression.

tryptophan and tyrosine, did not differ significantly among groups. Dihydroxyphenylalanine, a metabolite of tyrosine was higher in cases and trended towards significance at p=0.053. Isoleucine, a branched chain amino acid was higher in cases and trended towards significance at p=0.05. Anthranilic acid, a metabolite of tryptophan, was significantly elevated in MASLD (p=0.015). Other metabolites elevated in the MASLD included glutamate (p=0.004), histidine (p=0.024), proline (p=0.039), and the major gluconeogenic substrates; lactate (p=0.043), pyruvate (p= 0.001), and alanine (p=<0.001). Metabolites significantly reduced in the MASLD group included cinnamate (p=0.024), homocysteine (p=0.048) and N-acetyl-methionine (p=0.020). We also quantified 14 species of plasma acylcarnitines using LC-MS. Among these CAR 5.0 (p = 0.002) and CAR 16.0 (p = 0.048) were significantly greater in the MASLD group: Supplementary Table 3. A comprehensive list of all measured metabolites and acylcarnitines and are shown in Supplementary Tables 2, 3, respectively. Using the CAP of ≤ 267, sensitivity analyses revealed loss of significance for anthranilic acid, histidine, proline, cinnamate, and homocysteine. The gluconeogenic metabolites; lactate, pyruvate, and alanine remained significantly elevated in the case group. The acylcarnitine, CAR 5.0 remained elevated in the case group but CAR 16.0 lost significance.

Metabolite associations with BMI, steatosis and 24 h insulin dosage

Figure 3 depicts the top 25 metabolites that correlated with steatosis (Figure 3A), BMI (Figure 3B), or total daily insulin dosage (Figure 3C). Glutamate, pyruvate, alanine, valine, lactate, and CAR 5.0 correlated positively and significantly with BMI, steatosis, and 24h insulin dosage with variable results for other compounds. Orotate, and cinnamate correlated negatively and significantly with BMI, steatosis and 24h insulin dosage. Of the aromatic amino acids, only phenylalanine correlated positively with the steatosis score. Tyrosine correlated positively with BMI and 24h insulin dosage. Dihydoxyphenylalanine, a metabolite of tyrosine, also correlated positively with BMI and 24h insulin dosage. Proline correlated positively with BMI and steatosis and had a near significant positive correlation with 24h insulin dosage (p=0.058). Methylmalonate correlated positively with BMI and 24h insulin

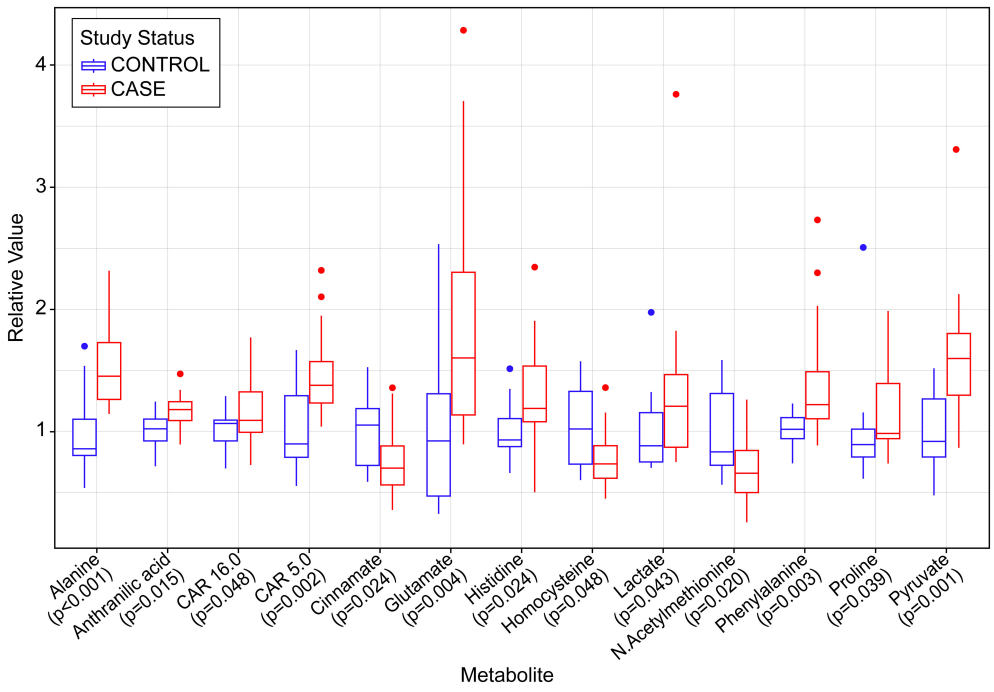

FIGURE 2
Boxplot displaying relative levels of metabolites that differ significantly between cases (red) and controls (blue, control values normalized to 1.00). Data are displayed via medians and interquartile ranges, with outliers denoted by points on the graph. P-values were calculated via the Welch two sample t-test for approximately normal distributed data according to the Shapiro-Wilk test or by the Wilcoxon rank sum test for data that were not normally distributed.

dosage and had a near significant positive correlation with steatosis (p=0.051).

Magnitude and fold change for individual metabolites

Figure 4 shows a Volcano plot determined by entry of 117 measured metabolites and 14 acyl carnitines into MetaboAnalyst 5.0 software. Alanine, pyruvate, glutamate, Car 5.0, and phenylalanine levels were notably higher in cases compared to controls with substantial positive fold change. Sensitivity analysis with a CAP cutoff ≤ 267 revealed the same directional and similar magnitude of these findings.

Pathway enrichment

We investigated whether certain functionally related metabolites were significantly enriched through hits compared with a database of 99 metabolic sets available in the library of the MetaboAnalyst 5.0 software (Figure 5). Of particular interest, the glucose-alanine cycle, phenylalanine and tyrosine metabolism, glutathione metabolism, alanine metabolism, tryptophan metabolism, cysteine metabolism and glutamate metabolism were significantly enriched in cases ($p < 0.05$). Sensitivity analysis with a CAP cutoff ≤ 267 revealed the same findings with similar magnitude.

Lipidomics

Limited lipidomic analyses were done using LC-MS. The results (Table 3) showed that ceramides ($p = 0.02$), diacylglycerols levels ($p = 0.0009$), and triacylglycerols ($p=0.0004$) were significantly higher in the MASLD group. Our metabolite profile included the non-esterified fatty acids; stearate, palmitate, laurate, and linoleate (Supplementary Table 2). Stearate was mildly elevated in cases versus controls (mean ± SD, 1.26 ± 0.44 *vs.* 1.00 ± 0.35) but missed significance at $p = 0.08$ while the others were very similar between groups. Sensitivity analyses showed that the data in Table 3 and the non-esterified fatty acid data was essentially not affected.

Multivariate regression model

Results from Table 4 show that elevated glutamate and phenylalanine levels predicted a greater steatosis score, while higher aspartate levels predicted a lower steatosis score. Table 5 shows that increased BMI was associated with higher fibrosis scores, while asparagine and sedoheptulose were associated with lower fibrosis scores.

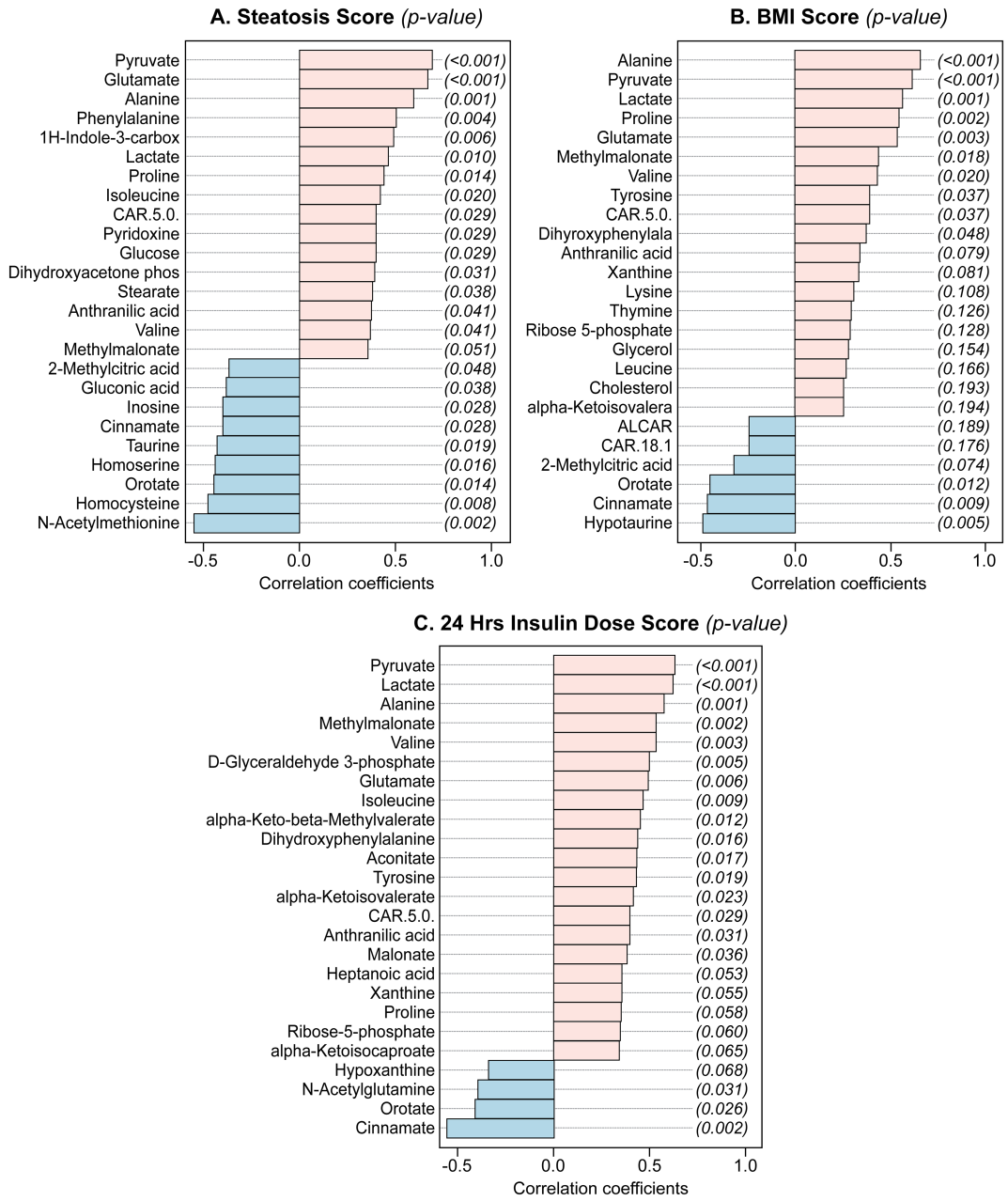

FIGURE 3
Top 25 metabolites as correlated to steatosis **(A)**, BMI **(B)**, and 24 h insulin dosing **(C)**.

Discussion

To our knowledge, this is the first study of MASLD in persons with T1D employing detailed metabolomic analyses. We found that over half of our participants had MASLD. Those with MASLD manifest features of insulin resistance including higher mean BMI and greater insulin dose requirements. Moreover, as discussed below our metabolite data was consistent with values associated with insulin resistance including elevated aromatic amino acids, metabolites involved in gluconeogenesis, acyl carnitines, ceramides, diacylglycerols and triacyclglycerols.

As compared to T2D, much less is known regarding the pathogenesis of MASLD in T1D. This is likely because of the presumed lower incidence of MASLD in T1D and exclusion of persons with T1D from MASLD studies. Over half (57%) of the recruited T1D participants in our study were found to have MASLD, with 24% in this case group having evidence of fibrosis (stage F2 or greater, i.e., fibrosis score 8.2 or greater). This is notable and suggests that the prevalence of MASLD in T1D, as we defined by steatosis on FibroScan, may be higher than reported in a meta-analysis that used a variety of criteria for the diagnosis of MASLD in persons with T1D (4). Long-term studies of patients with MAFLD

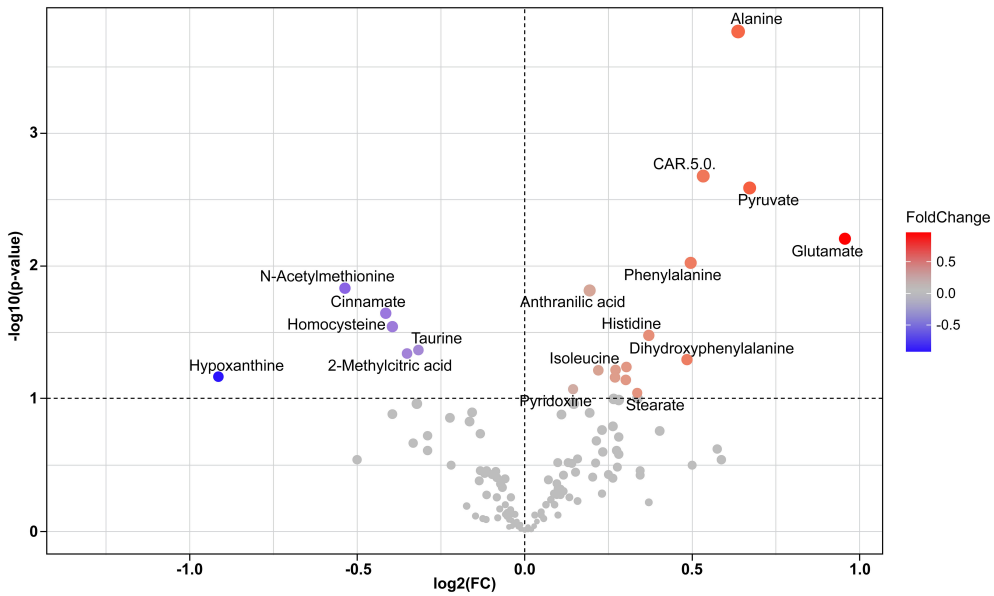

FIGURE 4
Compound levels depicted by Volcano plot. Upward and right direction favor greater levels in cases compared to controls. 117 metabolites and 14 acyl carnitines measured by LCMS were entered and analyzed using the MetaboAnalyst 5.0 software package.

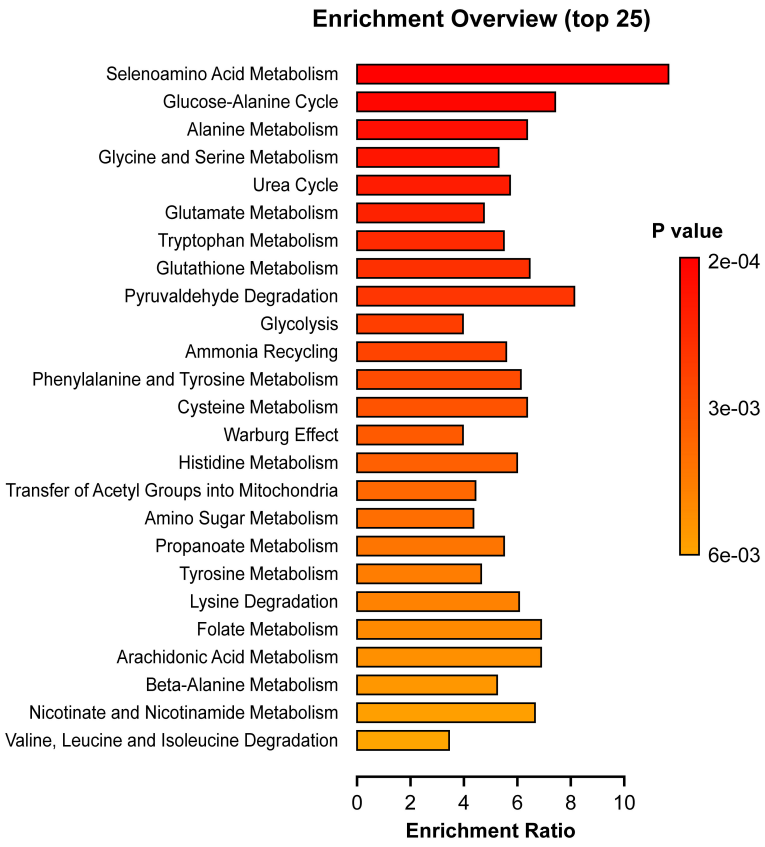

FIGURE 5
Pathway Enrichment Analyses. Depicted pathways differed significantly between cases and controls ($p < 0.05$). The enrichment ratio was determined using MetaboAnalyst 5.0 and calculated as the number of hits within a particular metabolic pathway divided by the expected number by chance.

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TABLE 3 Lipid classes determined by LC-MS.

Lipid species (nmol/ml)	Control [Median (interquartile range)] (n = 13)	Cases [Median (interquartile range)] (n = 17)	p-value	FDR p-value
Ceramides	4.54 (3.89 – 4.87)	5.40 (4.57 – 6.81)	**0.02**	**0.033**
Sphingomyelin	374.90 (315.30 – 403.90)	372.90 (325.20 – 440.60)	0.48	0.48
Diacylglycerols	33.16 (29.16 – 44.16)	51.20 (43.58 – 83.38)	**0.0009**	**0.0023**
Phosphatidylcholine	1184.0 (1125.0 – 1324.0)	1275.0 (1190.0 – 1548.0)	0.13	0.16
Triacylglycerols	531.70 (380.70 – 658.90)	789.60 (623.60 – 1695.0)	**0.0004**	**0.0020**

Differences between groups were determined using a Wilcoxon rank sum test. Data show median and interquartile range with significant differences indicated in bold type.

have confirmed that hepatic fibrosis stage F2 or higher is associated independently with liver-related mortality (15, 16).

Due to the complications of MASLD including cardiovascular and liver morbidity, mortality, and economic burden, it is important to better understand the pathogenesis of MASLD in T1D. Overall, we observed several similarities between the metabolic profile of the MASLD group in T1D and what is historically well recognized in persons with T2D. Notably our case group with MASLD exhibited significantly higher levels of alanine, proline, histidine, isoleucine, phenylalanine, glutamate, acyl carnitines (Car 5.0 and Car 16.0), ceramides, diacylglycerols and triacylglycerols, which are findings reminiscent of T2D with MASLD (9, 10, 17, 18). The MASLD group had higher levels of glucose, alanine, pyruvate, and glutamate compared to controls. These compounds represent key metabolites involved in the glucose-alanine cycle (Cahill Cycle), a pathway active in the presence of hepatic insulin resistance and found enriched in our pathway data (Figure 5). In this process, muscle glutamate reacts with pyruvate generating alanine and α-ketoglutarate catalyzed by alanine aminotransferase. Alanine can then be used by liver for gluconeogenesis (19, 20). Lactate was also greater in cases, which along with the elevated pyruvate, imply greater activity of the Cori Cycle. In this cycle, anaerobic glycolysis in muscle directs lactate to liver for reconversion to pyruvate used for glucose production contributing to the gluconeogenesis associated with MASLD. Also, phenylalanine and tyrosine metabolism were enriched in cases compared to controls consistent with reports that aromatic amino acids are increased with insulin resistance and obesity (21, 22). Moreover, we observed that the group with MASLD had higher levels of anthranilic acid, a metabolite of tryptophan, whose breakdown products are associated with obesity and insulin resistance (22).

Plasma levels of branched chain amino acids (BCAAs) have been shown to be elevated in obesity associated MASLD (9) due to impaired BCAA metabolism in the setting of mitochondrial dysfunction and hepatic inflammation (23). In this regard, it was suggested that elevated BCAAs may represent an adaptive response to inflammation and oxidative stress (10). In any case, there is a strong association of BCAAs with features of type 2 diabetes including insulin resistance, diabetes, and cardiovascular risk (24). Somewhat in contrast, our study of individuals with T1D revealed only a marginally significant increase in isoleucine: Supplementary Table 2, with non-significant increases in leucine and valine in cases versus controls: Supplementary Table 2.

We also found higher circulating levels of glutamate (Figures 2, 4) and enriched glutamate metabolism (Figure 5) in cases versus controls. We also observed that glutamate emerged as a predictor of steatosis in multivariate modeling (Table 4). However, we observed no difference in plasma glutamine, the dietary precursor of glutamate after mitochondrial conversion by glutaminase. There is evidence that glutamine supplementation may be preventative towards liver fat accumulation and the metabolic risk for cardiovascular disease (25). On the other hand, there is evidence that plasma glutamate may be a marker of liver fat accumulation, fibrosis, and cardiovascular risk (26, 27). Our current observation of higher glutamate in cases is consistent with this. Although glutamate is important in glutathione synthesis, a major antioxidant mechanism preventing hepatic inflammation and fibrosis (28), this may be a compensatory response (29). Finally, there is evidence that a higher glutamine-to-glutamate ratio is

TABLE 4 Multivariate modeling to predict steatosis.

Characteristic	Beta	95% CI[1]	p-value
Glutamate	67	48, 86	**<0.001**
Phenylalanine	72	42, 102	**<0.001**
Aspartate	-104	-162, -46	**0.001**

[1]CI = Confidence Interval.
When glutamate values increase by 1, we anticipate that the steatosis score will increase by 67 units. When phenylalanine values increase by 1, we anticipate that the steatosis score will increase by 72 units. When aspartate values increase by 1, we anticipate that the steatosis score will decrease by 104 units. p=statistical significance = <0.05.
The bold values are statistically significant with p <0.05.

TABLE 5 Multivariate modeling to predict fibrosis.

Characteristic	exp(Beta)	95% CI[1]	p-value
BMI, 5 Unit Increase	1.19	1.12, 1.26	**<0.001**
Asparagine	0.57	0.41, 0.80	**0.005**
Sedoheptulose	0.58	0.35, 0.95	**0.039**

[1]CI = Confidence Interval.
When BMI increases by 5, we anticipate a multiplicative change in their fibrosis score by 1.19. When asparagine increases by 1 unit, we anticipate a multiplicative change in the fibrosis score by 0.57. When sedoheptulose values increase by 0.58, we anticipate a multiplicative change in their fibrosis score by 0.58.
The bold values are statistically significant with p <0.05.

associated with decreased cardiometabolic risk while a higher glutamate concentration is associated with increased risk (30).

In our study the case group had a near significant (p=0.053) lower level of taurine compared with the controls: Supplementary Table 2 and taurine correlated negatively with steatosis (Figure 3a). Taurine is synthesized in the liver from cysteine and methinione metabolism (31) and it ameliorates hepatic steatosis by reducing reactive oxygen species, lipid accumulation, and preserving mitochondrial membrane potential (32).

The quantity and quality of accumulated lipids play a significant role in the pathogenesis of MASLD (33). In this regard, we observed that diacylglycerols, triacylglycerols and ceramides were significantly higher in the case group compared to controls. Prior lipidomic studies have shown sphingomyelin, ceramides, and dihydrate ceramides in plasma and liver biopsies of patients with MASLD and MASH (34). Ceramides are sphingolipids that accumulate in the liver and are involved in increased oxidative stress, mitochondrial dysfunction and cell apoptosis (33). Hepatic ceramides accumulation is believed to be involved in the pathogenesis of MASLD by interfering with signaling through disruption of the insulin receptor substrate and protein kinase B (35). Diacylglycerols accumulation is associated with MASLD through induction of protein kinase C (36).

Further, we found higher levels of isovalerylcarnitine (Car 5:0), a breakdown product of leucine, and L-Palmitoylcarnitine (CAR 16:0), a long chain acyl fatty acid derivative ester of carnitine, in cases compared to controls. Increased acyl carnitines suggest elevated mitochondrial fatty acid oxidation. This is consistent with the concept that MASLD in T1D, like T2D, is associated with both fat synthesis and increased fatty acid oxidation (37), although the later may be only compensatory. Our findings regarding Car 5.0 are consistent with Enooku et al. (38) who measured serum acylcarnitine species in 241 biopsy proven cases of MASLD and reported that acylcarnitine 5.0 positively correlated with steatosis (p = 0.056).

As shown in Figure 3, several compounds elevated in our case cohort and (27) were among the top metabolites correlated with steatosis, BMI, and insulin dosing. These metabolites include glutamate, valine, proline, and alanine. The aromatic amino acid, tyrosine was in the top correlating compounds for BMI and insulin dosage while phenylalanine was predictive of steatosis (Table 4). Also, we note that the glucose-alanine cycle and alanine metabolism, discussed above in relation to glucose production, were in the top three pathways enriched in cases (Figure 5). Selenoamino acid metabolism, the top enriched pathway (Figure 5) may be important in preventing hepatic oxidative damage, inflammation, and fibrosis by increasing the activity of glutathione peroxidase (39).

Limitations

There are several limitations to our current study. Our sample size was small and might have lacked power to detect significant differences between certain metabolites. We acknowledge that a larger study is needed to confirm metabolite perturbations in T1D and for direct comparison to T2D and non-diabetic individuals. But the strength of this study is that we examined a large number of metabolites and inferred pathways in a population (type 1 DM) where little was previously known and provide a basis for future work. Moreover, our metabolite data taken together with our metabolic pathway analyses imply differences between cases and controls despite the multiple listed comparisons. Another limitation is that did not carry out a full lipidomic analysis examining phospholipid subclasses, bile acids, and fatty acid levels, so it is possible that we may have missed other complex lipids that play a significant role in the pathogenesis of MASLD.

We acknowledge that insulin dosing is a surrogate rather than a direct measure of insulin sensitivity. We did not carry out more definitive euglycemic insulin clamp studies due to cost and burden on participants. We emphasize that persons with T1D produce essentially no endogenous insulin. So, unless there are absorption issues or differences in insulin breakdown, the dose required for control is a reasonable approximation of resistance. Of course, this dose needs to be considered relative to control achieved (e.g., HbA1c). But since cases took more insulin despite higher HbA1c levels it could be argued that they would have required even more insulin than controls if control were equalized.

The FibroScan is limited in detecting MASLD in participants with BMI >35 kg/m2 (40) so there could have been some overestimation of steatosis and fibrosis in some participants. We minimized this by using the XL probes for obese patients, a technique found to be more accurate for detecting greater than stage F2 fibrosis and cirrhosis (41). Our case and control groups were studied at about the same age, although the control group had a longer mean duration of diabetes. It is difficult to interpret this as related to steatosis and fibrosis scores, although the data is surprising in that, intuitively, we might expect that longer duration would lead to worse scores. Since our controls had lower scores, we might speculate that the differences between cases and controls would have been greater if durations were equal.

Conclusion

Our data support the concept that MASLD coincident with T1D is a distinctive health complication. Our data suggest that among individuals with T1D, MASLD is associated with features of insulin resistance and corresponding metabolite perturbations. Future work is needed to better characterize the pathways that contribute to the development and severity of MASLD in T1D and provide rationale for therapeutic interventions that might prevent or treat MASLD in T1D.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by Institutional Review Board, The University of Iowa. 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

AT: Conceptualization, Project administration, Software, Validation, Writing – original draft, Writing – review & editing. RM: Data curation, Methodology, Software, Visualization, Writing – review & editing. LW: Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – review & editing, Writing – original draft. DP: Writing – review & editing. HT: Data curation, Formal analysis, Methodology, Writing – review & editing. AM: Data curation, Writing – review & editing. JC: Data curation, Writing – review & editing. SS: Data curation, Writing – review & editing. BC: Data curation, Writing – review & editing, Methodology. NP: Writing – review & editing. BBC: Writing – review & editing. AS: Writing – review & editing, Methodology. PT: Formal analysis, Writing – review & editing, Methodology. DJ: Methodology, Supervision, Writing – review & editing, Visualization. AD: Supervision, Writing – review & editing, Funding acquisition. ET: Methodology, Supervision, Writing – review & editing. WS: Formal analysis, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing, Methodology.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article.: The study was conducted using an Internal grant from the Division of Endocrinology and Metabolism, at the University of Iowa Hospitals and Clinics. This study was also supported by the following grants: ET - NIHR01DK104998, DJ - NIH1RO1HL134738, WIS - NIH1R01DK123043, LW and PT - Clinical and Translational Science Award UM1TR004403, DP - American Heart Association predoctoral training grant (AHA 23PRE1020471), and the Iowa MSTP training grant (NIH T32 GM139776).

Acknowledgments

Research reported in this publication was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR002537. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We would also like to acknowledge the help of the following research personnel: Jesse Espinoza, Teresa Ruggle, Kimberly Sprenger, Jessica Valestin, Mary McCormick and Darby Donovan. We also acknowledge the support of the Metabolomic Core Laboratory Staff including Dr. T. Idil Evans and Dr. Reid Brown.

Conflict of interest

AS, has received research grants from Arrowhead, Gilead, Inventiva, Mallinckrodt, Mirum, Takeda, Sagimet, Merck, Intercept, AbbVie and Boehringer-Ingelheim.

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2025.1500242/full#supplementary-material

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 17 July 2025
DOI 10.3389/fendo.2025.1496671

OPEN ACCESS

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RECEIVED 15 September 2024
ACCEPTED 17 June 2025
PUBLISHED 17 July 2025

CITATION
Suzuki T, Sato T, Tanaka M, Nakata K, Endo K, Aida H, Kawaharata W, Hosaka I, Umetsu A, Ogawa T, Akiyama Y and Furuhashi M (2025) Treatment with pemafibrate ameliorates fatty liver index and atherogenic lipid profiles in Japanese patients with type 2 diabetes mellitus.
Front. Endocrinol. 16:1496671.
doi: 10.3389/fendo.2025.1496671

Treatment with pemafibrate ameliorates fatty liver index and atherogenic lipid profiles in Japanese patients with type 2 diabetes mellitus

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Background: Pemafibrate, a selective peroxisome proliferator-activated receptor α modulator, ameliorates hypertriglyceridemia. We investigated the effects of pemafibrate on steatotic liver disease (SLD) in relation to various atherogenic lipid profiles.

Methods: Thirty-nine Japanese patients with both type 2 diabetes mellitus (T2DM) and hypertriglyceridemia (men/women: 24/15, mean age: 58.2 years, median duration of diabetes: 5.0 years) were treated with 0.2 mg/day of pemafibrate for 12 months (M). SLD was estimated by fatty liver index (FLI), which is calculated by using waist circumference, body mass index and levels of triglycerides and γ-glutamyl transpeptidase.

Results: Treatment with pemafibrate significantly increased mean levels of high-density lipoprotein cholesterol (HDL-C) (baseline/3M/6M/12M: 46/55/55/54 mg/dL) and decreased median levels of triglycerides (baseline/3M/6M/12M: 211/112/99/98 mg/dL), non-HDL-C (146/128/125/121 mg/dL), small dense low-density lipoprotein cholesterol (45/33/30/30 mg/dL) and remnant-like particle cholesterol (8.1/2.6/2.3/2.4 mg/dL). There was no significant change in hemoglobin A1c level over time. FLI (mean ± standard deviation: 68.1 ± 21.9 *vs*. 39.6 ± 25.0, P < 0.001), but not FIB-4 index as a marker of hepatic fibrosis (median [interquartile range]: 1.04 [0.78-1.39] *vs*. 1.01 [0.68-1.36], P = 0.909), was significantly decreased by treatment with pemafibrate for 12M, and the proportion of patients with metabolic dysfunction-associated SLD (MASLD) was significantly decreased from 92.3% (baseline) to 61.5% (12M).

Conclusions: Pemafibrate ameliorates MASLD estimated by FLI in addition to various atherogenic lipid profiles in Japanese hypertriglyceridemia patients with T2DM in the past mean 5 years. An early intervention with pemafibrate might contribute to prevention of the development of MASLD and atherosclerotic cardiovascular disease.

KEYWORDS

pemafibrate, fatty liver index, metabolic dysfunction-associated steatotic liver disease, type 2 diabetes, atherogenic lipid profiles

Introduction

Atherosclerotic cardiovascular disease (ASCVD) is one of the major health problems worldwide (1). Low-density lipoprotein (LDL) cholesterol (LDL-C) has been shown as a therapeutic target for ASCVD (2). Various lipid abnormalities have also been reported to be targets as residual risks for ASCVD, and the candidates include small dense LDL (sdLDL) cholesterol (sdLDL-C) and remnant-like particle cholesterol (RLP-C) (3). sdLDL contains smaller amounts of apoprotein (Apo) B (ApoB) and ApoE, ligands for LDL receptors in hepatocytes, resulting in a longer stay of sdLDL-C than that of LDL-C in the blood (4). Furthermore, sdLDL infiltrates into the outer vascular space and can be easily oxidized, resulting in the progression of atherosclerosis (4). On the other hand, RLP-C is determined by intermediate metabolites resulting from the breakdown of lipoproteins such as very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) (5) and can induce ASCVD by being easily taken up by phagocytic cells and by having aggregating effects on platelets (6).

Nonalcoholic fatty liver disease (NAFLD) has been reported to be an upstream risk factor for atherosclerosis (7–9). New nomenclature of steatotic liver disease (SLD) including metabolic dysfunction-associated SLD (MASLD) has recently been proposed (10). Individuals with SLD complicated with diabetes mellitus (DM) are defined as patients who have MASLD or MASLD and increased alcohol intake (MetALD) (10). Although MASLD can be a potent risk for ASCVD (11) as well as its related diseases (12, 13), there are no effective and specific agents for treatment of MASLD. Fatty liver index (FLI) (14), which is calculated by using waist circumference (WC), body mass index (BMI) and levels of triglycerides (TG) and γ-glutamyl transpeptidase (γGT), has been established as a useful biomarker for detection of SLD (12, 15) as well as for risk estimation for the development of hypertension (16), DM (17), chronic kidney disease (18, 19) and ischemic heart disease (20). Thus, SLD is an upstream factor in cardiorenal metabolic diseases and may be a promising therapeutic target for ASCVD.

Pemafibrate, a selective peroxisome proliferator-activated receptor α (PPARα) modulator (SPARMα), has recently been developed for treatment of hypertriglyceridemia as a residual risk for ASCVD (21) as well as for improvement of NAFLD (22–25). The PROMINENT trial using patients with type 2 DM (T2DM) and hypertriglyceridemia revealed that treatment with pemafibrate did not reduce cardiovascular events over a median observation period of 3.4 years (22). In that trial, the recruited patients who had been treated with statins (96%, high-intensity dose: 69%) had mean levels of LDL-C as low as 79 mg/dL at baseline, and levels of non-HDL-C were not significantly changed by treatment with pemafibrate (22, 26). In a pooled analysis of the PROMINENT trial (27), levels of sdLDL-C estimated by the Sampson equation (28, 29), which has been validated by several cohorts (30–33), were not reduced by treatment with pemafibrate regardless of a reduction in TG level, which might be one possible reason why cardiovascular events were not reduced in the PROMINENT trial (22). In addition, the possibility that the relatively long duration of DM [≥10 years: 46.4% (22)] in the recruited patients affected the outcome due to the presence of already advanced latent atherosclerosis cannot be ruled out.

To address our hypothesis, we prospectively investigated the effects of pemafibrate on SLD assessed by FLI in relation to various atherogenic lipid profiles in Japanese patients with hypertriglyceridemia and T2DM who have a relatively short duration of DM.

Methods

This study was a prospective single-center observational study conducted in Japan. The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the human ethics committee of Natori Toru Internal Medicine and Diabetes Clinic (approval number: CR2022-01). Written informed consent was obtained from all of the subjects.

Study patients

Study patients were enrolled from outpatients attending Natori Toru Internal Medicine and Diabetes Clinic (Natori, Japan) who agreed to participate in the study. The inclusion criteria were as follows: 1) diagnosis of T2DM; 2) fasting serum TG > 150 mg/dL; 3)

serum creatinine < 2.5 mg/dL; 4) age ≥ 18 years old; and 5) Japanese race. The exclusion criteria included any of the following: 1) patients treated with drugs for hypertriglyceridemia including fibrates, omega-3 polyunsaturated fatty acids, eicosatetraenoic acid and docosahexaenoic acid; 2) patients treated with steroids; 3) presence of diseases that can affect the serum level of TG including nephrotic syndrome, Cushing's syndrome, inadequately controlled hypothyroidism, primary biliary cholangitis and obstructive jaundice; 4) unstable condition with progressive multiple organ damage; and 5) patients who were pregnant or potentially childbearing. According to the standard drug information, the recruited patients were treated with pemafibrate at a dose of 0.2 mg/day (0.1 mg twice daily) for 12 months.

Clinical examinations

The participants were examined every month to check their health status and the presence of any side effects of pemafibrate including any musculoskeletal, renal and hepatic events. Detailed laboratory parameters were measured before the start of treatment with pemafibrate and at 3 months, 6 months and 12 months after the start of treatment. Blood samples were collected in the morning in an overnight fasting condition.

Body weight was measured to the nearest 0.1 kg using a digital scale, and height was measured to the nearest 0.1 cm. BMI was calculated as body weight (kg)/(height [m])^2. WC was measured at the umbilical level in the late phase of expiration to the nearest 0.1 cm. Systolic blood pressure, diastolic blood pressure and pulse rate were measured by using a fully automatic measuring device (HBP-9020, Omron, Japan). A self-administered questionnaire survey was performed to obtain information on current smoking habit and alcohol drinking habit (≥ 1 time/week).

Measurements

Levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), TG, and RLP-C were measured by enzymatic assays. sdLDL-C concentration was directly measured by using a homogenous assay (sdLDL-EX SEIKEN; Denka Co., Tokyo, Japan) (34, 35). Lipoprotein (a) (Lp(a)) and apolipoproteins including ApoA1, ApoA2, ApoB, ApoC2, ApoC3 and ApoE were measured by turbidimetric immunoassay methods (BML, Inc., Tokyo, Japan). Lipoprotein fractions were measured by using a high-performance liquid chromatography (HPLC) method (BML, Inc., Tokyo, Japan) (36, 37).

LDL-C was calculated by using the Friedewald formula (38): TC − HDL-C − TG/5. Non-HDL-C was calculated by subtracting HDL-C from TC. TG-rich lipoprotein cholesterol (TRL-C), which is the same as remnant cholesterol reported in previous studies (39–41), was calculated by subtracting HDL-C and LDL-C from TC. Estimated glomerular filtration rate (eGFR) was calculated by the following equation for Japanese people (42): eGFR (mL/min/1.73m^2) = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$ × 0.739 (if female).

FLI and MASLD

Fatty liver index (FLI) was calculated by the following formula (14): $[e^{(0.953 \times \ln TG + 0.139 \times BMI + 0.718 \times \ln(\gamma GT) + 0.053 \times WC - 15.745)}]/[1 + e^{(0.953 \times \ln TG + 0.139 \times BMI + 0.718 \times \ln(\gamma GT) + 0.053 \times WC - 15.745)}] \times 100$. Although the cutoff value for SLD was originally reported as FLI ≥ 60 in Italian subjects (14), FLI ≥ 35 for men and FLI ≥ 16 for women were used for the definition of SLD in the present study as previously reported in Japanese subjects (15). FIB-4 index, a marker of hepatic fibrosis, was also calculated by the following formula (43): age (years) × aspartate aminotransferase (AST; IU/L))/(platelet count [10^9/L] × alanine aminotransferase [ALT; IU/L]$^{1/2}$).

MASLD was diagnosed by the absence of other discernible causes for hepatic steatosis and the presence of SLD with at least one of five cardiometabolic risk factors assessed by BMI, glucose management, blood pressure and levels of TG and HDL-C (10). The five cardiometabolic criteria include 1) BMI ≥ 23 or WC > 90/80 cm in Asian men and women; 2) fasting glucose ≥ 100 mg/dL, 2-h post-load glucose levels ≥ 140 mg/dL (no measurement in the present study), hemoglobin A1c (HbA1c) ≥ 5.7%, type 2 diabetes mellitus, or treatment for type 2 diabetes mellitus; 3) blood pressure ≥ 130/85 mmHg or specific antihypertensive drug treatment; 4) plasma TG ≥ 150 mg/dL or lipid-lowering treatment; and 5) plasma HDL cholesterol ≤ 40 mg/dL for men and ≤ 50 mg/dL for women or lipid-lowering treatment. MetALD was diagnosed by the presence of MASLD and average alcohol intake of 140–350 g/week [20–50 g/day] for women and 210–420 g/week [30–60 g/day] for men.

Statistical analysis

Numeric variables are expressed as means ± standard deviation (SD) for parameters with normal distributions and as medians [interquartile ranges] for parameters with skewed distributions. The distribution of each parameter was tested for its normality using the Shapiro-Wilk W test. Comparisons between two groups for parametric and nonparametric factors were performed by using Student's t-test and the Mann-Whitney U test, respectively. Paired categorical indices were statistically compared by McNemar's test. For comparison of two variables paired with time series correspondence, Wilcoxon single rank test was used. For comparison of three and more variables paired with time series correspondence, the Friedman test with Dunn's *post-hoc* test was used. A p value of less than 0.05 was considered statistically significant. All data were analyzed by using EZR (44) and GraphPad Prism version 9.5.

Results

Characteristics of the study patients

Characteristics of the 39 recruited patients (men/women: 24/15) at baseline are shown in Table 1. The mean age of the patients was 58.2 ± 13.1 years, and 61.5% of the patients were men. The mean

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TABLE 1 Characteristics of the recruited patients at baseline.

	All (n = 39)	Men (n = 24)	Women (n = 15)	*p*
Age (years)	58.2 ± 13.1	57.2 ± 14.5	59.7 ± 10.8	0.565
Body weight (kg)	74.9 ± 13.9	77.5 ± 14.4	70.9 ± 12.5	0.158
Waist circumference (cm)	95.3 ± 11.1	96.5 ± 10.1	93.4 ± 12.6	0.428
Body mass index (kg/m^2)	27.5 ± 4.0	27.2 ± 3.9	27.9 ± 4.3	0.573
Systolic BP (mmHg)	131 [120-138]	132 [119-137]	131 [129-152]	0.312
Diastolic BP (mmHg)	75 [68-86]	77 [68-86]	75 [70-86]	0.729
Pulse rate (bpm)	83 [72-91]	84 [71-90]	80 [73-92]	0.942
Current smoking habit	9 (23.1)	9 (37.5)	0 (0.0)	0.021
Alcohol drinking habit	4 (10.3)	4 (16.7)	0 (0.0)	0.260
Duration of diabetes (years)	5.0 [1.5-12.0]	7.0 [2.8-13.5]	5.0 [1.0-9.0]	0.205
Diabetic microangiopathy				
Retinopathy	23 (59.0)	13 (54.2)	10 (66.7)	0.662
Nephropathy	14 (35.9)	8 (33.3)	6 (40.0)	0.937
Comorbidity				
MASLD	36 (92.3)	24 (61.5)	12 (30.8)	0.404
MetALD	0 (0)	0 (0)	0 (0)	–
Hypertension	29 (74.4)	16 (66.7)	13 (86.7)	0.31
ASCVD	1 (2.6)	0 (0.0)	1 (6.7)	0.81
Anti-lipidemic drugs				
Statin	16 (41.0)	9 (37.5)	7 (46.7)	0.817
Ezetimibe	2 (5.1)	2 (8.3)	0 (0.0)	0.688
Anti-diabetic drugs				
Biguanide	27 (69.2)	16 (66.7)	11 (73.3)	0.934
Thiazolidinedione	1 (2.6)	0 (0.0)	1 (6.7)	0.810
DPP-4 inhibitor	17 (43.6)	10 (41.7)	7 (46.7)	1.000
SGLT-2 inhibitor	19 (48.7)	15 (62.5)	4 (26.7)	0.065
Imeglimin	2 (5.1)	1 (4.2)	1 (6.7)	1.000
GLP-1 receptor agonist	18 (46.2)	11 (45.8)	7 (46.7)	1.000
Insulin	6 (15.4)	5 (20.8)	1 (6.7)	0.461

Variables are expressed as number (%), means ± SD or medians [interquartile ranges].
ASCVD, atherosclerotic cardiovascular disease; BP, blood pressure; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, MASLD and increased alcohol intake; SGLT2, sodium-glucose cotransporter 2.

duration of DM was 5.0 [1.5-12.0] years. SLD was present in 32 patients (92.3%, men/women: 24/12) at determined by FLI ≥ 35 for men and FLI ≥ 16 for women, which were the cutoff values of SLD previously reported in Japanese subjects (15). Only 4 male patients had an alcohol drinking habit, and all of those patients had less than 30 g/day of alcohol equivalent. Subsequent interviews and clinical examinations revealed no evidence of alternative etiologies of SLD. Therefore, all of the patients with T2DM who had SLD were diagnosed as having MASLD but not MetALD. Only 1 female patient had a past history of ASCVD. Statins were used by 41.0% of patients (men/women: 9/7), and only 5.1% of those patients (men/women: 2/0) had been using ezetimibe.

Biochemical data at baseline are shown in Table 2. Levels of fasting glucose and HbA1c were 117 [107-140] mg/dL and 6.8 [6.4-7.4]%, respectively. Levels of TG and LDL-C were 211 [183-262] mg/dL and 108 [84-127] mg/dL, respectively.

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TABLE 2 Biochemical data at baseline.

	All (n = 39)	Men (n = 24)	Women (n = 15)	*p*
Biochemical data				
Fasting glucose (mg/dL)	117 [107-140]	119 [109-139]	117 [105-140]	0.862
Hemoglobin A1c (%)	6.8 [6.4-7.4]	6.9 [6.4-7.4]	6.8 [6.4-7.3]	0.783
Creatinine (mg/dL)	0.68 [0.57-0.79]	0.78 [0.63-0.91]	0.58 [0.49-0.74]	0.011
eGFR (mL/min/1.73m^2)	83.7 ± 24.0	88.3 ± 25.7	84.4 ± 22.0	0.965
CK (IU/L)	93 [71-135]	98 [74-164]	90 [58-126]	0.156
Liver-related variables				
AST (IU/L)	24 [21-35]	24 [21-33]	23 [19-23]	0.954
ALT (IU/L)	29 [22-49]	28 [22-50]	34 [21-48]	0.817
γGT (IU/L)	35 [27-52]	42 [29-67]	28 [24-40]	0.055
ALP (IU/L)	75 [65-92]	74 [62-79]	78 [71-104]	0.236
FLI	67.8 ± 21.9	68.4 ± 20.8	66.7 ± 24.5	0.831
FIB-4	1.04 [0.78-1.39]	1.00 [0.80-1.45]	1.09 [0.75-1.23]	0.870
Lipid-related variables				
TG (mg/dL)	211 [183-262]	196 [179-231]	234 [199-285]	0.103
TC (mg/dL)	194 [179-228]	184 [150-205]	222 [193-256]	0.013
HDL-C (mg/dL)	46 ± 10	44 ± 10	50 ± 7	0.091
LDL-C (mg/dL)	108 [84-127]	98 [78-114]	127 [102-155]	0.012
non-HDL-C (mg/dL)	146 [130-172]	138 [116-155]	167 [144-203]	0.018
sdLDL-C (mg/dL)	45.4 [37.3-65.0]	41.9 [37.1-53.9]	60.0 [40.8-86.7]	0.051
sdLDL-C/LDL-C	0.47 [0.40-0.57]	0.43 [0.39-0.48]	0.54 [0.42-0.59]	0.102
RLP-C (mg/dL)	8.1 [6.2-10.1]	7.5 [5.8-9.4]	8.5 [7.9-12.8]	0.088
TRL-C (mg/dL)	56 [50-68]	52 [46-62]	60 [52-78]	0.083
Lp(a) (mg/dL)	3.5 [3.0-8.3]	3.0 [3.0-5.1]	8.0 [4.3-12.9]	0.001
Lipoprotein fraction				
VLDL-C (mg/dL)	41 [33-49]	35 [32-45]	37 [32-46]	0.119
IDL-C (mg/dL)	12 [10-15]	12 [9-14]	13 [12-17]	0.050
HDL-C (mg/dL)	42 ± 10	40 ± 10	45 ± 8	0.091
LDL-C (mg/dL)	94 [73-116]	90 [67-109]	99 [86-135]	0.163
Apolipoprotein fractions				
ApoA1 (mg/dL)	142.4 ± 22.4	136.3 ± 24.0	152.1 ± 15.8	0.030
ApoA2 (mg/dL)	32.3 ± 4.9	31.4 ± 4.9	33.9 ± 4.7	0.113
ApoB (mg/dL)	98.0 [86.0-115.0]	92.5 [78.3-105.3]	115.0 [94.0-136.0]	0.019
ApoC2 (mg/dL)	6.0 [5.2-6.9]	5.5 [4.8-6.3]	6.6 [5.7-7.5]	0.046
ApoC3 (mg/dL)	13.8 [12.2-16.9]	13.2 [11.9-16.0]	16.1 [12.3-18.4]	0.209
ApoC3/ApoC2	2.4 [2.0-2.7]	2.4 [2.1-2.7]	2.3 [1.9-2.7]	0.212
ApoE (mg/dL)	4.7 [4.0-6.0]	4.3 [3.6-5.0]	5.5 [4.7-6.6]	0.337

Variables are expressed as number (%), means ± SD or medians [interquartile ranges].
ALP, alkaline phosphatase; ALT, alanine transaminase; Apo, apolipoprotein; AST, aspartate transaminase; CK, creatine kinase; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; FLI, fatty liver index; γGT, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate density lipoprotein cholesterol; Lp(a), lipoprotein (a); LDL-C, low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol; sdLDL-C, small dense low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; TRL-C, TG-rich lipoprotein cholesterol; VLDL-C, very low-density lipoproteins cholesterol.

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Changes in DM-related markers and side effects after the start of treatment with pemafibrate

Chronological changes in parameters after the start of treatment with pemafibrate are shown in Table 3. There was no significant change in HbA1c level after the start of treatment with pemafibrate, though fasting glucose level modestly, but significantly, decreased over time after the start of treatment with pemafibrate. Levels of creatinine and eGFR slightly, but significantly, decreased over time after the start of treatment with pemafibrate. Serum levels of creatine kinase, which is used to estimate the development of rhabdomyolysis, did not increase over time.

Changes in lipid-related parameters after the start of treatment with pemafibrate

Treatment with pemafibrate for 3 months significantly reduced the level of TG, and the effect was maintained until 12 months after

TABLE 3 Time course of physical and metabolic parameters.

	Baseline	3 months	6 months	12 months	*p*
Systolic BP (mmHg)	131 [120-138]	138 [127-148]	135 [128-149]	129 [116-137]	< 0.001
Diastolic BP (mmHg)	75 [68-86]	82 [75-86]	80 [70-88]	75 [69-82]	0.059
Pulse rate (bpm)	83 [72-91]	81 [75-88]	77 [70-85]	77 [72-88]	0.023
Biochemical data					
Fasting glucose (mg/dL)	117 [108-140]	111 [97-134]	106 [97-118]	100 [90-113]	0.003
Hemoglobin A1c (%)	6.9 [6.4-7.4]	6.7 [6.3-7.2]	6.6 [6.4-7.0]	6.6 [6.3-6.8]	0.810
Creatinine (mg/dL)	0.68 [0.57-0.79]	0.72 [0.59-0.96]	0.69 [0.59-1.00]	0.74 [0.60-1.04]	< 0.001
eGFR (mL/min/1.73m^2)	83.7 ± 24.0	78.2 ± 25.3	78.8 ± 25.3	76.8 ± 23.2	< 0.001
CK (IU/L)	93 [71-135]	111 [71-158]	99 [66-161]	108 [75-145]	0.853
Lipid-related variables					
TG (mg/dL)	211 [183-262]	112 [79-139]	99 [83-120]	98 [76-141]	< 0.001
TC (mg/dL)	194 [179-228]	185 [167-201]	181 [163-197]	179 [160-193]	0.002
HDL-C (mg/dL)	46 ± 10	54 ± 13	55 ± 12	54 ± 13	< 0.001
LDL-C (mg/dL)	108 [84-127]	104 [95-119]	101 [90-121]	100 [87-109]	0.114
non-HDL-C (mg/dL)	146 [130-172]	128 [116-146]	125 [108-145]	121 [107-140]	< 0.001
sdLDL-C (mg/dL)	45.4 [37.3-65.0]	33.3 [25.3-40.5]	30.0 [22.9-37.0]	30.3 [22.6-38.3]	< 0.001
sdLDL-C/LDL-C	0.47 [0.40-0.57]	0.30 [0.24-0.36]	0.28 [0.23-0.36]	0.29 [0.25-0.35]	< 0.001
RLP-C (mg/dL)	8.1 [6.2-10.1]	2.6 [1.8-4.1]	2.3 [1.7-3.5]	2.4 [1.7-3.3]	< 0.001
TRL-C (mg/dL)	56 [55-68]	35 [25-44]	32 [22-36]	30 [24-39]	< 0.001
Lp(a) (mg/dL)	3.5 [3.0-8.3]	5.4 [3.3-9.8]	6.3 [3.0-9.6]	6.1 [3.2-12.6]	0.003
Apolipoprotein fractions					
ApoA1 (mg/dL)	142.4 ± 22.4	157.0 ± 22.9	154.2 ± 24.8	151.0 ± 24.3	< 0.001
ApoA2 (mg/dL)	32.3 ± 4.9	40.6 ± 7.0	39.6 ± 6.6	39.8 ± 6.8	< 0.001
ApoB (mg/dL)	98.0 [86.0-115.0]	89.5 [76.8-99.0]	83.0 [73.5-93.0]	79.0 [74.0-92.0]	< 0.001
ApoC2 (mg/dL)	6.0 [5.2-6.9]	4.6 [3.6-5.7]	4.3 [3.3-5.3]	4.0 [3.4-5.0]	< 0.001
ApoC3 (mg/dL)	13.8 [12.2-16.9]	9.7 [7.0-11.1]	8.3 [6.6-9.6]	7.0 [6.0-9.3]	< 0.001
ApoC3/ApoC2	2.4 [2.0-2.7]	1.9 [1.8-2.1]	1.9 [1.7-2.2]	1.9 [1.7-2.0]	< 0.001
ApoE (mg/dL)	4.7 [4.0-6.0]	3.9 [3.4-4.5]	3.6 [3.3-4.4]	3.8 [3.2-4.2]	< 0.001

Variables are expressed as number (%), means ± SD or medians [interquartile ranges].
ALP, alkaline phosphatase; ALT, alanine transaminase; Apo, apolipoprotein; AST, aspartate transaminase; BP, blood pressure; CK, creatine kinase; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; FLI, fatty liver index; γGT, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate density lipoprotein cholesterol; Lp(a), lipoprotein (a); LDL-C, low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol; sdLDL-C, small dense low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; TRL-C, TG-rich lipoprotein cholesterol; VLDL-C, very low-density lipoproteins cholesterol.

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the start of treatment (Figure 1A). Levels of non-HDL-C were modestly, but significantly, decreased by treatment with pemafibrate for 12 months (Figure 1B), whereas LDL-C levels were not significantly changed over time (Figure 1C). Levels of HDL-C were significantly increased by treatment with pemafibrate (Figure 1D).

Analysis of lipoprotein fractions measured by an HPLC method showed that a significant reduction in VLDL cholesterol (VLDL-C) in the first 3 months after the start of treatment with pemafibrate remained during the whole observation period (Figure 1E). IDL cholesterol (IDL-C) significantly and gradually decreased during the follow-up period (Figure 1F).

Other atherogenic lipid parameters, including sdLDL-C (Figure 1G), sdLDL-C/LDL-C, RLP-C (Figure 1H) and TRL-C, significantly decreased after the start of treatment with pemafibrate (Table 3). Levels of Lp(a) significantly increased after the start of treatment with pemafibrate, though the absolute values of Lp(a) at baseline and after the start of treatment (median: 3.5-6.3 mg/dL) were relatively low [reference value: < 30 mg/dL (45–47)]. As for apolipoproteins, treatment with pemafibrate significantly increased

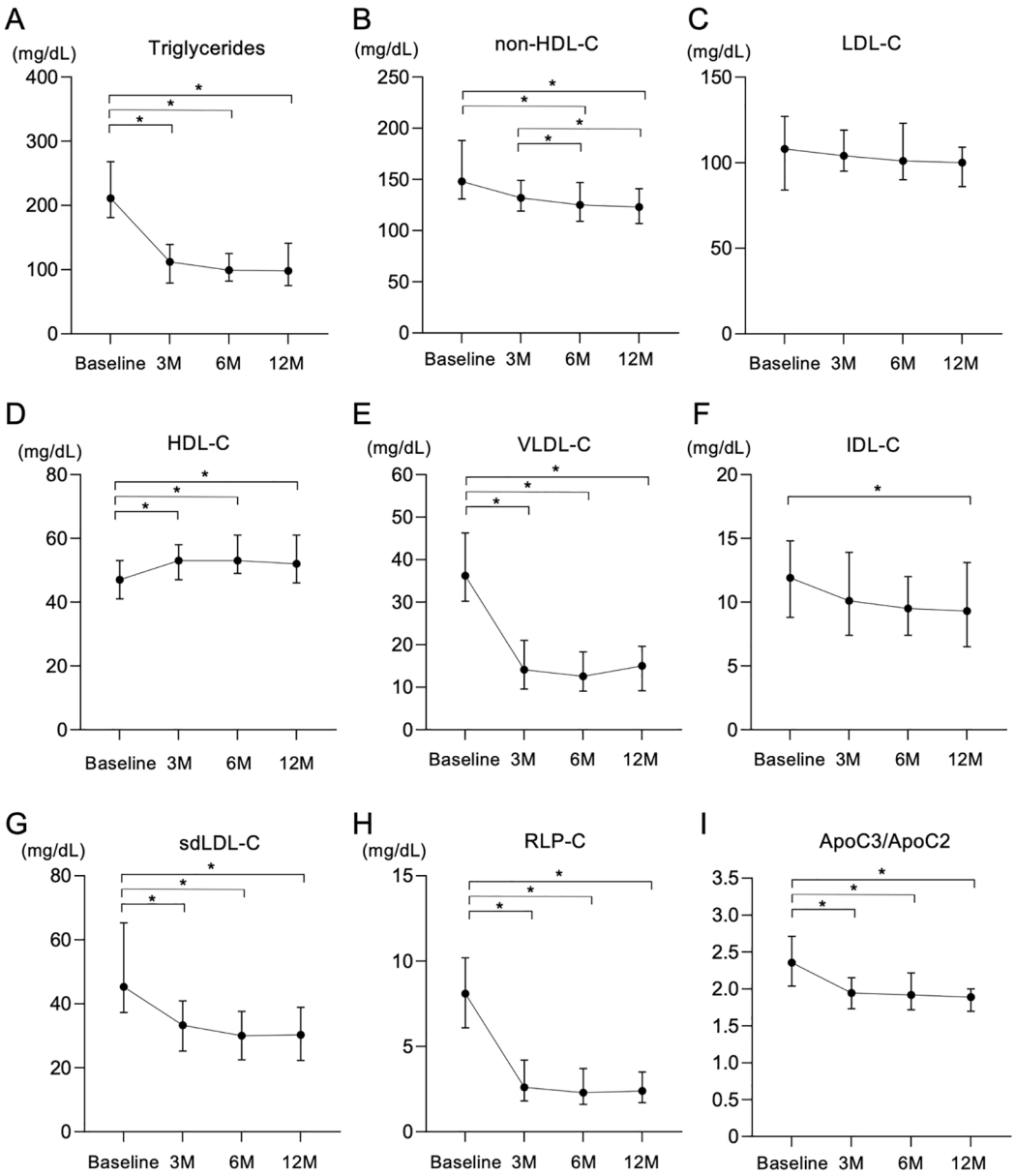

FIGURE 1
Changes in lipid-related parameters after treatment with pemafibrate for 12 months. **(A-I)** Changes in lipid-related variables including triglycerides (TG) **(A)**, non-high-density lipoprotein cholesterol (non-HDL-C) **(B)**, low-density lipoprotein cholesterol (LDL-C) **(C)**, high-density lipoprotein cholesterol (HDL-C) **(D)**, very low-density lipoprotein cholesterol (VLDL-C) **(E)**, intermediate-density lipoprotein cholesterol (IDL-C) **(F)**, small dense low-density lipoprotein cholesterol (sdLDL-C) **(G)**, remnant-like particle cholesterol (RLP-C) **(H)** and the ratio of apolipoprotein C3 to apolipoprotein C2 (ApoC3/ApoC2) **(I)** after treatment with 0.2 mg/day of pemafibrate for 12 months (M). Data are presented as medians with interquartile ranges. *p < 0.05 by the Friedman test with Dunn's *post-hoc* test.

levels of ApoA1 and ApoA2 and significantly decreased levels of ApoB, ApoC2, ApoC3 and ApoE. The ratio of ApoC3 to ApoC2 (ApoC3/ApoC2) significantly decreased after the strat of treatment with pemafibrate (Figure 1I).

Changes in liver-related markers after the start of treatment with pemafibrate

FLI, an index of hepatic steatosis, was significantly decreased by the treatment with pemafibrate for 12 months (Figure 2A). The improvement of FLI was observed in both men (Figure 2B) and women (Figure 2C). All of the constituent elements to calculate FLI including WC (Supplementary Figure S1A), BMI (Supplementary Figure S1B) and levels of TG (Figure 1A) and γGT (Supplementary Figure S1C) were significantly decreased by treatment with pemafibrate for 12 months. These results indicate that pemafibrate, primarily known as a TG-lowering agent, also improved all components of FLI.

There was no significant change in FIB-4 index, an index of hepatic fibrosis, after the start of treatment with pemafibrate, though the absolute value of FIB-4 index (median: 0.78-1.39) was low at baseline (reference value: < 1.3) (Supplementary Figure S1D). After treatment with pemafibrate for 12 months, the percentage of patients with MASLD was significantly decreased from 92.3% to 61.5% (Figure 2D).

Discussion

The present study showed that treatment with pemafibrate for 12 months significantly ameliorated FLI as a marker of hepatic steatosis as well as various lipid profiles as potent atherosclerotic risk factors including levels of TG, non-HDL-C, sdLDL-C, TRL-C, RLP-C, VLDL-C and IDL-C in Japanese patients with T2DM at a clinical practice level. It has been reported that pemafibrate has beneficial effects on hepatic function with a lower frequency of hepatic impairment than other PPARα agonists (48, 49) and that

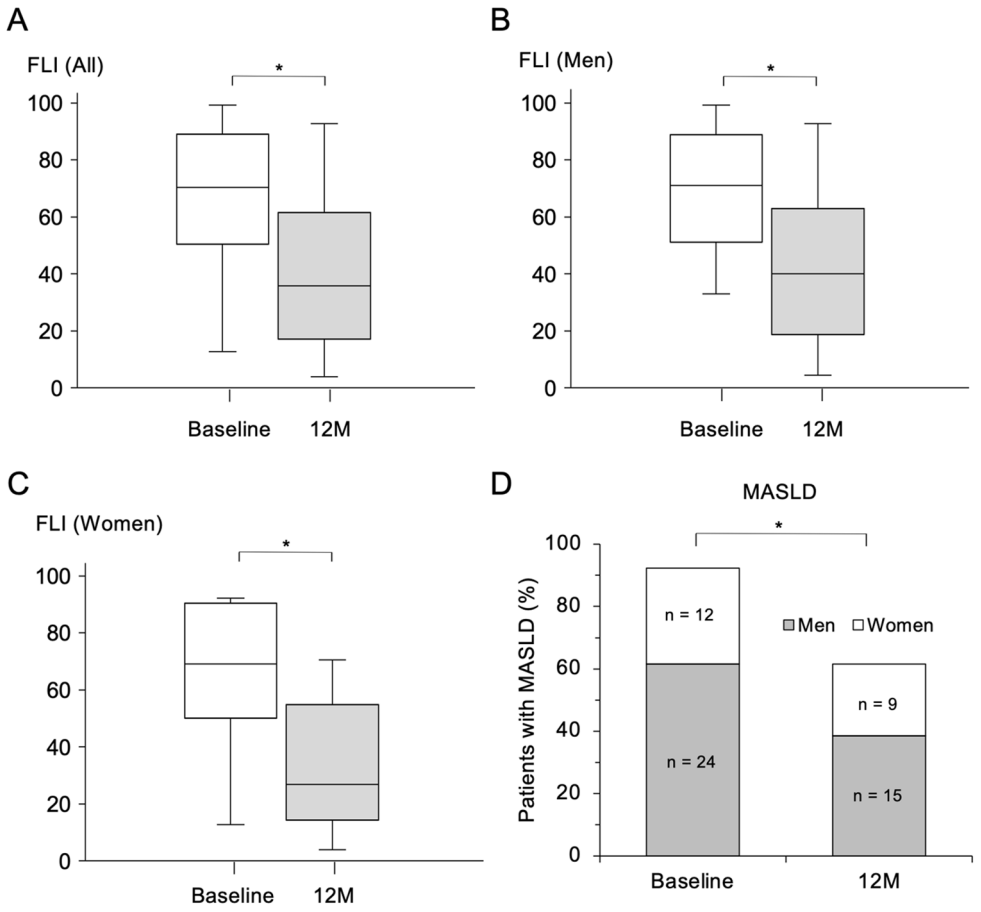

FIGURE 2
Change in FLI and prevalence of MASLD after treatment with pemafibrate for 12 months. **(A-C)** Comparisons of fatty liver index (FLI) before (at baseline) and 12 months (M) after the start of treatment with 0.2 mg/day of pemafibrate in all of the recruited patients (n = 39) **(A)** and in male patients (n = 24) **(B)** and female patients (n = 15) **(C)**. Data are presented as box-and-whisker plots. *p < 0.001 by Wilcoxon's signed-rank test. **(D)** Comparison of the prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) before the start of treatment (at baseline) and that at 12 M after the start of treatment with pemafibrate. *p < 0.001 by McNamer's test.

pemafibrate decreases markers of liver dysfunction including ALT and γGT and markers of liver fibrosis including FIB-4 index and the AST to platelet ratio index (50–57). Treatment with pemafibrate not only reduced the levels of TG, which may contribute to atherosclerosis partly via inflammation (9), but also markedly decreased the prevalence of MASLD estimated by FLI in the present study. However, there was no significant change in FIB-4 index, presumably due to the small number of patients with advanced liver fibrosis in the present study. It has been reported that pemafibrate increases fibroblast growth factor 21 (FGF21) (58), a favorable hepatokine. FGF21 analog is currently in the clinical trial stage and has been shown to increase adiponectin, a favorable adipokine, and to ameliorate insulin resistance in humans (59). An increased circulating level of FGF21 might be involved in the results of the present study.

It has been reported that the PPARα-activating effect of pemafibrate is more than 2,500-fold stronger than that of fenofibrate, a conventionally specific PPARα agonist, and that even a dose of 0.2 mg/day of pemafibrate can affect lipid metabolism (60). Indeed, levels of TG as well as ApoB, which reflects an atherogenic lipoprotein (61), were significantly reduced over time after the start of treatment with 0.2 mg/day of pemafibrate in the present study. The findings indicate at least one of the plausible reasons why levels of sdLDL-C and RLP-C, which are highly associated with levels of TG and ApoB (62–64), were significantly and dramatically reduced by treatment with pemafibrate. Although LDL-C was not changed by treatment with pemafibrate in the present study, the sdLDL-C/LDL-C ratio was consistently decreased (Table 3), suggesting that pemafibrate affects the level of sdLDL-C rather than the total LDL-C level. A recent study using data from the Copenhagen General Population Study to simulate the PROMINENT trial showed that the lack of cardiovascular benefit in the original trial might be explained by a simultaneous increase in LDL-C and ApoB despite a reduction in remnant cholesterol (41). On the other hand, in the present study, there was a decrease in level of TRL-C, which is equivalent to remnant cholesterol, without accompanying an increase in level of LDL-C or ApoB (Table 3). Although clinical outcomes were not investigated in the present study, these changes in lipid variables may have potential effects on the prevention of atherosclerosis.

Evaluation of the effects of pemafibrate on cardiovascular outcomes was conducted in the PROMINENT trial using statin-treated patients with T2DM who had a relatively long duration of DM (≥10 years: 46.4%), mild-to-moderate hypertriglyceridemia (fasting TG ≥ 200–499 mg/dL) and HDL-C levels ≤ 40 mg/dL (22, 26). The trial was discontinued after treatment for 16 weeks (partially 48 weeks) due to a lack of sufficient reduction in cardiovascular events by treatment with 0.2 mg/day of pemafibrate compared to the treatment with a placebo in the interim analysis (22). On the other hand, in the present study, we prospectively investigated changes in lipid and metabolic factors, adverse events and indices of liver damage over time for 12 months (52 weeks) in patients with T2DM who had a relatively short duration of DM (median duration: 5.0 years), although the number of study participants was small. It is difficult to compare the results of the PROMINENT trial designed as an event-driven study with a placebo control group and the results of the present study showing a rapid and long-term improvement in FLI and atherogenic risk factors. In a subgroup analysis of the PROMINENT trial, there was a trend toward fewer primary composite endpoint in patients with duration of diabetes < 10 years than in those with duration of diabetes ≥ 10 years (22). Furthermore, the number of patients with NAFLD was also decreased by treatment with pemafibrate in the PROMINENT trial (22). It is possible that earlier intervention with pemafibrate suppresses the progression of atherosclerosis as well as MASLD.

The level of Lp(a), a potential atherogenic factor (65), increased after the start of treatment with pemafibrate in the present study. It has been speculated that PPARα agonists increase the VLDL receptor in the liver and that Lp(a) can be taken up by hepatocytes, resulting in a decrease in Lp(a) (66). It has recently been shown in a crossover study that treatment with pemafibrate for 6 months decreased Lp(a) by -17.8% (20.4 ± 30.3 to 19.1 ± 23.9 mg/dL) (67). Although the precise reason for the increase in Lp(a) in the present study is not clear, the clinical significance of a subtle increase in Lp(a) (3.5 to 6.1 mg/dL) would be small since the cutoff value of Lp(a) as an atherogenic risk factor has been reported to be 30–50 mg/dL (45–47).

It has been reported that PPARα agonists downregulate the expression of ApoC3 (68–71), a possible factor that inhibits lipoprotein lipase activity (72), and that pemafibrate also decreases ApoC3 (73), which was consistent with the results of the present study. ApoC2, which promotes the function of lipoprotein lipase, was also decreased after the start of treatment with pemafibrate in the present study. However, the ApoC3/ApoC2 ratio was reduced by treatment with pemafibrate (Figure 1I) as previously reported (73, 74), possibly leading to an increase in lipoprotein lipase activity and a decrease in the level of TG. Further studies are needed to determine whether pemafibrate affects the regulation of both ApoC2 and ApoC3 and, if so, how pemafibrate affects the regulation.

Treatment with pemafibrate slightly, but significantly, decreased renal function in the present study, as was also observed in the PROMINENT trial (22). The change in renal function has been reported to be reversible and recovered by discontinuation of treatment with pemafibrate (75). The level of creatine kinase was not increased over time by treatment with pemafibrate, and rhabdomyolysis was not confirmed in the present study. In addition, during the follow-up period, there was no significant change in HbA1c, but there was a slight decrease in fasting glucose. These findings are consistent with the results of a previous study showing that pemafibrate is selective for PPARα with less adverse effects than those of conventional fibrates (67).

It is noteworthy that pulse rate in the enrolled patients was significantly decreased by treatment with pemafibrate (Table 3). It has been shown that an increase in resting pulse rate (or heart rate) is associated with autonomic dysfunction in patients with T2DM (76). While the effects of pemafibrate on reducing pulse rate have not been established yet, the finding in the present study suggests

that pemafibrate has the potential to improve autonomic nervous dysfunction independently of glycemic management in patients with T2DM. Further studies are warranted to investigate the causal relationship and underlying mechanisms between pemafibrate treatment and cardiac autonomic dysfunction in patients with T2DM.

There are several limitations in the present study. First, the sample size was small, and no placebo group was included because the present study was designed as a single-center prospective and real-world study conducted at a clinical practice level. Second, detection of SLD estimated by FLI as a surrogate marker, but not diagnosis by invasive liver biopsy samples and additional image modalities, was used in the present study. Third, since only Japanese patients were enrolled, the results obtained in the present study might not be applicable to other races. Fourth, although this study was conducted in patients with T2DM and hypertriglyceridemia as a high-risk group for ASCVD, the impact of pemafibrate should be evaluated in several conditions including less glycemic management and severe liver damage. Finally, while this study could not address the underlying mechanisms by which pemafibrate improves MASLD and atherogenic lipid profiles, its potential involvement in interorgan crosstalk as proposed in the emerging concepts of cardiovascular-kidney-metabolic syndrome (77) and the liver-spleen axis (78) warrants further investigation.

In conclusion, pemafibrate ameliorates MASLD estimated by FLI in addition to various atherogenic lipoid profiles in Japanese hypertriglyceridemia patients with T2DM. An early intervention with pemafibrate might contribute to prevention of the development of MASLD and ASCVD.

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 human ethics committee of Natori Toru Internal Medicine and Diabetes Clinic. 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

TOS: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. TAS: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. MT: Data curation, Formal analysis, Writing – review & editing. KN: Data curation, Writing – review & editing. KE: Data curation, Writing – review & editing. HA: Data curation, Writing – review & editing. WK: Data curation, Writing – review & editing. IH: Data curation, Writing – review & editing. AU: Data curation, Writing – review & editing. TO: Data curation, Writing – review & editing. YA: Data curation, Writing – review & editing. MF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

ToS received honoraria from Kowa Co. Ltd. TaS and MF received research grants and honoraria from Kowa Co. Ltd.

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2025.1496671/full#supplementary-material

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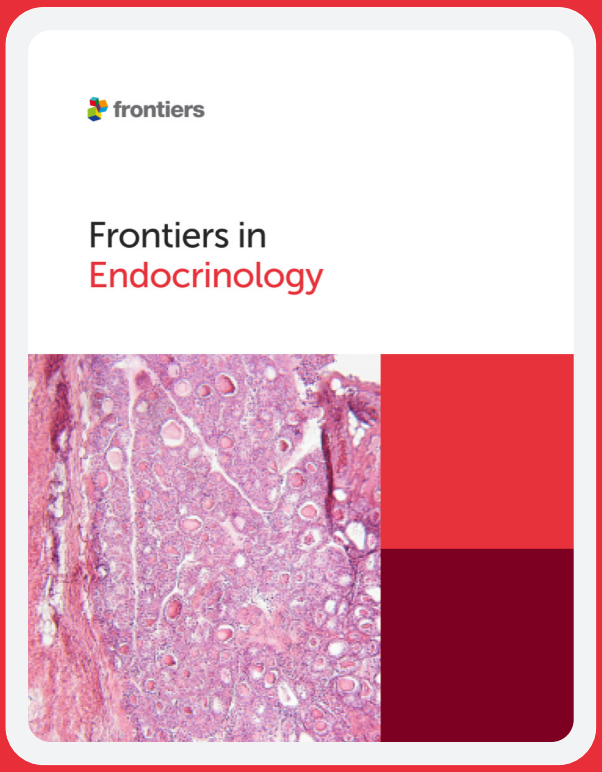

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